DEVELOPMENT OF A DIATOM INDEX FOR WETLAND HEALTH

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ABSTRACT

In a semi-arid country like South Africa, the availability and quality of water has always played an important part in determining not only where people can live, but also their quality of life. The supply of water is also becoming a restriction to the socio-economic development of the country, in terms of both the quality and quantity of what is available. To conserve, and improve the quality of what is already available, different monitoring techniques have to be implemented.

Water quality monitoring has traditionally been by the means of physico-chemical analysis; this has more recently been augmented with the use of biomonitoring techniques. However, since the biota commonly used to indicate aquatic conditions are not always present in wetlands; this study tested the use of diatoms as bio-indicators in wetlands.

Diatom samples were collected from thirteen wetlands in the Western Cape Province, and cells from these communities were enumerated and diatom-based indices were calculated using version 3.1 of OMNIDIA. These indices were useful for indicating water quality conditions when compared to the measured physico-chemical parameters. In addition, most diatom species found were common to those found in riverine environments, making the transfer of ecological optima possible.

The objective of the study was to provide a preliminary diatom-based index for wetlands, however, given the relatively small study area and the strong bias towards coastal wetlands it was deemed inadvisable to construct such an index, instead several indices are recommended for interim use until further research that more comprehensively covers wetlands in South Africa has been conducted. It is thus the recommendation of this study that more data is collected for comparison to other wetlands and that in the interim, indices such as SPI be applied for routine biomonitoring of these environments.

ABBREVIATIONS

- **AGU**   Agulhas

- **BDI**   Biological Diatom Index (Lenoir and Coste, 1996)

- **BOD**   Biological oxygen Demand

- **CCA**   Canonical Correspondence Analysis

- **COD**   Chemical Oxygen Demand

- **DEAT**   Department of Environmental Affairs and Tourism

- **DO**   Dissolved Oxygen

- **DRI**   Drift Sands

- **DWA**   Department of water Affairs and Forestry

- **EC**   Electrical Conductivity

- **EM**   Electron Microscope

- **EPA**   Environmental Protection Agency

- **EPI**   Eutrophication \ Pollution Index (Dell'Uomo, 1996)

- **FRAI**   Fish Response Assessment Index

- **FAII**   Fish Assemblage Integrity Index

- **GCV**   Glen Cairn Vlei

- **GDI**   Generic Diatom Index (Coste and Ayphassorho, 1991)

- **HAI**   Habitat Assessment Index

- **IHAS**   Invertebrate Habitat Assessment System

- **IHI**   Index of Habitat Integrity

- **KEN**   Kenilworth

- **LGV**   Lange Vlei

- **LM**   Light Microscope
- **LOT**   Lotus River

ABBREVIATIONS Continued.....

- **LPV** Little Princess Vlei

- **MFU** eMfuleni

- **MIRAI** Macro-Invertebrate Response Assessment Index

- **NBPAE** National Biomonitoring Programme for Aquatic Ecosystems

- **NTU** Nephelometric Turbidity Units

- **NWA** National Water Act (act 36 of 1998)

- **RHP** River Health Programme

- **RPM** Revolutions per Minute

- **RTV** Rietvlei

- **SASS** South African Scoring System

- **SEM** Scanning Electron Microscope

- **SHE** Scheifele and Schreiner's index (Scheifele and Schreiner, 1991)

- **SPI** Specific Pollution sensitivity Index (Coste in CEMAGREF, 1982)

- **TDI** Trophic Diatom Index (Kelly and Whitton, 1995)

- **TDS** Total Dissolved Solids

- **TWQR** Target Water Quality Range (DWAF, 1996)

- **VEGRAI** riparian Vegetation Response Assessment Index

- **WAT** Watanabe index (Watanabe *et al*., 1986; Watanabe, 1990)

- **WHI** Wetland Health and Integrity Research Programme

- **WRC** Water Research Commission

- **WVV** Wildevoël Vlei

- **ZDV** Zand Vlei

- **ZRV** Zoar Vlei

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CHAPTER ONE

INTRODUCTION AND STUDY AIMS

1.1 Introduction

Water is, and has always been a precious but scarce commodity. It is one of the most vital natural resources for all life on earth. The availability and quality of water has always played an important part in determining not only where people can live, but also their quality of life. Even though there always has been plenty of water on earth, water has not always been available when and where it is needed, nor is it always of suitable quality for all uses.

Approximately seventy percent of the surface of the planet is covered with water, and this is water found in the oceans, rivers, lakes and estuaries, with polar ice-caps and clouds also forming part of this seventy percent (Davies and Day, 1998). Although this seems like a lot of water, 97% of this is the salt water found in oceans, a further 2.2% is found in the glaciers and ice-sheets which when melted will only cause the sea levels to rise. Therefore, the proportion of water that is fit for human consumption (freshwater) makes up less than one percent of the total water covering the earth, and is made up of ground, surface, and atmospheric water (Davies and Day, 1998).

Atmospheric water serves as the earth's source of precipitation, and thus the only renewable water supply sustaining freshwater, estuarine, and terrestrial ecosystems (Davies and Day, 1998). Freshwater provides many benefits, such as water for drinking, industrial production, and irrigation. However, due to the development of cities and the increasing population numbers, municipal and industrial uses of water are rapidly increasing worldwide, thus exhausting the already limited supply of freshwater.

With an average annual rainfall of 450 mm (Davies and Day, 1998), as compared to a world average of 860 mm and an average annual evaporation of 1 100 mm to 3 000 mm, which is well in excess of the annual rainfall, South Africa may be considered a semi-arid country. The disproportional spread of rainfall throughout the country (dry in some areas e.g. plateau, and wet in others e.g. south east coastal areas) results in almost half of the population (mostly in rural areas) not having access to potable water (Davies and Day, 1998).

In 2004 the National State of the Environment Report of South Africa revealed that the country's existing freshwater resources are almost completely utilised and under strain (South Africa, 2004). Based on the population growth and economic expansion rates anticipated at that time, the report pointed out that it is unlikely that the estimated demand on water resources in South Africa will be sustainable.

The report envisaged that water will gradually become the limiting resource and will become a major constraint to the future socio-economic development of the country, in terms of both the quantity and quality of what is already available.

Population growth, increased economic activity and intensification of land use practices all lead to increased water demand, and an increasing degradation of the resource (South Africa, 2004, and Taylor *et al.,* 2007a). At present many water resources are polluted by industrial effluents, domestic and commercial sewage, acid mine drainage, agricultural run-off and litter (South Africa, 2004). The country's industrial, domestic and agricultural users are highly dependent on a reliable supply of water; as a result, large volumes of water are now being transferred from both inside the country and from neighbouring countries (the Lesotho highlands project) to supply the rapidly growing demands of industrial and urban centres (South Africa, 2004).

With water being such a limited resource in South Africa, new policies on how to manage the country's aquatic resources had to be developed and implemented. In 1998, the Department of Water Affairs and Forestry (DWAF now DWA) developed the South African National Water Act, number 36, (DWAF, 1998) which states that a water resource must be protected, conserved, managed and controlled in an equitable and sustainable manner for the benefit of all mankind. This act was founded on the principles of efficient service delivery and sustainable use of water resources, with primary requirements of the act being resource quality monitoring, assessment and a national information system in support of decision-making (DWAF, 1998). Thus, to ensure the productivity of these aquatic resources, the quality of water needs to be monitored, on a regular basis.

Water quality is a term used to describe the aesthetic, biological, chemical, as well as the physical properties of water that determine the sustainability and protection of aquatic ecosystems (DWAF, 1996).

The aesthetic properties describe the parameters that can be observed by senses, such as taste, litter, algal bloom, and odour. Biological properties describe the biodiversity (community structure) of the system; chemical properties include dissolved oxygen, conductivity as well as the presence and concentration of dissolved salts, pH, and metals, whereas physical properties of water include temperature and turbidity (DWAF, 1996). Thus water quality monitoring is a process whereby the above-mentioned properties of water are monitored and maintained at levels required to protect aquatic ecosystems.

Based on the properties mentioned above, water quality monitoring can be divided into biomonitoring and physico-chemical monitoring. Biomonitoring is a site-specific quantitative or qualitative process describing the biological status of aquatic systems, based on the reference (unimpacted) condition of the biological communities inhabiting a specific site (DWAF, 1996). It is a process whereby the ecological condition of a resource is studied by examining how the organisms living in a particular environment interact with their surroundings (Hohls, 1996). Physico-chemical monitoring on the other hand, is also a site-specific qualitative or quantitative process; however, it describes the physical and chemical status of the aquatic systems based on the presence as well as the concentration of specific variables (DWAF, 1996).

The monitoring of physico-chemical parameters of water quality is fundamental to the management of surface water as well as to the protection of aquatic biota (Damásio *et al.,* 2007). Traditionally, in South Africa, the quality of water has always been monitored by measuring the magnitude of physical attributes and the concentration of chemical substances in the water (Day, 2000). Although very accurate, these analyses only reflect conditions at the exact time of sampling, and due to the vast number of pollutants that may be present in the water, chemical analysis becomes very expensive and time consuming because there is no one standard method which can test for the presence of all the pollutants. Furthermore, the most toxic substances occur in minute quantities, often below the detection limits of even the most sophisticated analytical techniques (DWAF, 1996). These factors therefore led to the development of the National Biomonitoring Programme for Aquatic Ecosystems (NBPAE) by DWAF, the Water Research Commission (WRC) and the Department of Environmental Affairs and Tourism (DEAT) in 1996 (Minne, 2003).

The NBPAE makes use of biological indicators such as aquatic invertebrate communities, fish communities, and the riparian vegetation, to assess the condition or health of an aquatic system (Kleynhans, 1999).   The objectives of this programme were to design a programme which will monitor the integrity (health) of aquatic ecosystems throughout the country and thus provide information that can be used to manage water resources and aquatic ecosystems (Minne, 2003). The main advantage of this biological approach is that it examines organisms (such as fish and aquatic macro-invertebrates) whose exposure to water and any pollutants therein is continuous, and thus reflects the actual impacts (both long and short-term) of pollutants on the ecosystem (Hohls *et al*., 1996, and U.S.EPA, 2002a). This biomonitoring process is based on the fact that changes observed on the community structure after disturbances are the result of the competitive selection of the most tolerant species (Damásio *et al.*, 2006).

Physico-chemical monitoring therefore, studies the magnitude and concentration of variables available to organisms living in the environment, whereas biomonitoring is the study of the behavioural and physiological response of living organisms to their environment. Figure 1.1 below shows the current structure of water quality monitoring techniques carried out in the country.

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                        │ Water quality studies │
                        └─────────────────────┘
                  ┌──────────────┴──────────────┐
        ┌───────────────────────────┐    ┌──────────────┐
        │ Physico-chemical variables │    │ Biomonitoring │
        └───────────────────────────┘    └──────────────┘
         ┌──────────┴──────────┐                │
    ┌─────────────┐  ┌──────────────┐   ┌──────────────────┐
    │ • Conductivity│  │ ▪ pH          │   │ • FRAI, FAII     │┐
    │ • Nutrients   │  │ ▪ Turbidity   │   │ ▪ IHAS, SASS     │├ RHP
    │ • % Oxygen    │  │ ▪ Temperature │   │   & MIRAI        ││
    │ • Chem. & Org.│  │ ▪ Total       │   │ ▪ HAI & IHI      ││
    │   compounds.  │  │   dissolved   │   │ ▪ VEGRAI         │┘
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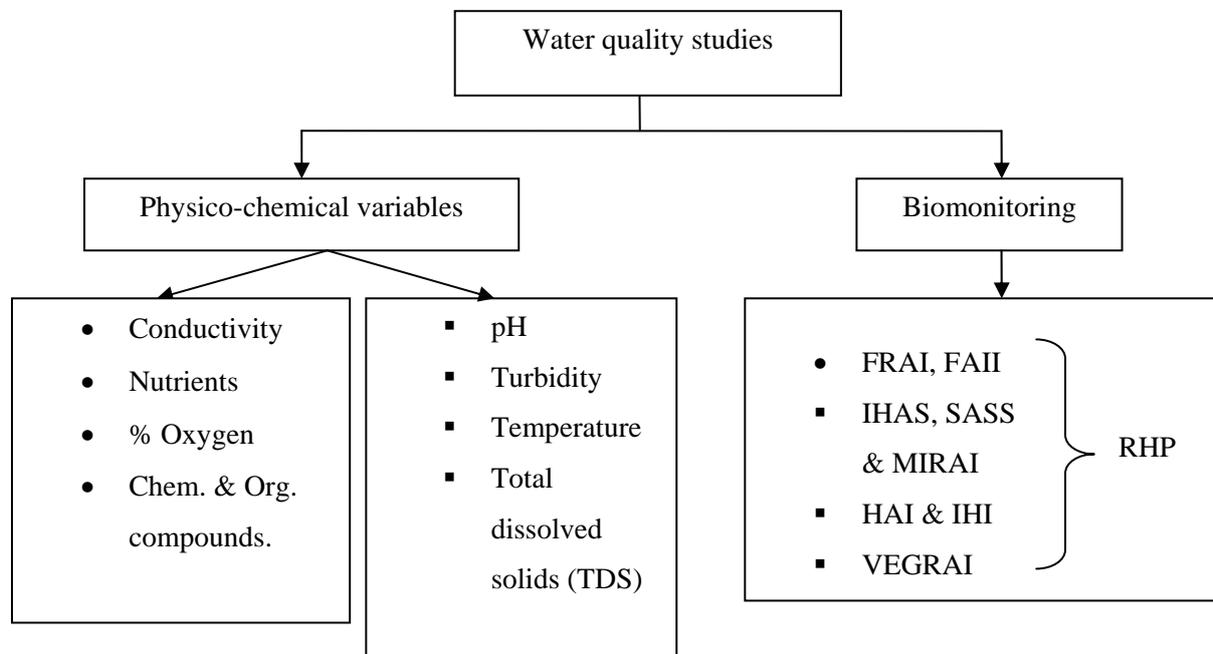
Figure 1.1 The structure of the current water quality monitoring techniques in South Africa.

The biomonitoring techniques listed in figure 1.1 form part of a national monitoring programme known as the River Health Programme (RHP), which was developed by DWAF, the WRC and DEAT in 1994. The river health programme uses in-stream and riparian biological communities (e.g. fish, invertebrates, and vegetation) to monitor the response of aquatic organisms to multiple disturbances (DWAF, 2008) in their environment. The rationale is that the integrity or health of the biota inhabiting the river ecosystems provides a direct and integrated measure of the health of the river as a whole (DWAF, 2008). Therefore, since fish are relatively long-lived and mobile, they make good indicators of long term influences on the general condition of aquatic systems, whereas macro-invertebrates which are short-lived make good indicators of short-term aquatic stressors (Dallas, 2005).

The Fish Response Assessment Index (FRAI) and the Fish Assemblage Integrity Index (FAII) make use of fish communities to assess the integrity of a system, whereas Invertebrate Habitat Assessment System (IHAS), Macro-Invertebrate Response Assessment Index (MIRAI), and the South African Scoring System (SASS) make use of aquatic invertebrate communities to measure the health of an aquatic system. The integrity or health of a system can also be measured by means of the riparian Vegetation Response Assessment Index (VEGRAI), which monitors changes in the structure and function of riparian vegetation (Dallas, 2005).

The Habitat Assessment Index (HAI) and Index of Habitat Integrity (IHI) on the other hand are used to assess the number and severity of anthropogenic perturbations (such as pollution, water abstraction, building of weirs, and dams, as well as biotic factors, such as the presence of alien plants and aquatic animals) and the damage they potentially inflict on the habitat integrity of the system (Dallas, 2005). The success of these biomonitoring techniques relies on the availability of biota (such as fish and macro-invertebrates) commonly used to indicate aquatic conditions; thus limiting their application in systems such as wetlands where the environment is rather different.

The nature (seasonal and annual variations) of the hydrology of wetlands plays an important role in the type of biota found within these systems (Chipps *et al.,* 2006). This heterogeneous nature makes it difficult for the identification of common indicator species (such as fish, and aquatic invertebrates) in these systems (Brazner *et al.*, 2007). However, even though they have a different environment, wetlands are an important water resource worldwide.

A wetland is any part of the landscape where the accumulation of water occurs often and long enough to influence the plants, animals and the soil occurring in that area (DWAF, 2004). Thus ecosystems such as bogs, coastal lakes, estuaries, floodplains, mangroves, marshes, mires, moors, pans, peat lands, seeps, sloughs, springs, swamps; vlei's and wet meadows can be included in the term wetland (DWAF, 2004). Wetlands make up approximately 6% of the world's land surface (DWAF, 2004), and they are found in every climate, from the tropics to the frozen tundra. The South African National Water Act, no 36 of 1998 states that a wetland is "that land which is transitional between a terrestrial and an aquatic system, where the water table is usually at or near the surface or where the land is periodically covered with shallow water and usually inhabited by hydrophytic vegetation" (DWAF, 1998). Wetlands are areas of intimate relationship between the land and water, with distinctive hydrological (e.g. water storage), biogeochemical (e.g. removal of elements), and physical (habitat) properties, as well as intricate biological food web compositions found nowhere else (Cowan, 1995).

Wetlands moderate water quality and quantity, they reduce floodwater velocity and therefore lessen the damage caused by floods, particularly erosion (U.S. EPA, 2002a). They act as filters by trapping sediments and nutrients, and in addition trap pollutants such as heavy metals and pesticides, thereby improving the quality of the water (Swanepoel *et al*., 2007, and U.S. EPA, 2002a). Furthermore, wetlands have diverse ecological attributes and provide important ecosystem services such as water storage, biogeochemical cycling and the maintenance of biodiversity and biotic productivity (Stevenson *et al.,* 2002), and they also sustain habitats containing large numbers of endemic and threatened species (Cowan, 1995).

Wetlands also act as important sources of crustaceans, fish and other food for people, they provide housing materials and medicinal plants, wetlands may also provide a source of water that sustains agriculture, industries, towns and cities (Cowan, 1995).

Some wetlands provide an area where marine, fresh water and terrestrial animals interact, therefore, supporting an enormous variety of biota with some of these organisms surviving nowhere else (Cowan, 1995). Therefore to guarantee the conservation of the biodiversity found within wetlands, these aquatic ecosystems have to undergo regular assessments to help ensure their equitable management and sustainable use.

Although wetlands have many important ecological benefits, their uses were overlooked, and without affording these systems any kind of conservation or protection by law, they were perceived as impediments to development, as productive land suitable for agriculture, and practices such as draining and infilling as well as other forms of destruction were deemed accepted worldwide (Cowan, 1995, U.S. EPA, 2002a, and DWAF 2004).

In South Africa alone, almost 35-50% of the wetlands were lost (Swanepoel *et al*., 2007) or severely degraded as a result of unsustainable social and economic pressures where these ecosystems were viewed as excellent systems for water abstraction, drainage, grazing, sewage waste disposal, mining (including peat mining), and cultivation (DWAF, 2004). The importance of these systems was realised only recently through the Convention on Wetlands of International Importance especially as Waterfowl Habitat, held in Ramsar, Iran in 1971, now commonly known as the Ramsar Convention.

The Ramsar convention is a governmental treaty that provides the structure for international collaboration for the preservation of wetland habitats (Cowan, 1995). The objectives of which are to stem the worldwide loss of wetlands; to ensure effective conservation and management (wise use) of all wetlands, to promote special protection of listed wetlands as well as to promote the implementation of parties' obligations under the convention (Cowan, 1995 and DWAF, 12004).

As a signatory of the Ramsar convention, South Africa is bound to incorporate wetland conservation into its state policy and to ensure active measures are taken to meet the requirements of the convention (Cowan, 1995). Thus it can be said that the Ramsar convention bought about a "paradigm shift" in terms of how wetlands were perceived prior to the convention. Wetlands are now considered to be the third most important systems that can sustain life on the planet (Cowan, 1995).

Many wetlands do not have a channelled bottom and the water may be dispersed in several discrete areas within the wetland. In addition, water is usually shallow and may not be deep enough to support fish and other large organisms. In such an environment which might be dominated by microscopic organisms, it is difficult if not impossible to use the current biomonitoring techniques such as FRAI and SASS to assess the integrity of the wetland.

Thus for South Africa to comply with both national and international legislation, methods for monitoring the quality of all aquatic resources (especially wetlands) must be developed and implemented so as to maintain the sustainability and thus ensure the conservation of these resources (Bowd, 2005). A number of water quality monitoring techniques already exist; however, most of these techniques were developed for the assessment of riverine ecosystems. Therefore to study the water quality in areas such as wetlands, another technique which uses microscopic organisms for monitoring is proposed.

The bioassessment of wetlands is based on the premise that the biota found within the wetland will integrate the effects of both short-term and intermittent stressors and thus reflect the overall biological integrity of the wetland (U.S. EPA 2002a). There is a large amount of information available on methods which were developed globally for the assessment of wetlands, but there is no single method which can be applied to every situation (there is no standard), especially for water quality studies. Bioassessments using macroinvertebrates and algae have been well tested for rivers and streams, but the study is still in its developmental stages in wetlands (Weilhoefer and Pan, 2007). This study therefore, focuses on the use of algae, especially diatoms to infer water quality. The use of algae as indicators has been studied for almost a hundred years now (Kitner and Poulíčková, 2003) but its implementation has been rather slow; especially in the developing countries.

Algae (including diatoms) and other microscopic organisms attached to submerged surfaces occur in most shallow aquatic habitats where there is sufficient penetration of light. In most wetlands, these aggregations of algae known as periphyton grow attached to submerged substrata such as sediment, woody and herbaceous plants and rocky substrata. Because of their high dispersal rates, rapid growth rate and their direct response to environmental changes, algae provide the first indication of changes and are thus one of the most widely used indicators of biological integrity and physico-chemical conditions in aquatic ecosystems (Kwandrans *et al.*, 1998, U.S. EPA, 2002b).

Algae play important roles in wetland function and can be valuable indicators of biological integrity and ecological conditions of wetlands (U.S. EPA, 2002b). However, since diatoms (a group of algae) are ubiquitous in nature, they can be tested as indicators of wetland water quality, and if successful, used to infer wetland integrity. Studies of diatoms which were conducted in wetlands have thus far shown strong correlations between changes in physical and chemical parameters with diatom composition (Lane, 2007).

The first written record of diatoms (published in the *Philosophical Transaction*s, by the Royal Society of London) was the description of "pretty branches, composed of rectangular oblongs and exact squares adhering to the roots of the pond weed *Lemna",* which were observed in 1703 by an unknown Englishman using a simple microscope (Minne, 2003).

These "pretty branches" were later described as the diatom *Tabellaria flocullosa* (Minne, 2003). Diatom taxonomy increased towards the end of the 18th century; however, the interest in diatoms soared during the latter half of the 19th century when microscopes became readily accessible (Minne, 2003). Diatoms, which constitute approximately 40% of any algal community, are unicellular, occasionally filamentous algae belonging to the group Bacillariophyceae, characterized by having a cell wall composed of silica (Sládeček, 1986). They are characterised by chloroplasts containing the auxiliary photosynthetic pigment known as fucoxanthin, giving diatoms their yellowish-brown colour. They are estimated to contribute at least twenty percent of the global annual primary productivity (Lopez *et al.,* 2005).

Diatoms are found throughout most aquatic, sub-aerial and terrestrial habitats, and they have been classified into different groups based on their preferred habitat as shown in table 1.1 below.

Table 1.1 Classification of diatoms based on their different habitats, (adapted from Minne, 2003 and Taylor, 2007b).

Habitat	Description
Endolithon	Usually found on, and penetrating into soft (often calcareous) rock and in pores and crevices.
Endopelon	Grow in mucilage tubes and motile species living beneath the surface on sandy shores.
Endophyton	Occur in cavities within various macroscopic plants
Endopsammon	Live beneath the surface of sand in lakes and estuaries.
Endozoon	Grow within animals.
Epilithon	Occurs in areas where water movements are sufficiently strong, such as in streams and rivers.
Epipelon	Found moving on and in the surface sediment, they grow on sand and silt in areas of slow moving water in streams and rivers.
Epiphyton	Attach to macrophytic plants.
Epipsammon	Occur on and between sand particles.
Epizoon	Attach to shells and surface of animals

Other diatom species are also frequently found growing on artificial substrates such as ships, hulls, or on any other object placed in the water, this is known as fouling (Minne, 2003). As micro-organisms, diatoms lack dispersal barriers (Finlay *et al*., 2002), and may be transported by wind, aerosols, by wading birds and may even survive passage through insect's digestive tracts. Furthermore, many hundreds of thousands of cells may be produced within a few square centimetres of a wetland environment and this also adds to the ease with which they are dispersed (Finlay *et al*., 2002).

The implication of this is that an area with more or less homogenous water quality will have more or less homogenous diatom communities, i.e. even if discrete pools within a wetland are spatially dispersed but still share similar properties, a single diatom sample from any one of the pools should provide an adequate reflection of the water quality in the wetland as a whole (Finlay *et al*., 2002).

Diatom communities react rapidly and specifically to changes in environmental conditions such as eutrophication, organic enrichment, salinisation and they are the most sensitive indicators of changes in pH (Battarbee *et al.,* 1997, and Rott *et al.,* 1998). Their rapid growth rates enable experimental manipulation of environmental conditions to determine cause-effect relationships between diatomic response and specific environmental stressors (U.S. EPA, 2002b). These organisms which occur in all types of aquatic ecosystems are primary producers and actively integrate nutrients and other components of water quality (Morales *et al.,* 2001).

The use of diatoms for bioassessment in wetlands may provide a valuable tool to infer water quality, based on the following advantages of diatoms:

- The ecological optima and tolerances for many diatom species are well documented (Morales *et al.,* 2001).
- Diatom assemblages are regulated by environmental conditions, such as the availability of nutrients (Pan *et al*., 1996).
- Each species in an association has its own particular ecological requirements (U.S .EPA, 2002b).
- They are diverse and are represented in various habitats within an aquatic ecosystem and its surrounding drainage basin (Morales *et al.,* 2001).
- These organisms are very easy to sample and identify, and permanent records can be made from each sample collected (Round, 1991, and 1993, and Morales *et al.,* 2001).
- They differ from fish and macro-invertebrates in that, in general, they do not need any specialised food, habitat, depth or velocity of water (Round, 1993).

As the cell walls of diatoms are composed mostly of silica, they can remain preserved for years (Morales *et al.,* 2001), and thus they provide a year round approach for assessing the ecological integrity of wetlands, thus providing a basis for developing regulatory decisions when other organisms are not present (U.S.EPA, 2002b). Furthermore, when removed in a core from the sediment, these preserved cell walls or frustules may also be used to trace the history of a wetland (Taylor *et al.,* 2005).

In addition to the above mentioned advantages, the use of diatoms as indicators of water quality is also cost effective (Lane, 2007). Since a general water quality indicator should be easy to use, readily accessible and generally not expensive, diatoms are therefore, a valuable addition to a water quality monitoring programme.

The use of diatoms to support decision making in fresh water management has increased over the past two decades, with the development of diatoms indices which are now used to provide information on nutrients, acidification, eutrophication, organic pollution and general water quality. However, according to Kelly *et.al.,*(2008), these approaches are appropriate at determining the intensity of particular types of pollution, and are not suitable for assessing ecological status (the quality of the structure and functioning of the ecosystem) as they do not allow for the comparison of a water body with that expected in the absence of anthropogenic disturbance.

Diatoms are an integral constituent of the aquatic biota, thus the multiplication or inhibition of species living at any time at a given locality, is influenced by changes in the physico-chemical conditions of the surrounding waters, thus changing the percentage composition of certain species within a community (Taylor *et al.*, 2006). The physico-chemical parameters influencing the growth and thus, distribution of diatoms have been extensively studied to identify conditions of optimum growth (Patrick, 1971, de Almeida and Gil, 2001, Nishikawa *et al.*, 2006, and Montagnes and Franklin 2001), thus, factors such as light, the presence of trace elements, temperature and the availability of vitamins are important for the growth of diatoms.

In their studies Montagnes and Franklin (2001), and Patrick (1971) both showed that temperature is important in the growth rate of diatoms, whereas, in 2005 Leblanc *et al.* showed that trace metals such as Fe, and Zn, are important for growth, and that their limited availability will result in the modification of the diatom cell size, or species composition. Although these parameters are important, it is however factors such as salinity, pH, oxygen availability, and the availability of nutrients that account for a considerable part in the composition of diatom communities (van Dam, 1974). Agreeing with van Dam, de Almeida and Gil (2001) also confirmed that conductivity, pH and chemical oxygen demand (COD) are the most influential variables in the distribution of diatoms. Thus, their ubiquitous nature, diversity as well as the specificity of their individual taxa, makes diatoms good indicators of ecological conditions (Brazner *et al.,* 2007).

The potential applicability of diatoms as indicators of ecological conditions has resulted in the extensive study of their biology, ecology, and community structure. As discussed earlier in the chapter, studies on these organisms have shown their applicability as indicators of environmental variables in different fresh water ecosystems, from lakes and ponds (Lim *et al.,* 2001), wetlands (Chipps *et al.,* 2006 and Lane, 2007), to rivers and streams (Kelly *et al.,* 1995, Kwandrans *et al.,* 1998, Taylor, 2004) as well as on dry systems where they can be used to infer past events (US.EPA, 2002b).

The possible use of diatoms as indicators of water pollution was demonstrated by researchers such as Cholnoky (1968), who studied the significance of oxygen and organic nitrogen content of water in the distribution of diatoms, as well as Hustedt (1939), who developed a pH-classification system for diatoms and, in 1957 studied the influence of organic pollution on the composition of diatom communities (van Dam, 1974).

In South Africa, the use of diatoms as indicators of water quality was first studied during the early 1950s to the late 1980s by Cholnoky, Archibald and Schoeman, who described many species occurring throughout South African rivers (Taylor, 2004). They were again only recently studied in depth in 2002 by Bate *et al.* (Taylor *et al.*, 2006). Diatoms have since been studied extensively in South African rivers, with prospects of developing a diatom index to be introduced into the NBPAE as an addition to the current biomonitoring techniques (DWAF, 2008).

The design of software programmes for the calculation of diatom indices has also facilitated the use of diatom based biomonitoring methods. According to Taylor (2004), the functioning of diatom indices is based on the fact that in a sample from a body of water with a particular level of determinant (e.g. salinity), diatom taxa with their optimum close to that level will be most abundant. He continues to explain that an estimate of the level of that determinant in the sample can be made from the average of the optima of all the taxa in that sample with each sample weighted by its abundance, meaning that a taxon that is found frequently in a sample has more influence on the result than the one that is rarely found. The index therefore is expressed as the mean of the water quality optima (the tolerance limits of diatoms to water quality variables) of the taxa in the sample, weighted by the abundance of each taxon. One such program which was designed for the calculation of diatom indices is OMNIDIA (Lecointe *et al.*, 1993).

OMNIDIA can calculate up to seventeen indices, where some of the indices are based on the weighted average of the Zelinka-Marvan equation (1961) shown below:

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

Where **aj** = abundance (proportion) of species **j** in sample,
vj = indicator value and
sj = pollution sensitivity of species **j**.

Each diatom species used in the calculation/equation is assigned two values; the first value reflects the tolerance or affinity of the diatom to a certain water quality (good or bad) while the second value indicates how strong (or weak) the relationship is (Taylor *et al.*, 2006).

The performance of the indices depends on the values given to the constants **s** and **v** for each taxon and the values of the index ranges from 1 to an upper limit equal to the highest value of **s** (Kelly *et al.*, 1995). These values are then, in addition, weighted by the abundance of the diatom in the sample i.e. how many of the particular diatom in the sample occurs in relation to the total number counted. According to Denys, (2004) abundance-weighted averages of the species indicator values provide a more integrated basis for site comparisons, condition estimates and trend monitoring of water.

The Omnidia database contains over 12 000 diatom taxa (together with synonyms), out of which the ecological sensitivity and indicator values are characterised for about 1800 (Ács *et al.*, 2004). Some of the indices included in the database are listed below:

➢ **SHE** = Schiefele-Schreiner index (Schiefele and Schreiner, 1991), categorises 386 species into 7 groups according to their trophic state and pollution resistance.

➢ **WAT** = This is the index by Watanabe (Watanabe *et al.*, 1986), its other name is DAIpo (Diatom Assemblage Index to organic pollution), which classifies 226 taxa on the basis of their pollution tolerance (biological oxygen demand).

The following indices are based on the Zelinka-Marvan (1961) equation:

➢ **DES** = Descy's (1979) index, classifies 106 species into 5 sensitivity classes.

➢ **SLA** = the index of Sladeček (1986), which classifies 323 species into 5 sensitivity classes.

➢ **L&M** = this is the index by Leclercq and Maquet (1987), and it classifies 210 species into 5 sensitivity categories.

➢ **ROT** = Rott's index (Rott, 1991), has five sensitivity classes, primarily on the basis of saprobiological preferences.

➢ **IDAP** = Diatom Index Artois-Picardie (Prygiel *et al.*, 1996), was developed for the French Artois-Picardie region, and it classifies diatom species into five categories.

➢ **SPI** = Specific Pollution Sensitivity index (Coste in Cemagref, 1982), uses every species from the database and categorises into five sensitivity groups.

➢ **GDI** = Generic Diatom Index: (Coste and Ayphassoro, 1991). This index uses five sensitivity classes, for which diatoms need to be identified only at the genus level. It uses every freshwater species and genera from the database.

➢ **BDI** = Biological diatom Index (Lenoir and Coste, 1996). This is also primarily a practical index, as it treats the morphologically related taxa as one group and composes so-called associated taxa.

This index was also standardised from sampling through sample preparation to microscopical analyses (identification and enumeration) it uses 209 species from the database.

➢ **CEE** = the index of Descy and Coste (1991), uses 208 species.

➢ **EPI-D** = Eutrophication Pollution Index Diatoms (Dell'Uomo, 1996), classifies into five categories.

➢ **TDI** = Trophic Diatom Index (Kelly, 1998), classifies into five sensitivity categories. This index is widely used in the United Kingdom, it is appropriate for the qualification of strongly polluted waters, where wastewater input is significant.

The %PT (Pollution Tolerant Taxa %) is connected to the TDI index; and it gives the percentage of pollution tolerant taxa in the given sample (Kelly *et al.,* 1995).

Of all the above mentioned indices, most of the work has been done on the Specific Pollution sensitivity Index, the Generic Diatom index, the Biological Diatom Index, as well as on the Trophic Diatom Index (Kelly *et al.,* 1995). The SPI and GDI were originally developed as indices of organic pollution, whereas TDI was developed as an index for measuring inorganic nutrient concentrations (Kelly *et al.,* 1995). The SPI is the most comprehensive index with values of *s* and *v* available for over 1300 species whereas GDI is based only on 44 genera (Kelly *et al.,* 1995). GDI is the easiest index to use as it only requires identification to the genus level, thus, making it useful for providing initial indication of a polluted aquatic system (Taylor, 2004). BDI has a better relationship to water quality and as mentioned above, only requires the identification of 209 key taxa; this index has a high level of reliability due to the extended period and wide geographical range of testing (Taylor, 2004).

It is highly important for indices to be designed in a way which makes it possible for the data to be interpreted into information useful for management purposes (Kelly *et.al* 1998), hence the development of the Omnidia software, which calculates index scores for all the indices.

Table 1.2 Diatom index scores indicating different water quality classes (adapted from de la Rey *et. al.,* 2004).

Class	Index score
High Quality	>17
Good Quality	15-17
Moderate Quality	12-15
Poor Quality	9-12
Bad Quality	< 9

Table 1.2 above shows the interpretation of the index scores as calculated by Omnidia. The scores range from zero to twenty, (with the exception of TDI) where a score of zero indicates bad quality water whereas, and score of twenty indicates high quality / pristine water (de la Rey *et. al.,* 2004).

The TDI scores range from zero to a hundred, where a score of 0 indicates low nutrient concentrations and a score of 100 indicates high nutrient concentrations (Kelly, 1998). In addition, %PT estimates the influence of organic pollution on the indication of eutrophication at the studied sites. Since taxa generally tolerant to organic pollution are usually abundant at sites with elevated levels of phosphorus, %PT is calculated as the sum of cells belonging to these taxa (Kelly, 1998). As a result, %PT is an indicator of the reliability of the TDI as a measure of eutrophication at a site, where values lower than 20% of the total count indicate that organic pollution is either absent, or its effects are mild (Kelly, 1998).

In a country where the demand for water is beginning to exceed its supply, South Africa is definitely no exception to the global need for good quality water (Bate, 2004). As established earlier in the chapter, every drop counts, hence all water resources must be protected and preserved for future sustainability. Therefore, regular monitoring of aquatic systems is required for the evaluation of general water quality (Taylor *et al*., 2006).

Thus, in addition to maintaining the overall integrity of the system, one of the objectives of water quality monitoring is to manage and minimise the frequency of pollutant-oriented problems, thus supplying water of appropriate quality to serve agricultural, commercial, and domestic purposes (Boyacioglu, 2006).

Water quality monitoring, has traditionally measured the magnitude and concentration of physico-chemical variables in aquatic systems (Taylor *et al*., 2006). Therefore, since these variables are influenced by processes (such as precipitation, evaporation, drought, agricultural run-off, storm water drainage and other factors) that take place in the system, physico-chemical monitoring becomes a "snapshot" of the quality of water at the time of sampling. Thus, since the possibility of measuring the multitude of physico-chemical stressors that could affect aquatic ecosystems was deemed both ecologically and economically unfeasible (U.S. EPA, 2002a), other monitoring methods were developed. One such method is the biomonitoring technique which monitors the response of living organisms to their environment.

These monitoring techniques were initially developed for riverine ecosystems where they proliferated and evolved rapidly in accordance with the greater research and conservation attention given to these systems compared to wetlands (Bird, 2009), which were considered to be of no ecological or economical importance.

However, since the Ramsar Convention brought a turning point in the conservation of wetlands, these ecosystems now benefit from having monitoring techniques initially developed for rivers forming the foundation for their own assessment techniques (U.S. EPA, 2002a). The above statement is also true for this study, in which diatom indices (which were initially developed for riverine assessments) were tested for their applicability in South African wetlands.

Diatoms are virtually found everywhere, and they respond rapidly to fluctuations in physico-chemical variables, as well as to disturbances (such as floods or droughts) occurring in their environment, thus their presence and abundance should reflect current ecological conditions, as well as infer the effects of previous drainage disturbances (Mayer *et al*., 2001).

Since the biota commonly used for biomonitoring (fish and aquatic macroinvertebrates) may not always be present in wetlands, this study proposes using diatoms as indicator species for water quality monitoring in South African wetlands.

1.2 Aims of the study

The objectives of this study are to document the distribution of diatoms in different wetlands as well as to correlate diatom community structure to water quality variables in the selected wetlands. Furthermore, it is to test for the applicability of European and other diatom indices in South African wetlands.

The main aim of the current study is to contribute to the development of a wetland water quality assessment technique for use in South Africa.

Therefore to achieve the above mentioned objectives, the following two questions were asked:
- Since diatoms are virtually found everywhere, are the diatoms found in wetlands the same as those commonly found in rivers? and
- Can the diatom indices currently used in rivers and streams be applied successfully in wetlands?

1.3. References

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CHAPTER TWO

MATERIALS AND METHODS

2.1 Study sites

All diatom samples were collected from palustrine wetlands around the Western Cape area. The sites sampled are found within the Cape flats and southern suburbs of the Cape Town metropolitan area, and along the Agulhas plains, on the south coast of the province.

Figure 2.1 below, shows the estimated location of the sampling sites, whereas Table 1 & 2 in Appendix A, give detailed information such as GPS (latitude and longitude) co-ordinates, the date of sampling as well as the type of substratum sampled.
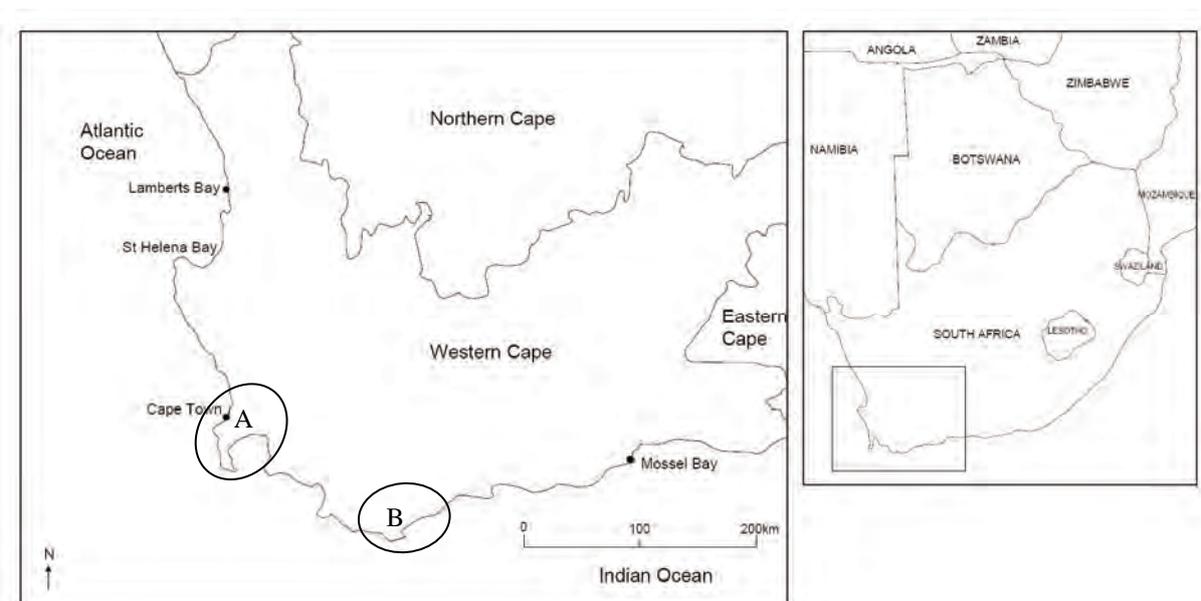
Figure 2.1 The estimated location of the wetlands sampled within the Western Cape area, South Africa. A: Cape Town Metropolitan Area B: Agulhas plains. (Map adapted from Bird, 2009).

Circle A on the map represents twelve wetlands sampled within the greater Cape Town metropolitan area, the sampled wetlands are Die Oog, Drift Sands, eMfuleni, Glen Cairn Vlei, Kenilworth, Lange Vlei, Little Princess Vlei, Lotus River, Rietvlei, Wildevoël Vlei, Zand Vlei and Zoar Vlei, and circle B-represents sites sampled along the Agulhas plains.

2.2 Sampling procedure

Samples were collected from diatom communities growing on either aquatic macrophytes or on sediments, however, where both substrates were found, then a sample from each substrate was collected. Diatoms were collected from three habitats (emergent vegetation, submerged vegetation, and open water) in each sampling site. The only systems that were excluded from the study were those that were completely dry at the time of sampling.

For ease of data analysis, the wetlands sampled were divided into two groups based on the number of water samples collected per year, as shown in Table 2.1 below.

Table 2.1 The two groups of wetlands based on the number of water samples collected per year.

	Abbreviation of wetland	Name of wetland	Number of water samples
Samples from the City of Cape Town.	OOG	Die Oog	Water samples were collected once every month for a period of three years.
	GCV	Glen Cairn Vlei	
	LGV	Lange Vlei	
	LPV	Little Princess Vlei	
	WVV	Wildevoël Vlei	
	RTV	Rietvlei	
	ZDV	Zand Vlei	
	ZRV	Zoar Vlei	
Samples from the WHI programme.	AGU	Agulhas	Water samples were collected once-off on the day of diatom sampling.
	DRI	Drift Sands	
	eMFU	eMfuleni	
	KEN	Kenilworth	
	LOT	Lotus River	

The analysis of water samples from the City of Cape Town were carried out in their scientific laboratory according to standard methods for water quality analysis. These water samples were collected on a monthly basis (as stipulated in table 2.1 above), for a period of three years (from January 2005 to December 2007), for the measurement of variables such as conductivity, dissolved oxygen, nutrients, pH, temperature and turbidity, and water samples from the other wetlands were collected concurrently with the sampling of diatoms.

The analysis of water samples (collected between August and September) from the WHI Programme were carried out in the WHI laboratory according to the methodology stipulated in section 2.2.1 below (physico-chemical analysis).

2.2.1 Physico-chemical analysis (adapted from Bird, 2009)

In situ physico-chemical parameters were measured during the day, at a standardized depth of 30cm at each habitat where diatoms were also sampled; producing three sets of measurements per wetland. The average for the three habitats was calculated and the mean value was used for further analysis.

The physico-chemical variables were measured with different instruments, where a Crison pH25 meter was used to measure pH, a Crison OXI45 oxygen meter was used to measure the dissolved oxygen ($mg.L^{-1}$), a Crison CM35 conductivity meter was used to measure the electrical conductivity ($\mu S.cm-1$); and a Hach 2100P turbidimeter was used to take turbidity (NTU) readings from the water column at two randomly selected points in the wetland.

Water column nutrient concentrations were measured at each site to facilitate the comparison of wetlands based on their trophic status. At each site, five (1L) surface water samples were randomly collected, and these samples were combined to make up one 5L sample.

This bulk 5L sample was carefully mixed and sub-sampled to obtain a 200ml sample which was analysed for nutrient concentrations (NO_2, NO_3, NH_4, and PO_4) using a Lachat Flow Injection Analyser, as described below:

- NH_4 was measured using Lachat's QuikChemR Method 31-107-06-1, based on the Berthelot reaction in which indophenol blue is generated;
- NO_3 and/or NO_2 were estimated using Lachat's QuikChemR Method 31-107-04-1-E, in which NO_3 is converted to NO_2 and diazotized with sulfanilamide to form an azo dye; and
- PO_4 was measured by forming an antimony-phospho-molybdate complex using QuikChemR Method 31- 115-01-1.

Table 2.2 The approximate detection limits of tested nutrients (adapted from Bird, 2009).

Nutrient	Detection limit
NO3 and NO2	2.5 μg.l-1 N
NH4	5 μg.l-1 N.
PO4	15 μg.l-1 P

Diatom samples were collected by Matthew Bird of the WHI programme, as well as Dr. Bill Harding of DH Environmental Consulting in Cape Town. Thus, the sampling methodology outlined in section 2.2.2 below is based on the standard diatom sampling methods.

2.2.2 Collection of diatom samples

As mentioned in chapter one, diatoms grow everywhere, they are found growing on rocks, plant material, suspended in the water, and even growing on sediments, thus, due to such a variety of substrata, the method employed for collecting samples was based on the type of substratum sampled (Round, 1993). Diatom samples were collected from communities growing on macrophytic plants as well as those growing on sediments, therefore, only these two methods of sampling are discussed in this paper. The methods described in this section are based on Taylor *et.al.* (2007a).

2.2.2.1 Sediment sampling

Diatoms are motile organisms, moving by a mechanism which combines the secretion of mucus and the movement of actin filaments. They live and move on the surface of the sediment and their mucous secretions is what binds the sediment particles together forming a delicate biofilm at the sediment/water interface. This biofilm may be distinguished as a golden-brown sheen on the surface of the sediment, and when it is disturbed it gently peels away leaving sediment of a different colour underneath.

A flat scraper (like the type for removing paint) was used to lift the biofilm or the top layer of the sediment, the diatom material was then carefully transferred into an appropriately labelled sample bottle and the process was repeated until sufficient material was collected (about 5-10 'scoops' of sediment were collected per wetland, and this was then preserved with 70% ethanol).

2.2.2.2 Sampling in shallow water

A large syringe was attached to the upper end of a flexible latex tube with the contact end of the latex tubing cut at an angle to allow for oblique contact with the sediment material. The diatom material was then carefully sucked up and transferred into a correctly labelled sample bottle and the procedure was repeated until adequate material was collected.

2.2.2.3 Sampling from aquatic macrophytes

Sampling techniques for aquatic macrophytes were based on whether they were emergent or submerged.

a. *Sampling from emergent macrophytes*

The stem of the macrophyte was cut off with a knife above the water line, a plastic bottle was then inverted over the remainder of the stem and the stem was cut off slightly above the point where it emerged from the sediment. The bottle was then inverted and taken out of the water. This process was repeated until five stems were randomly collected from different plants. The diatoms on the stems were dislodged into a tray by vigorously brushing the stems with a small brush. The diatom suspension (from all five stems) was then transferred to a labelled sample bottle.

b. Sampling from submerged macrophytes

Replicates from five different plants each consisting of a single stem plus associated branches were placed in a plastic bag together with 50ml of the wetland water. The plastic bag was tied and its contents were vigorously shaken and squeezed, and the resulting brown suspension was poured into a labelled sample bottle.

2.2.3 Preservation of diatom samples

To prevent bacterial growth and preserve diatom material, in samples that were not processed within twenty four hours, ethanol was added to reach a final concentration of 20% by volume.

2.3 Processing of diatom samples

Although live diatom material can be used for superficial analysis of the diatom community, it is preferable to use cleaned material since it can be archived, and referred to at a later stage.

2.3.1 Cleaning of samples

Diatom samples contain organic material, thus, for the accurate identification of diatoms, it is absolutely imperative that samples be cleaned and all unwanted material removed that may obscure the view and interfere with the identification process. In this study, although modified, the process of cleaning diatom samples was based on a method using potassium permanganate and hot hydrochloric acid, described by Taylor *et al.* (2007a).

Due to the release of hazardous gases during sample processing, it is vital for the cleaning of diatom material to be carried out in a fume cabinet.

The diatom samples were allowed to settle overnight, the supernatant was then discarded and the remaining sample was thoroughly mixed. Two milliliters of the sample were transferred into a clean test tube, and an equal amount of saturated potassium permanganate was added to the sample, the solution was mixed then left overnight to allow for the oxidation of the organic material present in the sample. The remaining original sample was not discarded, but kept throughout the preparation stage in case the processing of more slides should be required (Kelly *et al.*, 1998).

After twenty-four hours, two milliliters of concentrated hydrochloric acid (32%) were added to the test tubes with the sample solution, and the test tubes were placed in a beaker with water. The beakers were placed on a hot plate to speed up the process of the digestion of potassium permanganate by the hydrochloric acid. The solutions were slowly heated until they cleared to a straw yellow colour.

To check if the oxidation reaction was complete, and that no organic material remained in the sample, a few drops of hydrogen peroxide were added. Foaming of the solution indicated the presence of organic material; in this case, the samples had to be placed in the beaker with water and boiled again. When oxidation was complete, the samples were left to cool to room temperature before they were centrifuged.

After cooling, the samples were vigorously swirled with the aid of a vortex (to re-suspend the diatoms while causing heavier particles to remain in the bottom of the test tube), transferred into 10ml centrifuge tubes (filled with distilled water where the solution was less than 10ml) and the solutions were rinsed by centrifuging with distilled water for ten minutes at two thousand five hundred revolutions per minute (2500 rpm).

 After centrifugation the supernatant was decanted and the remaining solution was rinsed with distilled water. With every rinsing stage, the supernatant was poured off in a single movement to prevent the loss of diatom material. After decanting the supernatant, about 2ml of distilled water was added to the tube and the sample material was mixed to loosen small diatom and sand particles adhering to the bottom of the tube. The tube was then filled with distilled water to the 10ml mark, and the process of centrifuging and rinsing was then repeated four times. After the last rinse, half of the supernatant was decanted, and the remaining solution was transferred into a small, labeled glass storage vial.

2.3.2 Preparation of slides

A few drops of prepared diatom material was transferred to a test tube and diluted with distilled water until it appeared cloudy. A single drop of 10% ammonium chloride was added to neutralize electrostatic charges on the suspended particles and to reduce aggregation, allowing the material to spread evenly on the cover slip. The solution was then placed on a clean, dry cover slip (previously cleaned with ethanol) and allowed to dry out overnight at room temperature. After all the water had evaporated, the dried cover slips coated with diatoms were placed on a hot plate (~350°C) for 2-3 minutes to burn off excess moisture and to sublimate the residual ammonium chloride.

The slides were allowed to cool to room temperature, and were examined under 400 x magnification to check if the diatom concentration on the cover slip was correct. Ideally there should be between 10 and 40 valves visible per field (5-20 valves visible at a 1000x magnification). Where the concentration of the solution was found to be too high, or too low, the process was repeated using a more or less concentrated solution depending on the desired outcome. After reaching the desired concentration, the cover slip was placed on a hot plate at a lowered heat (90-120ºC) and the slides were mounted in Pleurax (with a refractive index of 1.73; Hanna, 1949), produced at the North-West University by Dr. J.C. Taylor.

Two drops of Pleurax were placed on the cover slip using a pipette, the mounting media was then allowed to settle, and a glass slide previously cleaned with ethanol was lowered onto the cover slip, inverted and heated until the mountant boiled and all the solvent evaporated. The slide was then removed from the hot plate and allowed to cool; it was then labeled with details such as the database code (sample number) and the date of collection.

2.3.3 Preparation for Scanning Electron Microscopy

The cleaned diatom material was filtered through a 2.0 µm polyester membrane filter placed inside a Swinnex 13 Millipore, and the material was filtered by pushing air through the filter with a syringe. The filter membrane was examined under a light microscope to ensure that there was adequate material for examination. The membrane was then mounted on an aluminium microscope stub with carbon tape and coated with gold palladium, and examined with an FEI QUANTA ESEM 2000 at a voltage between 10 and 15kV.

2.3.4 Archiving

The archiving of diatom samples is important as it allows for future preparation of slides for light and electron microscopy, as well as to facilitate future analysis, cross referencing and verification by other workers (Kriel, 2008). All diatom material was archived; a portion of the unprocessed original sample was transferred to a small plastic vial, preserved with ethanol and stored. After slide preparation, the remaining portion of the cleaned material in the glass vials was also preserved with ethanol (reaching a final concentration of more than 20% by volume) to prevent the growth of microorganisms.

Slides provide a permanent historical record of water quality conditions at a site, thus they were stored in the diatom herbarium found in the botany department at the Potchefstroom campus of the North-West University.

2.3.5 Diatom identification, enumeration and data analysis

a. Identification

Diatoms were identified using an oil-immersion lens at 1000x magnification (100x objective in combination with a 10x eyepiece). The morphological characteristics of these diatoms were used to identify them to the genus and species level. The taxa of diatoms were identified according to Taylor *et al.* (2007b) and Lange-Bertalot, (2000). From each sample, three to four cells of the dominant species (more than 5% of the count) were photographed for verification of identification and for the production of plates, using a Nikon 80i compound light microscope equipped with a 100x 1.4 N.A plan upper oil immersion lens. Differential interference contrast was used to obtain contrast in images. The images were captured with a Nikon D.S –U2, 5 megapixel digital camera using version 2 of the Nikon N/S elements software. Diatoms that were too small and difficult to identify using the light microscope were identified by scanning electron microscopy.

b. Enumeration

Diatom valves were counted until a minimum of 400 valves and at least (where possible) ten different taxa were observed. According to Prygiel *et al*. (2002) enumeration beyond 400 valves has shown no significant effect on diatom index scores, thus a count of 400 valves ensures semi-quantitative data from which ecological conclusions can be made. However, where a count of 400 cannot be reached, then 300 valves can still be used for index calculations (Prygiel *et al*., 2002).

A 1000x magnification was used to count all the diatom cells that were positively identified in a field of view, these cells were then documented before proceeding along a vertical (or horizontal) transverse to the next field on the slide.

During the enumeration process no distinction was made between a diatom frustule and a diatom valve due to the difficulty in identifying between intact frustules and isolated valves. Broken valves were included in the count only if more than 50% of the valve was still intact; valves on the margin of the field of view were only identified and counted when more than 50% of the valve was visible within that field of view. Species appearing in girdle view were only included in the count if they could be accurately identified.

c. Data analysis

As mentioned above, the frequency of presence of each taxon (species) was calculated by counting 400 valves per sample. After enumeration, a spreadsheet of the species total and relative abundance (taxon abundance / total abundance x 100) was created using Microsoft excel 2003. The results were then imported into version 3.1 of the OMNIDIA (Lecointe *et al*., 1993), database, which was used to calculate several different diatom indices.

The diatom indices calculated in the study were:

- **SPI** = Specific Pollution Sensitivity index (Coste in Cemagref, 1982),
- **GDI** = Generic Diatom Index: (Coste and Ayphassoro, 1991).
- **TDI** = Trophic Diatom Index (Kelly, 1998),

SPI and GDI index values were transformed to the scale from 0 to 20 (as shown in table 1.2, in Chapter 1), indicating the quality range from polluted to clean waters.

Since the environmental variables data did not show a normal distribution, thus all environmental variables' data except for pH were log transformed, and non-parametric statistical methods were employed to represent the general water quality conditions in the different wetlands. The median, 25%-75% quartiles and range data was calculated for each of the environmental variables as well as diatom indices, and the results were presented graphically in Chapters three and four respectively.

In order to assess the degree with which the different indices used in the study responded to changes in the environmental variables, statistical analyses (correlation analyses, and the production of graphs) were carried out with Statistica version 9 (Statsoft, 2009), and standard two-way, correlation analyses were employed, using a Pearson's correlation coefficient of $p<0.05$.

Since changes in the diatom community may lead to changes in diatom index scores (Kriel, 2008), an ordination technique was used to determine the response of diatom species to general water quality variables. Canonical Correspondence Analysis (CCA) was used to determine the major patterns of diatom species distribution in relation to the measured environmental variables.

Canonical Correspondence Analysis (CCA) is a multivariate method developed to explain the relationships between biological community of species and their environment (ter Braak *et al*., 1995). It is similar to other ordination techniques, in that it provides an integrated description of species-environment relationships (ter Braak, 1986). Thus, CCA was carried out with version 4.5 of CANOCO (ter Braak, 1986), and CCA biplots were drawn with CanoDraw for windows.

2.4 References

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CHAPTER THREE

RESULTS AND DISCUSSION – PHYSICO-CHEMICAL VARIABLES

3.1 Introduction

To maintain aquatic systems functioning, these systems have to be monitored and managed not only to sustain humans, but also to support and sustain their own unique biodiversity.

In this chapter, an overview of the results obtained from the measurements and analyses of physico-chemical variables will be given in section 3.3, which is divided into three sub-sections based in the type of variable measured. This is followed by section 3.4, which gives a discussion based on the measured environmental variables (results in sections 3.3).

The purpose of this chapter is to describe the importance of monitoring these environmental variables and to briefly describe the effects that these variables may have on the health and integrity of aquatic ecosystems. Although this is a brief overview, chapter four gives detailed discussions of the relationship between these environmental variables and the overall structure of diatom communities encountered in the studied wetlands.

3.2 Water quality analysis in South Africa

As mentioned in chapter one, properties of water can be divided into four groups, the aesthetic, biological, chemical as well as the physical. The aesthetic properties describe the properties of water such as taste, odour, and litter, the biological properties describe the biodiversity of the system, and the chemical properties include dissolved oxygen, conductivity as well as the presence and concentration of dissolved salts, pH, and metals, whereas the physical properties of water include temperature and as mentioned earlier, turbidity (DWAF, 1996).

It is crucial therefore, to monitor these variables regularly so that physiological disruptions or loss of species composition can be prohibited. Thus, as a result, the Department of Water Affairs and Forestry has developed what is known as the Target Water Quality Range (TWQR) for South African aquatic ecosystems (DWAF, 1996).

The Target Water Quality Range was created as part of the South African Water Quality Guidelines (DWAF, 1996). These guidelines act as a foundation for developing resources to inform water users about the physical, chemical, biological and aesthetic properties of water (DWAF, 1996).

Thus, "the TWQR is a range of concentrations and levels (of physico-chemical variables) within which no measurable adverse effect is expected on the overall health of the system" (DWAF, 1996). The TWQR of the variables measured in this study are given in table 3.1 below.

Table 3.1 TWQR values for fresh, inland, aquatic systems.

Water quality variable			Natural values	TWQR
Nutrients	Nitrogen	Ammonia, ammonium, nitrates and nitrites	Below 0.5 mg.L^{-1}	Below 15% deviation
	Phosphorus	Orthophosphates	Below 5000 mg.L^{-1}	Below 15% deviation
Physico-chemical variables		Conductivity	Not Available	Below 15% deviation
		Dissolved oxygen	80-120% saturation	80-120% saturation
		pH	4-11	Below 5% deviation
		Temperature	5-30˚C	Below 10% deviation
		Turbidity (TSS)	Below 100 mg.L^{-1}	Below 10% deviation

Thus, regular monitoring of these aquatic systems, and ensuring that the concentration of physico-chemical variables are maintained within the levels stipulated in the TWQR will ensure the protection of aquatic organisms living within these systems (DWAF, 1996).

3.3 Physico-chemical variables

The interactions of physico-chemical factors within natural conditions may determine the growth, and hence the survival of organisms (Yamaguchi *et al*., 1991 in Nishikawa *et al*., 2006), thus drastic or random fluctuations in these variables may cause severe disruptions to the ecological and physiological functions of aquatic organisms (DWAF, 1996), which may lead to the loss of key species and ultimately the entire community structure.

Physico-chemical monitoring is a site-specific qualitative or quantitative process describing the physical and chemical status of the aquatic systems based on the presence as well as the concentration of specific variables (DWAF, 1996). Some of the variables measured in the studied wetlands include ammonia, dissolved oxygen, electrical conductivity, nitrates, pH, phosphates, temperature as well as turbidity.

3.3.1 System variables

Aquatic organisms usually adapt to the natural seasonal cycles of temperature, pH and dissolved oxygen, and as a result, these constituents regulate essential ecosystem processes such as migration and spawning (DWAF, 1996).

a. **Temperature**

Temperature is an important variable in water, as it affects the rates of chemical reactions, thus affecting the metabolic rates of organisms living in that water (Davies and Day, 1998, and DWAF, 1996). The above statement is also true for diatoms, whose reproductive rate, and hence succession, is also influenced by temperature (Patrick, 1971).

Figures 3.1 and 3.2 below show the median values of temperature in the different wetlands.

Figure 3.1 and 3.2 below show that the temperature values were well within the permissible TWQR values stipulated in table 3.1. From figure 3.1 it can be seen that the temperature range in the eight wetlands was almost similar, with the exception of Lange Vlei (LGV), whose temperature ranged between 10 and 20˚C, thus being the only wetland with the lowest temperature range, giving the assumption that the temperature at this wetland remained almost constant throughout the study, whereas the other wetlands had inconsistent temperatures. Although temperature values were maintained within acceptable ranges, figure 3.1 also shows Wildevoël Vlei (WVV) and Zand Vlei (ZDV) to have had the widest variability in temperature values.
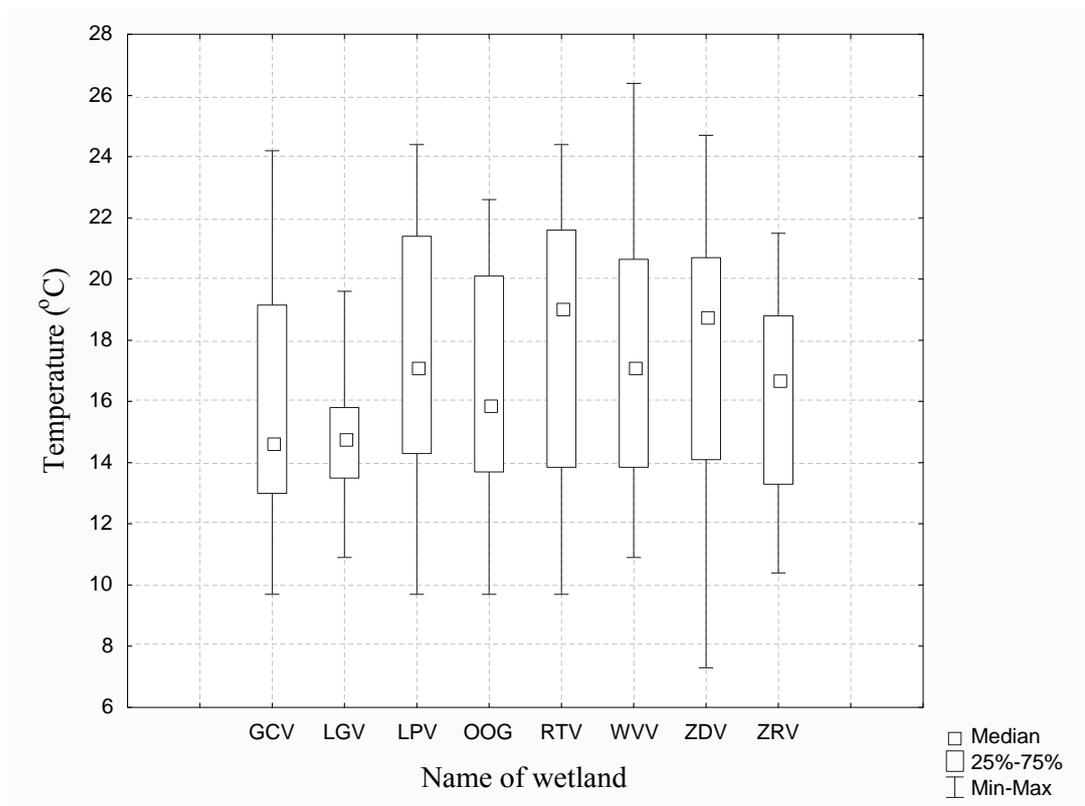
Figure 3.1 Median temperature (˚C) values of data collected over three years.
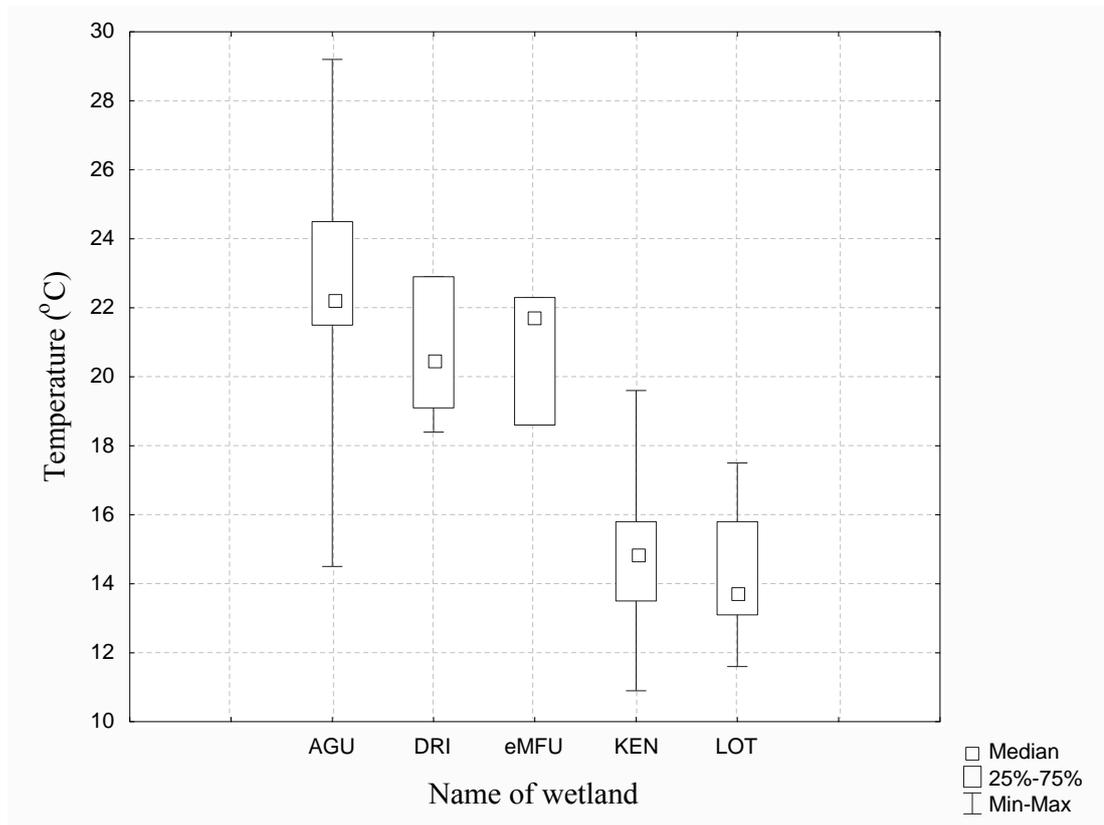
Figure 3.2   Median Temperature (˚C) values of data collected once off.

In figure 3.2, it is very clear that Kenilworth (KEN) and Lotus River (LOT) had the lowest temperature range in comparison to the other three wetlands, with Agulhas (AGU) showing the highest median value of 22.2˚C, and temperatures reaching 28˚C at some sites, followed by eMfuleni (eMFU) and Drift Sands (DRI) with maximum temperatures of 22.1 and 23˚C, respectively.

When comparing the two groups of wetlands (figure 3.1 and 3.2) it can be seen that AGU (in figure 3.2) had the largest range of high temperatures (between 18 and 29˚C). This large range of high temperatures at Agulhas might be influenced by the warm Agulhas current along the south coast of South Africa (DWAF, 1995), where this wetland is situated. Since all the other wetlands are found within the Cape Town Metropolitan area, which is located along the west coast of South Africa, their temperatures are influenced by the cold Benguela current (DWAF, 1995), hence, the slightly lower temperatures measured in these wetlands.

b. <u>Dissolved oxygen</u>

Dissolved oxygen analysis is a measure of the amount of gaseous oxygen (O_2) dissolved in an aqueous solution (Davies and Day, 1998). Oxygen is an essential element to all forms of life; thus, its concentration in water is one of the most important abiotic factors determining the survival of aquatic organisms (DWAF, 1996).

Figures 3.3 and 3.4 below; show the median DO % values in the different wetlands.

By looking at the 25%-75% quartile on the graph in figure 3.3, it can be seen that in RTV, WVV and ZDV the level of dissolved oxygen remained almost constant (within recommended levels) throughout the study, whereas DO levels were below 80% in the other wetlands.

The figure also shows that although levels remained constant at WVV, there were incidences where levels of DO fluctuated between a very low 0% (anoxic stage) and extremely high 220% (eutrophic). It can also be seen that levels of dissolved oxygen remained below 60%, at LPV, OOG, and ZRV, with levels at ZRV fluctuating between 20 and 60% saturation throughout the duration of the study.
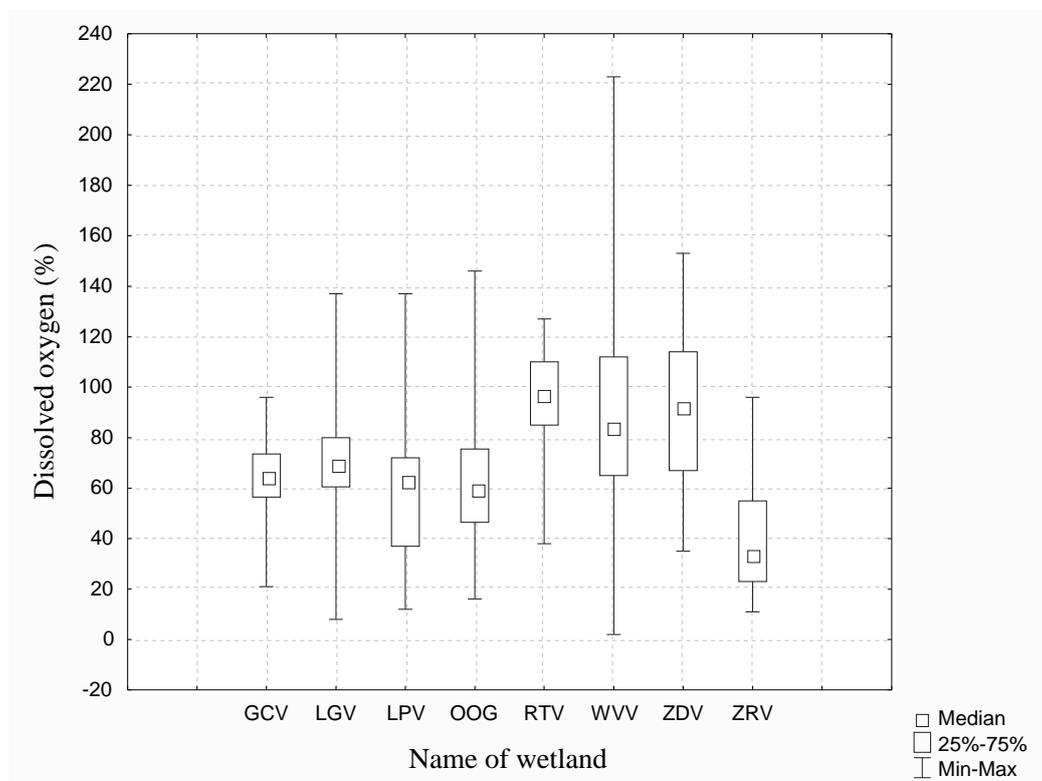
Figure 3.3 Median Dissolved Oxygen saturation values of data collected over three years.
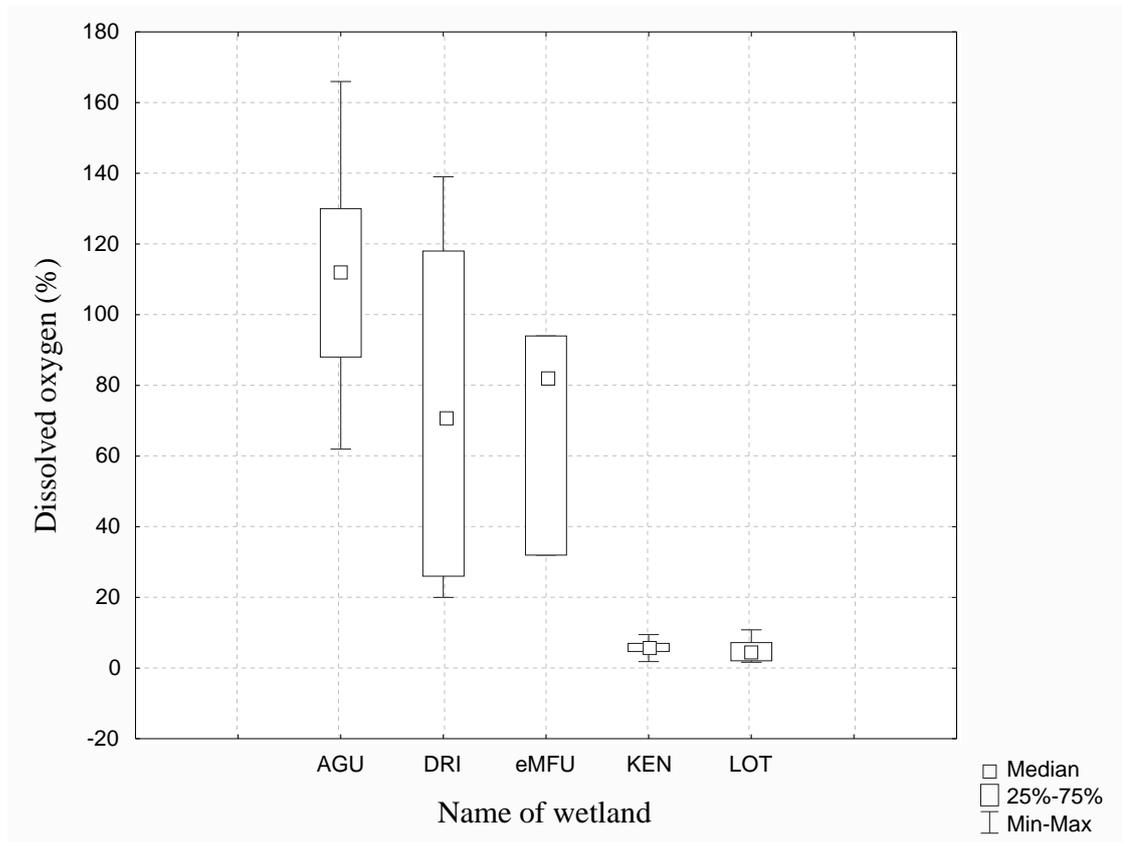
Figure 3.4 Median Dissolved Oxygen saturation values of data collected once off.

In figure 3.4 it can be seen that oxygen saturation levels were within the TWQR values at Agulhas (AGU) wetland only. The graph also shows a great variability in the oxygen saturation levels at Drift Sands (DRI) and eMfuleni (MFU) where the oxygen levels at some sites were below 40% saturation. From observing figure 3.4, it can also be seen that KEN and LOT had the lowest saturation levels, with values ranging between 1.88 & 9.49% at Kenilworth, and 1.63 & 10.79% at Lotus River, with median values of 6.06% as well as 5 % saturation in Kenilworth and Lotus River respectively.

c. **pH**

The pH of a sample of water is a measure of the concentration of hydrogen ions [H^+] in that water (Davies and Day, 1998). The pH of water determines the solubility (amount that can be dissolved in the water) and biological availability (amount that can be utilized by aquatic life) of chemical constituents such as nutrients (phosphorus, nitrogen, and carbon) and heavy metals (lead, copper, cadmium) (Davies and Day, 1998).

Figures 3.5 and 3.6 below; show the median pH values in the different wetlands.
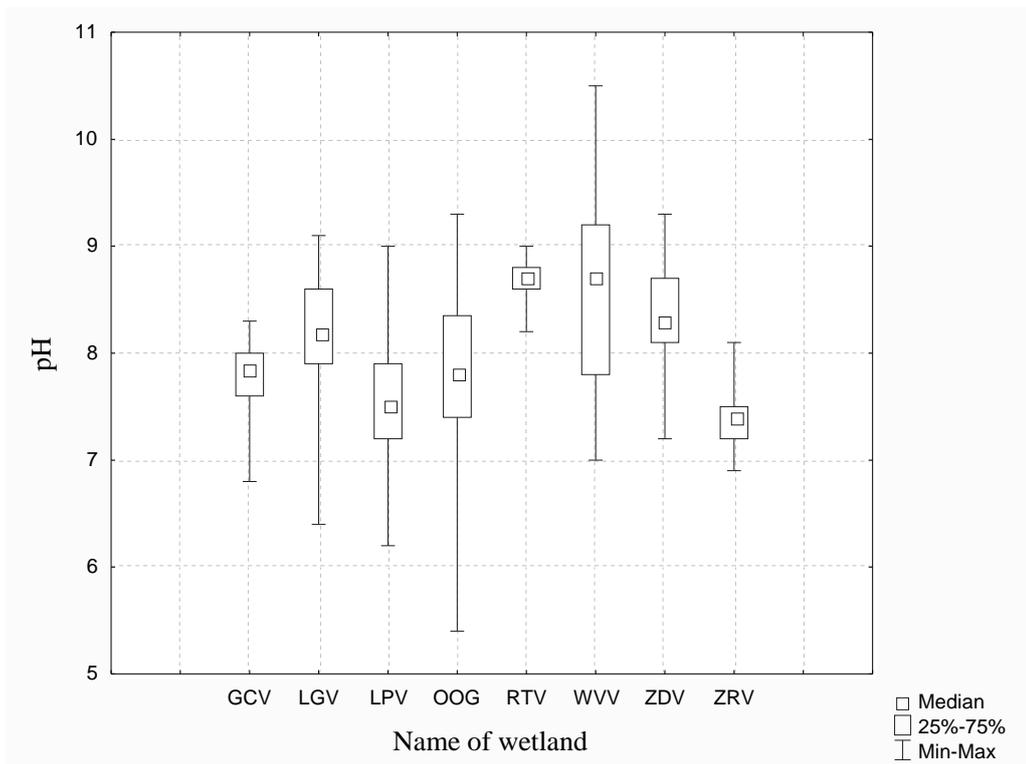
Figure 3.5 Median pH values of data collected over three years.
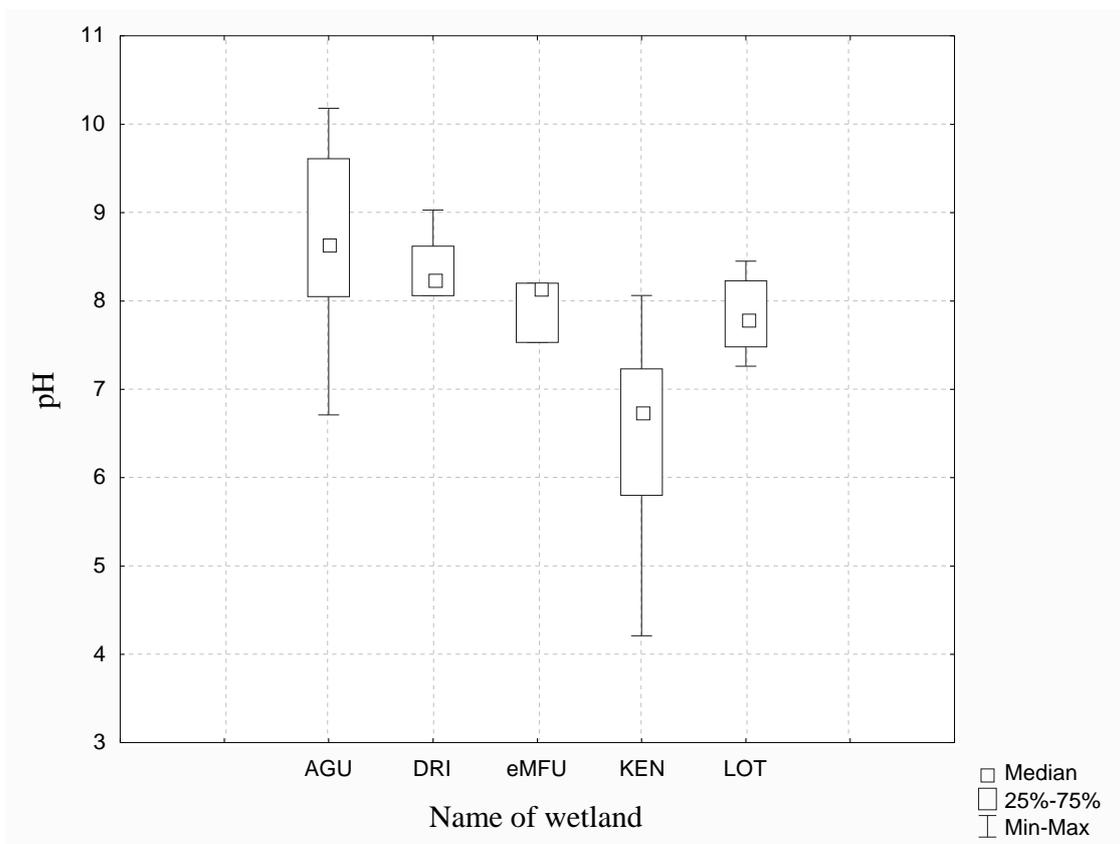
Figure 3.6 Median pH values of data collected once off.

Based on figure 3.5 above, it can be seen that Glen Cairn Vlei (GCV), Rietvlei (RTV) and Zoar Vlei (ZRV) were the only wetlands, whose pH remained almost constant throughout the study, with pH values ranging between 7 and 8.5 at GCV, and between 8.3 and 9.2 at RTV, and 6.8 and 7.6 at ZRV.

Although all the other wetlands show a wide variability in pH values, it can be seen that WVV had the highest fluctuations in pH, with values ranging between 7.0 and 10.5, with the maximum value being high above the permissible levels as can be seen in table 3.1 above.

In figure 3.6, it can be seen that although the maximum pH values are above the recommended levels, pH values at DRI, MFU, and LOT, remained constant throughout the wetlands, with values ranging between 8 and 9 at DRI, and between 7.5 and 8.2 at MFU and 7.2 and 8.5 in Lotus River. Agulhas and Kenilworth (KEN) on the other hand, had the widest range of pH values, with some sites at Kenilworth showing acidic environments (min pH of 4).

3.3.2 Inorganic constituents

Factors such as electrical conductivity and turbidity may cause toxic effects at extremely elevated concentrations; however, they are regarded as system characteristics since their natural concentrations depend on geochemical, physical and hydrological processes occurring in that particular environment (DWAF, 1996).

a. Electrical conductivity

Electrical Conductivity [measured in micro-Siemens per centimeter (μS.cm^{-1})] is a measurement of the ability of an aqueous solution to carry an electrical current (Davies and Day, 1998).

Figures 3.7 and 3.8 below show the median EC values in the different wetlands.
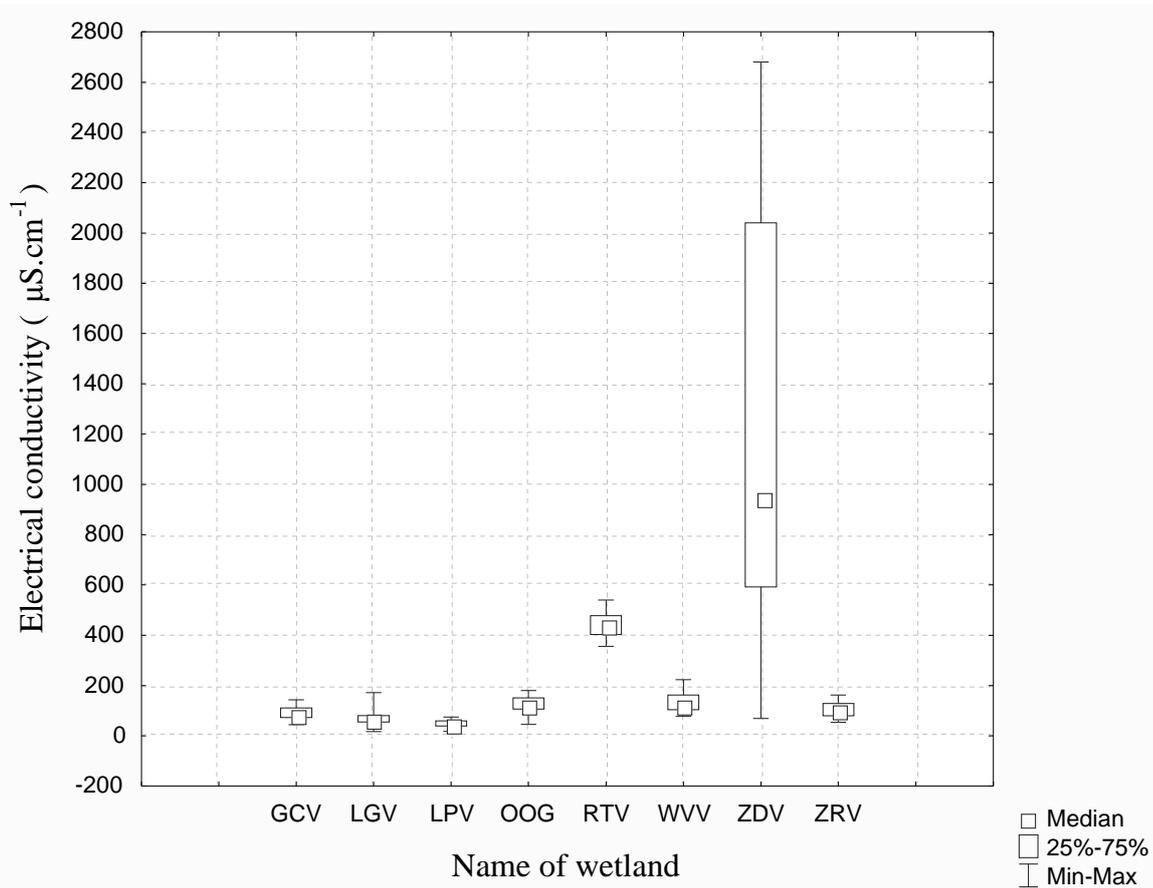
Figure 3.7 Shows the median electrical conductivity (μS.cm $^{-1}$) values of data collected over three years.
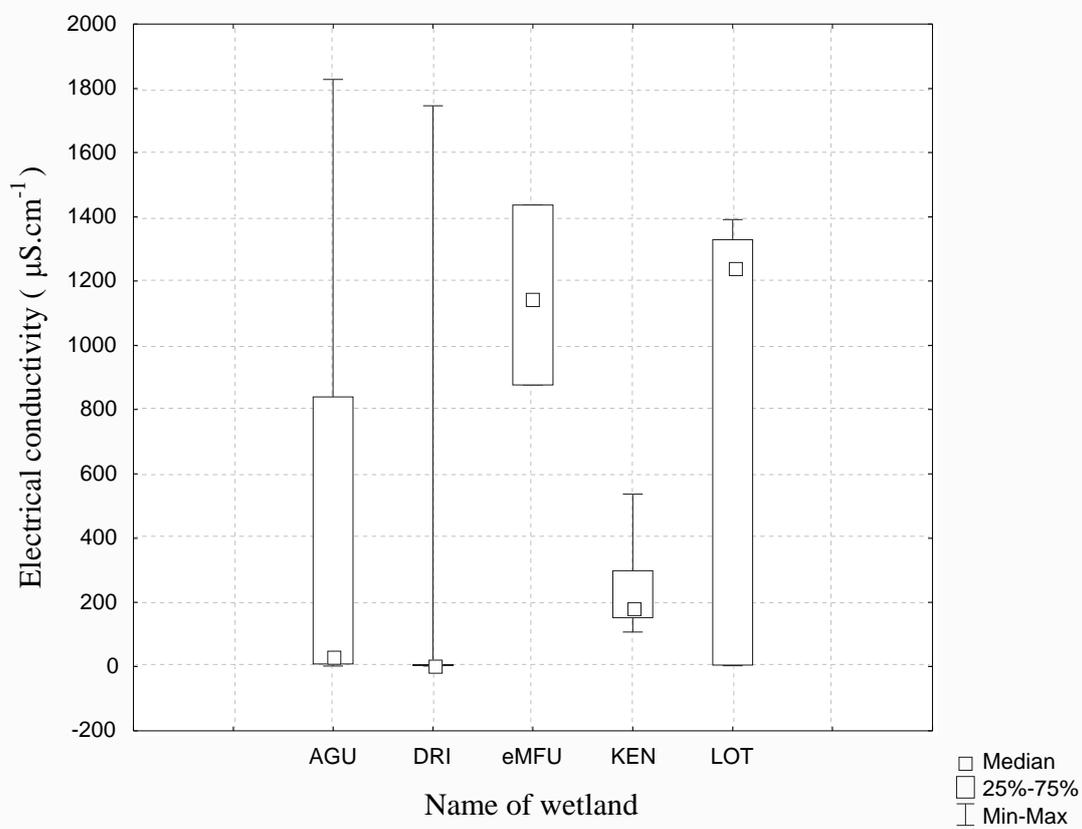
Figure 3.8 Shows the median electrical conductivity (μS.cm $^{-1}$) values of data collected once off.

From figure 3.7 it can be seen that Zand Vlei (ZDV) was the only wetland which had the highest and most inconsistent EC scores, with readings ranging between 0 and 2800 µS.cm $^{-1}$. The EC of the other seven wetlands remained constant throughout the study, with EC values at Rietvlei (RTV), ranging between 200 and 600 µS.cm $^{-1}$, whereas in the other wetlands, EC remained below 200 µS.cm $^{-1}$.

According to figure 3.8 above, of all the wetlands, Drift Sands had the lowest EC; with a median score of 3.8 µS.cm $^{-1}$. From this figure it can also be seen that EC readings were inconsistent in the other four wetlands, with Agulhas (AGU) and Lotus River (LOT) showing the widest inconsistency.

b. <u>Turbidity</u>

Turbidity is a measure of the degree to which water loses its transparency due to the presence of suspended particulates (DWAF, 1996). The suspended particles interfere with sunlight penetration by scattering the light, thus decreasing the photosynthetic activity of primary producers (Davies and Day, 1998).

Figures 3.9 and 3.10 below show the median turbidity values in the different wetlands.
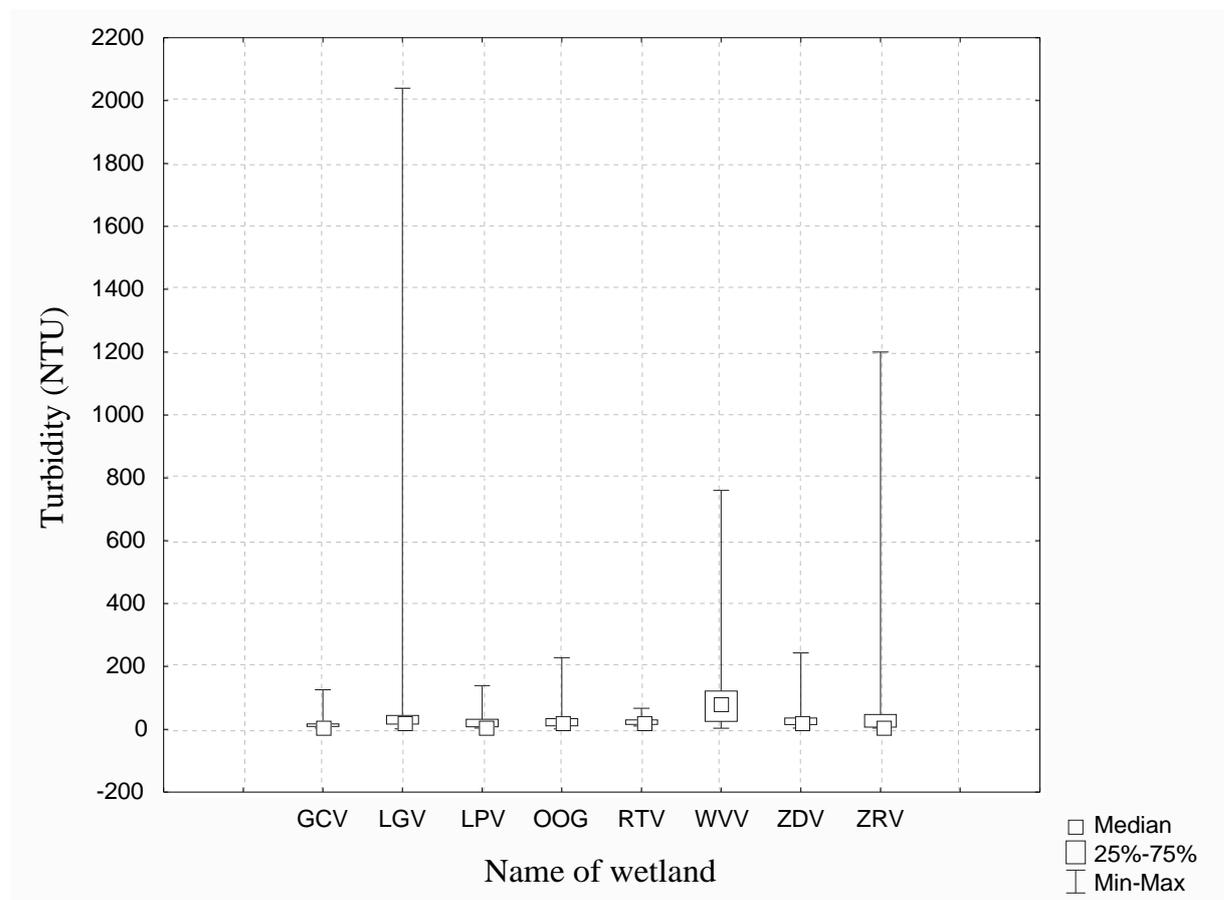
Figure 3.9 Median turbidity values of data collected over three years.

With the exception of Lange Vlei (LGV), Wildevoël Vlei (WVV) and Zoar Vlei (ZRV), whose turbidity ranged between 0 and 2050 NTU, (figure 3.9), the turbidity in all the other wetlands remained below 300 NTU throughout the study. With maximum levels of 2050 NTU, the water in LGV has exceptionally high turbidity levels.

In Figure 3.10 below, it can be seen that turbidity values were below the recommended TWQR scores, where the median turbidity levels ranged between 2 and 3.75 NTU, and maximum values ranged between 5 and 50 NTU, with AGU showing the highest values. When not stagnant, wetlands have low flow rates (when compared to rivers), therefore their environment allows for the deposition of suspended material, and the re-suspension of deposited material is usually low.
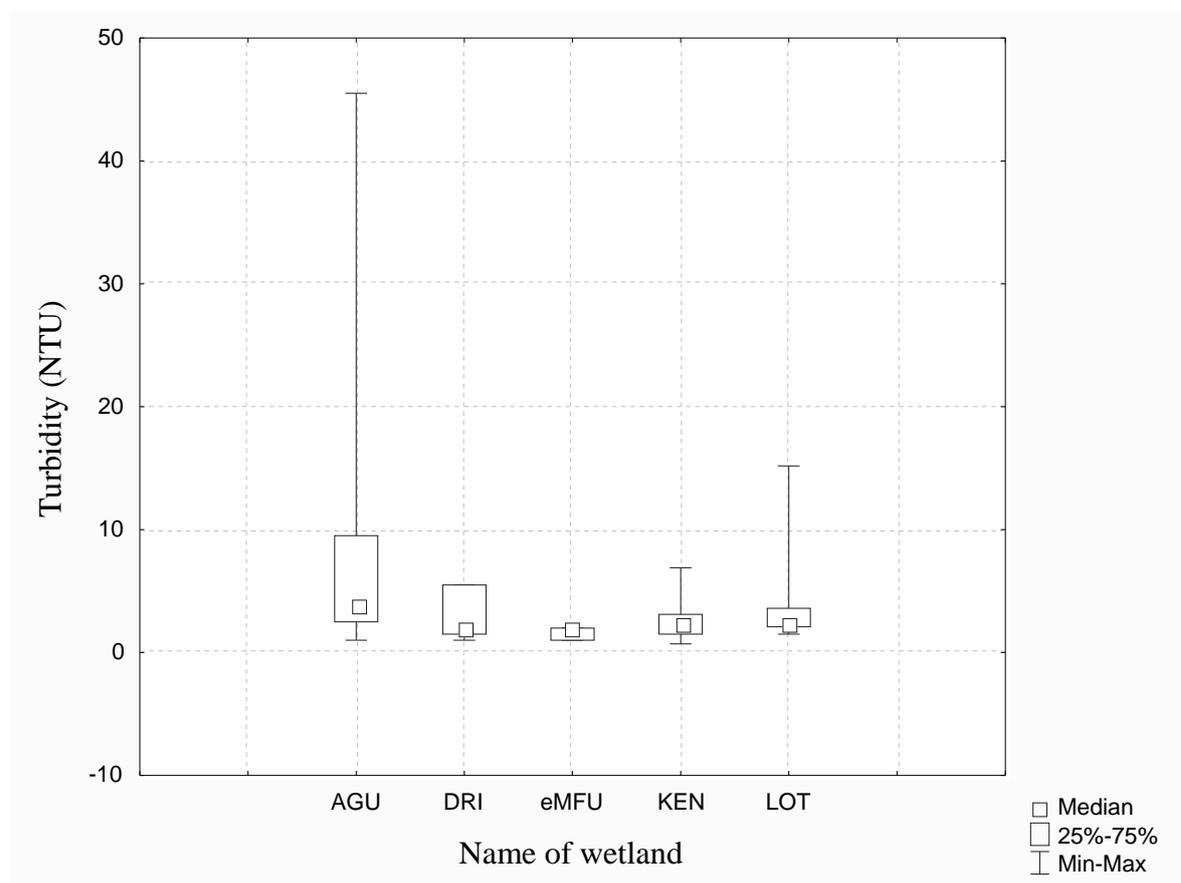
Figure 3.10 Median turbidity values of data collected once off.

3.3.3 Nutrients

The term nutrient refers broadly to those chemical elements essential to life on earth, the elements required for normal plant growth and reproduction. Although constituents such as inorganic nitrogen (nitrites, nitrates, and ammonium) and inorganic phosphorus (ortho-

phosphates) are generally not toxic, they may stimulate eutrophication if present in excess amounts (DWAF, 1996).

a. **Nitrogen**

Nitrogen is one of the most abundant elements in nature and it is an essential constituent of most biological and biochemical processes (Davies and Day 1998). It may be present in both organic and inorganic forms in aquatic systems. Organic nitrogen is found in proteins, and is continually recycled by plants and animals; and inorganic nitrogen includes all the major inorganic nitrogen components such as nitrates (NO_3^-), nitrites (NO_2^-), ammonium ions (NH_4^+) and ammonia (NH_3).

The figures below (figure 3.11 to 3.15) show the concentrations of the different components of inorganic nitrogen in the different wetlands.

Figure 3.11 shows the median ammonia levels. In this figure it can be seen that, with the exception of Die Oog (OOG) with a median value of 1.5 mg.L^{-1}, and maximum levels of 3.79 mg.L^{-1}, the median levels of ammonia in all the other wetlands are below the acceptable concentration of 0.5 mg.L^{-1}. However, from closely observing the graph it can also be seen that concentrations of ammonia did not remain constant throughout the duration of the study, as some high concentration of ammonia were recorded at GCV, LGV and WVV.
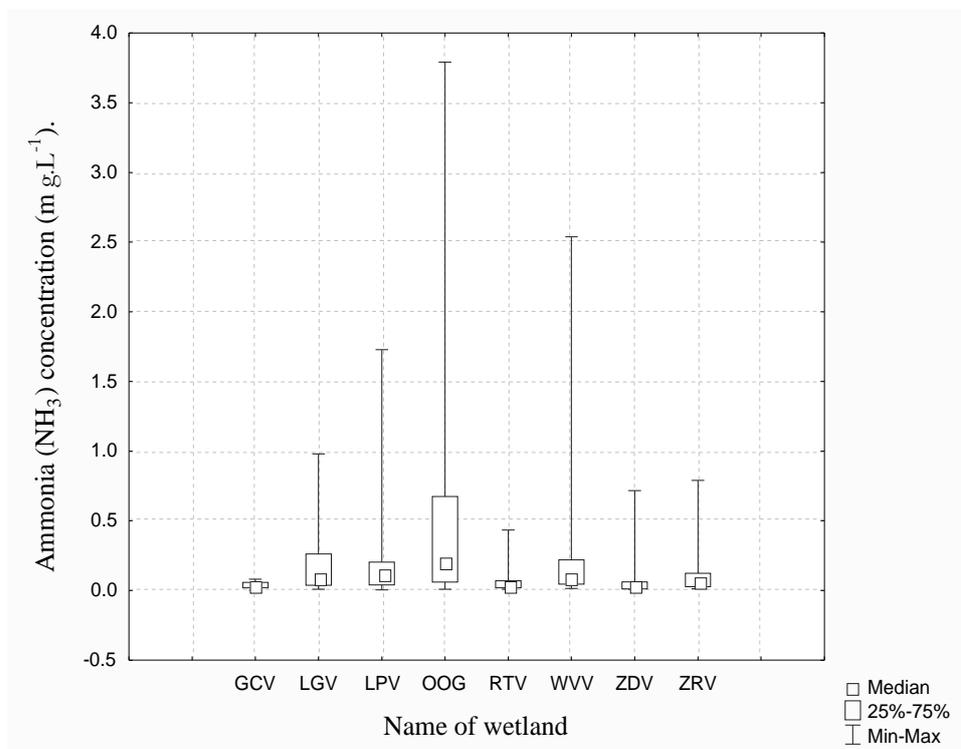
Figure 3.11 The median ammonia (NH3) values of data collected over three years.
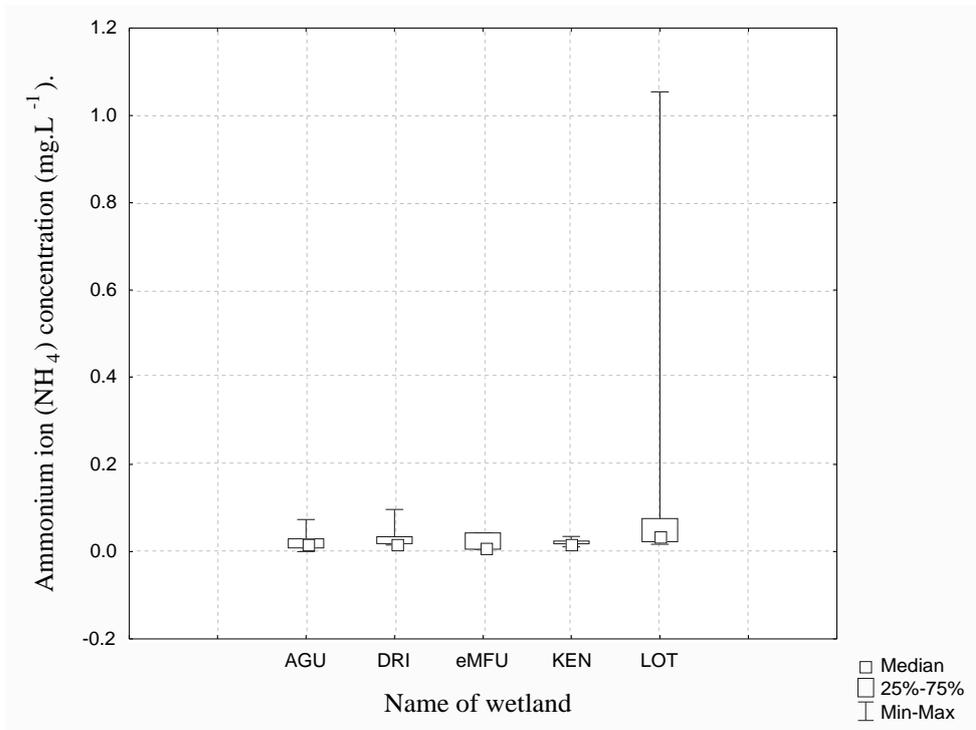
3.12 Median ammonium ion (NH4) values of data collected once off.

Figure 3.12 above, shows the ammonium ion concentrations. This figure shows the median levels of ammonium ion concentration to be below the TWQR levels, with the lowest concentration measured in AGU, and maximum ammonium levels at Lotus River.
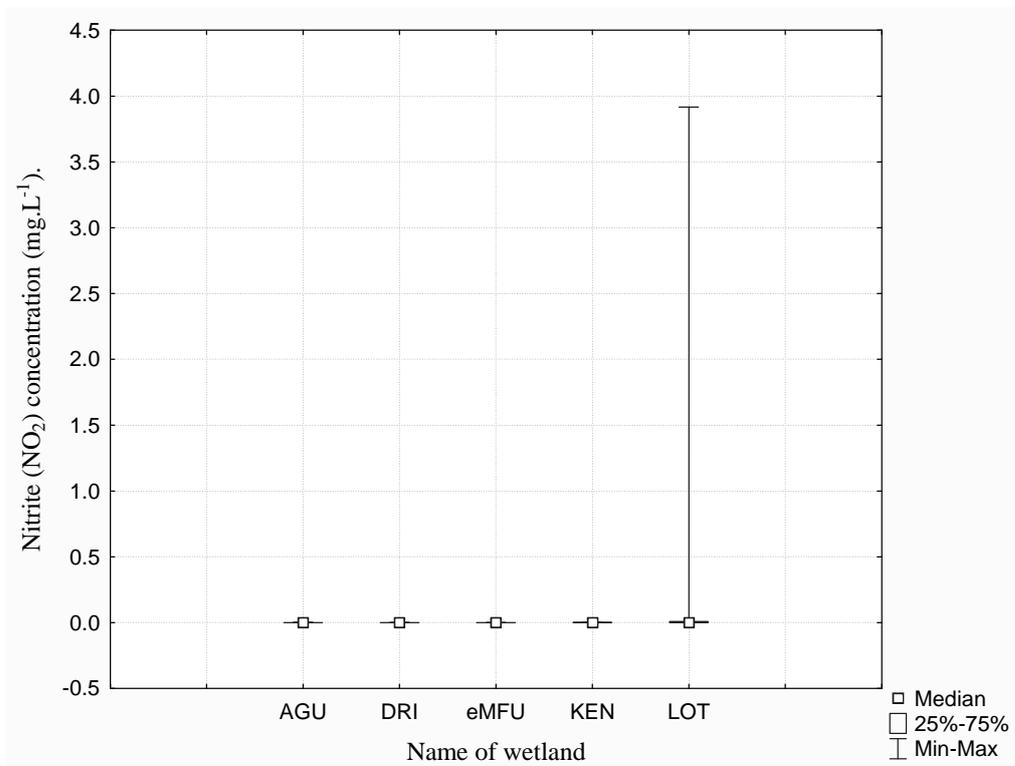
Figure 3.13 Median nitrites (NO2) values of data collected once off.

Although the median nitrite values in figure 3.13 above are all within the acceptable limits, and almost within the same range, with the lowest reading of 0.005 mg.L⁻¹, at Agulhas, some sites in Lotus River had high nitrite concentrations when compared to the other four wetlands.

Figure 3.14 below, shows the concentration of nitrates in the different wetlands.
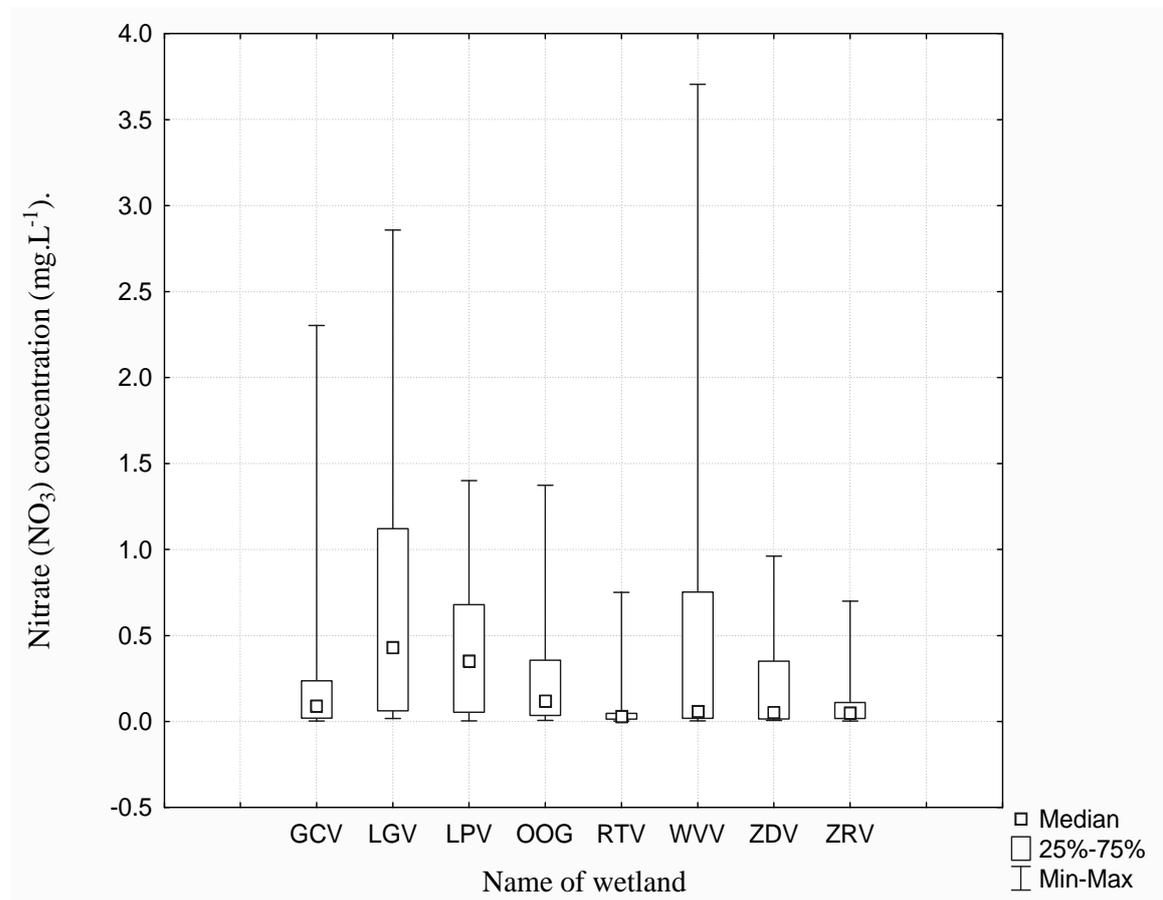
Figure 3.14 The median nitrate (NO3) values of data collected over three years.

From figure 3.14, it can be seen that median nitrate concentrations of all the wetlands were below the TWQR recommended levels, whereas maximum concentrations were exceptionally higher at GCV, LGV, LPV, OOG and WVV.

Rietvlei had the lowest range of concentrations, with the lowest value of 0.009 mg.L⁻¹, and a median value of 0.03 mg.L⁻¹, whereas Lange vlei had the widest range of concentrations within its individual sites, with the lowest concentration recorded at 0.017 mg.L⁻¹ (which is still higher than the lowest concentration recorded at Rietvlei), a median value of 0.4295 and the highest value of 2.081 mg.L⁻¹. Another wetland which had high levels of nitrates at some of its sites is WVV, whose maximum nitrate levels were recorded at 3.709 mg.L⁻¹.
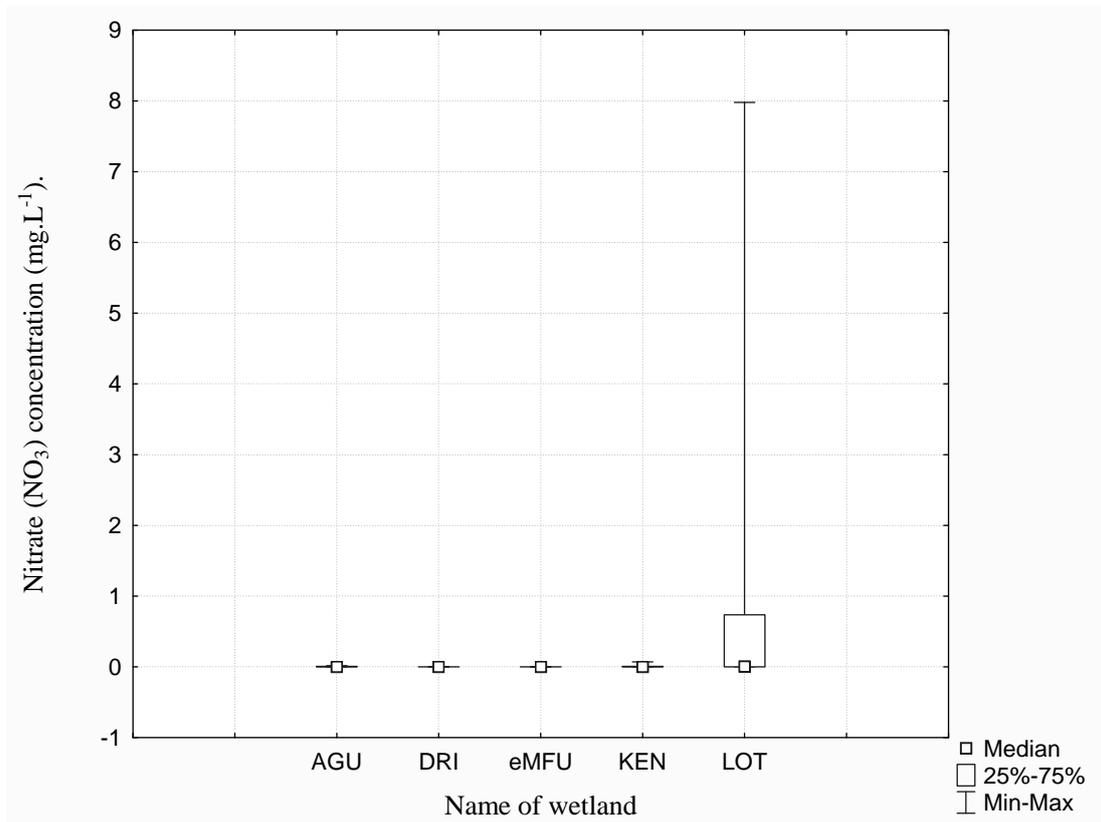
Figure 3.15 Median nitrate (NO3) values of data collected once off.

In figure 3.15 above, it can be seen that the concentration of nitrates at AGU, DRI, eMFU, and KEN remained constant through these wetlands with concentrations below 0.1 mg.L⁻¹, in all the four wetlands. The highest variability was recorded at Lotus River (LOT), which had a median value of 0.00628 mg.L⁻¹, and a maximum concentration of 8.0 mg.L⁻¹.

b. **Phosphorus**

Phosphorus is one of the key elements necessary for the growth of plants and animals (Davies and Day, 1998). The element phosphorus can occur in nature in forms such as orthophosphates (commonly known as phosphate), metaphosphates (or polyphosphate) and organically bound phosphate, with each compound containing phosphorus in a different chemical formula (DWAF, 1996). Of the three forms of phosphates, orthophosphate (PO_4), which is formed by the oxidation of phosphorus, is the most abundant dissolved inorganic form in aquatic environments (DWAF, 1996).

Figures 3.16 and 3.17 below show the median phosphate concentrations in the different wetlands.
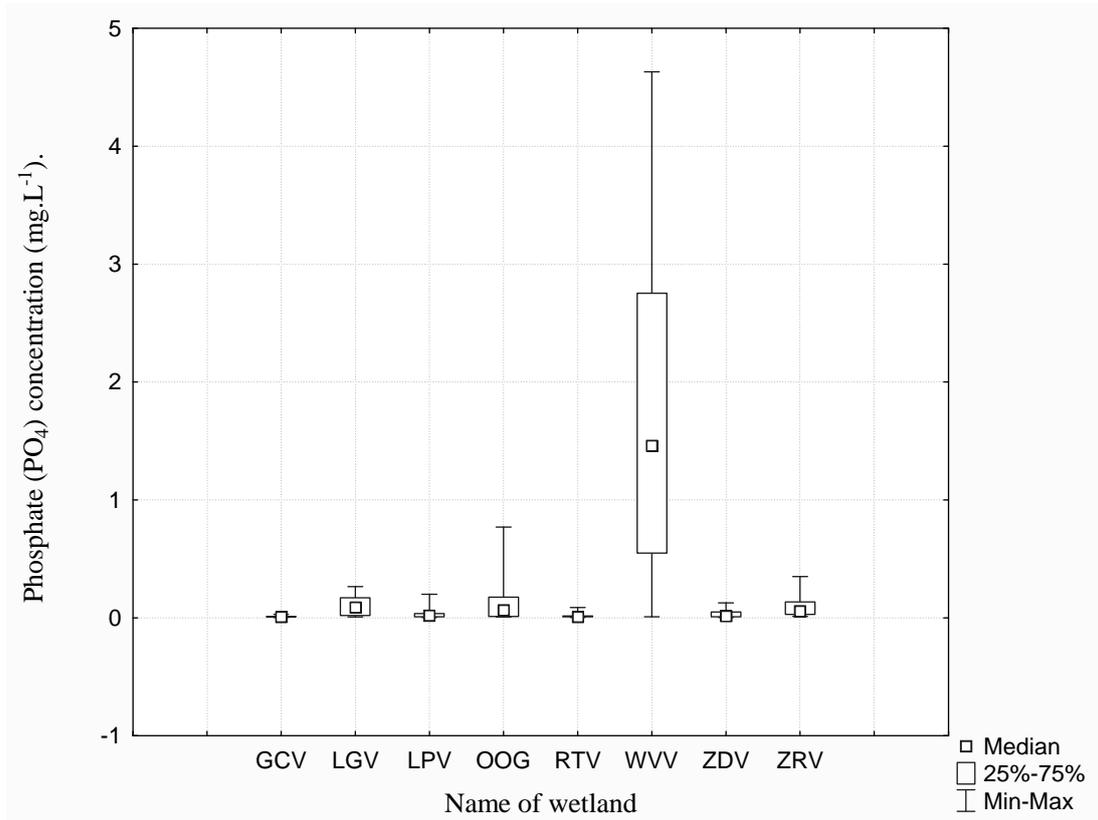
Figure 3.16 The median phosphate (PO4) values of data collected over three years.
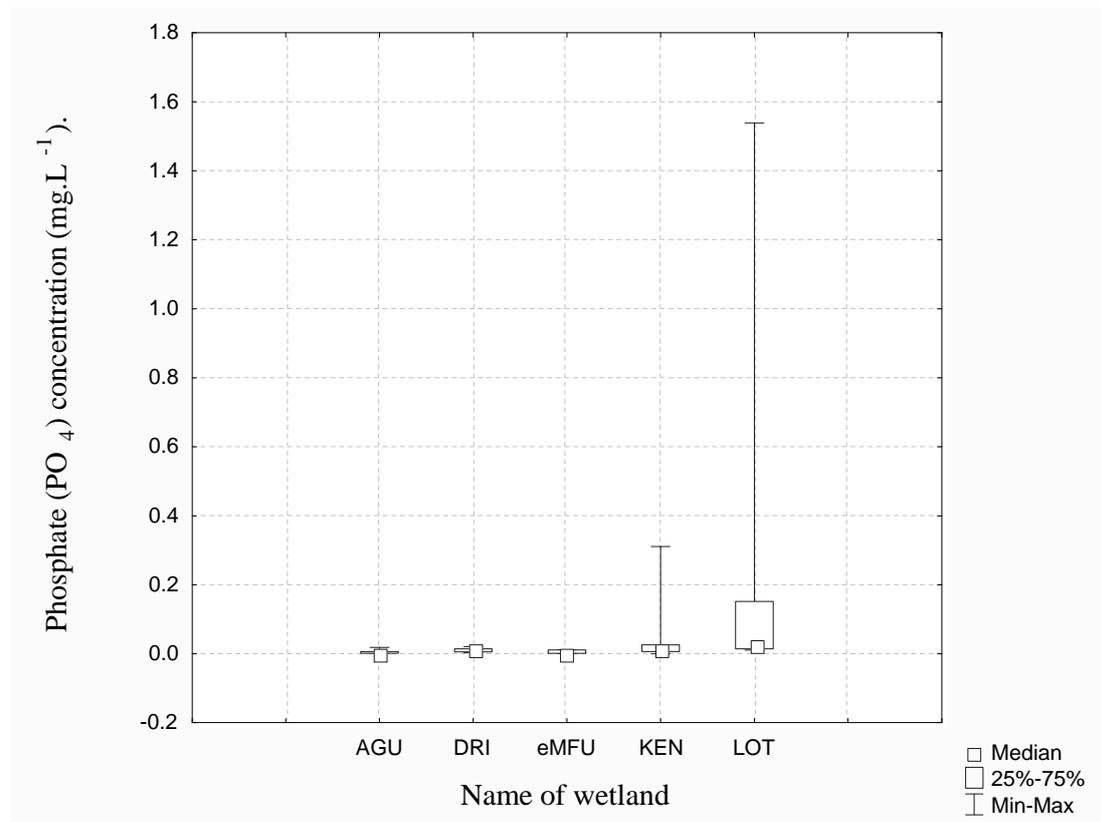
Figure 3.17 Median phosphate (PO4) values of data collected once off.

Based on these two figures above, it can be seen that Wildevoël Vlei (WVV) and Lotus River (LOT) had the highest phosphate concentrations. In figure 3.16, it can be observed that Wildevoël Vlei (WVV) had the highest phosphate values when compared to the other wetlands. From this figure, it can be seen that the large range of values at WVV with a median of 1.46 mg.L^{-1}; suggest that the phosphate concentrations in this wetland, fluctuated between 0 and 5 mg.L^{-1} throughout the study.

Figure 3.17 shows the same trend as with both the nitrites and nitrates measured in this group of wetlands. From this figure, it is clear that Lotus River had the highest phosphate values when compared to the other four wetlands. The phosphate concentration in the four wetlands ranged between 0 and 0.04 mg.L^{-1}, thus remaining constant throughout the wetlands. The large variability observed at Lotus River suggests that the concentration of phosphates was not constant throughout the wetland.

3.4 Discussion

The physical, chemical, and biological composition of water affects the ability of aquatic environments to sustain healthy ecosystems, thus, from section 3.3 above, it can be seen that a decline in water quality and quantity of a particular system will result in the distress of the organisms living within that system as well as the loss of ecosystem services.

The deviation from natural levels of environmental variables measured in this study, were monitored with the application of TWQR's. The TWQR for most of these variables, (with the exception of pH, temperature and turbidity), recommended that the maximum variation from natural levels should not be more than 15%. As mentioned in section 3.3, the TWQR is a range of concentrations and levels (of physico-chemical variables) within which no measurable adverse effect is expected on the overall health of the system (DWAF, 1996).

From the results obtained in section 3.3, it can be seen that in all the wetlands, pH and temperature values were within natural ranges (see table 3.1) for South African inland waters (DWAF, 1996). The pH of natural waters is determined by a variety of factors such as the geology, precipitation (acid rain), biotic and anthropogenic (agricultural, industrial, and mining effluents) activities (Davies and Day, 1998).

According to the South African Water Quality Guidelines, the rate of change of pH depends on the buffering capacity of the water, with the carbonate-bicarbonate system being the most important buffering system in fresh waters (DWAF, 1996). Therefore, even though the natural pH in surface waters ranges between 4 and 11 (table 3.1), most of South Africa's fresh water systems are well buffered, and the pH ranges between 6 and 8 (DWAF, 1996, and Davies and Day, 1998), as it can be seen in some of the studied wetlands.

However, as it was the case in KEN (figure 3.6), pH values lower than 4 can be found in some parts of the southern and south-western Cape, where very dilute sodium-chloride dominated waters are poorly buffered because they contain virtually no bicarbonate or carbonate (DWAF, 1996).

pH determines the availability and toxicity of chemical constituents (Davies and Day, 1998), it influences water chemistry as well as the type of organisms found in aquatic environments, as a result, it is considered to be one of the most important water quality parameters (DWAF, 1996).

The temperature in the studied wetlands can successfully sustain aquatic organisms since the levels measured in all these wetlands were within the temperature levels found in natural inland waters as shown table 3.1 above. Temperature is another essential variable which is important in the regulation or triggering of many physiological processes in aquatic organisms (DWAF, 1995), furthermore, it also plays a major role in the concentration of dissolved oxygen, as higher temperatures reduce the solubility of dissolved oxygen in water (Davies and Day, 1998), thus decreasing its concentration and thus its availability to aquatic organisms.

Oxygen naturally dissolves in water by diffusion from the surrounding air, by aeration, and as a waste product of photosynthesis (DWAF, 1996). The amount of dissolved oxygen naturally fluctuates diurnally, as a result of photosynthesis (which requires light, thus occurs only during daylight hours), respiration and decomposition (both occurring throughout the day), and also fluctuates seasonally, as a result of biological productivity and temperature (DWAF, 1996). The fluctuation of dissolved oxygen in natural waters is between 80 and 120% saturation, with levels below the saturation level indicating depleted oxygen, whereas values higher than this indicate eutrophication (DWAF, 1996).

Levels of dissolved oxygen saturation are influenced by the diffusion of oxygen from the surrounding air, temperature, and diurnal, as well as seasonal fluctuations. The turbulence of the water also increases the rate at which oxygen dissolves in water (DWAF, 1996). Some of the wetlands studied, had extremely low levels of dissolved oxygen (with lowest levels of 2% saturation encountered at WVV), which might be due to the fact that most wetland environments have stagnant waters, where there is very little water flow and turbulence (U.S EPA, 2002). Sine oxygen dissolves better in colder water, the levels of DO at KEN and LOT were expected to be higher when compared to the other wetlands, however, these two wetlands have the lowest levels of DO. These low levels of DO in these two wetlands might be a result of other factors such as low primary production, high respiration rates, as well as the presence of organic matter.

Organic matter may occur as a result of the death and decay of plant matter, or as a result of anthropogenic inputs such as domestic, sewage or industrial waste (Braid and Nawn, 2009). High levels of organic matter are oxidised by bacteria in the presence of oxygen and other oxidising agents found in water, thereby further reducing the concentration of dissolved oxygen (Braid and Nawn, 2009).

One of the major descriptors of water quality is the total amount of material dissolved in that sample of water (Dallas and Day, 2004). Factors such as the weathering of rocks, the dissolution of minerals in soils and decomposing plants, as well as evaporation and rainfall may influence the total amount of dissolved solids in the water (DWAF, 1996). The quantity of substances dissolved in water may be measured in one of three forms: as, salinity, electrical conductivity (EC), or total dissolved solids (TDS), where salinity measures the total amount of salts (saltiness of water) dissolved in the water, and conductivity measures the ionic activity of a solution in terms of its capacity to transmit an electrical current, whereas, TDS measures the total quantity of materials dissolved in that water (Davies and Day, 1998, and Dodds 2002).

Thus TDS is a measure of the total amount of organic and inorganic, as well as ionised and un-ionised material dissolved in a sample of water (DWAF, 1996). Since the electrical conductivity of water is a function of the number of ions in solution, and most of the material dissolved in water is ionic, this means that TDS and conductivity are closely correlated, thus conductivity can be used to give an estimate of the amount of total dissolved solids (TDS) or the total amount of dissolved ions in the water (DWAF, 1996, and Davies and Day, 1998). Therefore, the higher the concentration of ions in the water, the higher its electrical conductivity will be.

Wetlands such as AGU, eMFU, KEN, LOT and ZDV had the highest variability in EC values when compared to the other wetlands. Since ZDV was one of the wetlands whose water quality was monitored over three years, the high variability in EC values in this wetland may be a result of natural factors such as precipitation and evaporation, whereas the high variability in EC values within the other wetlands might have been influenced by pollution, or anthropogenic activities.

The transparency or clearness of water is considered to be another good measure of water quality. Turbidity is a measure of the degree to which water loses its transparency due to the presence of suspended particulates (DWAF, 1996). These suspended particles interfere with sunlight penetration by scattering the light, thus reducing the photosynthetic activity of primary producers (Davies and Day, 1998), hence lowering the oxygen concentration. Since it is considered to be equivalent to the measure of the concentration of suspended solids, turbidity is usually correlated with the concentration of suspended solids (DWAF, 1996).

As mentioned in the previous section (section 3.3), the low levels of turbidity measured in some of the studied wetlands (with the exception of WVV) are believed to be influenced by the low flow rates found within wetlands. However, since the erosion of land surfaces by wind and rain is a continuous and natural process, soil particles derived from land surfaces are one of the major suspended material found in most natural waters (DWAF, 1996), thus, the high variability measured at WVV, may be a result of soil erosion.

Some of the key processes of wetlands include nutrient retention (Kotze, 2009), thus bearing this in mind, wetlands are expected to have increased levels of nutrients.

Nitrogen, which may be present in both organic and inorganic forms in aquatic systems, is one of the most abundant elements in nature and an essential constituent of most biological and biochemical processes (Davies and Day 1998). In the previous section (section 3.3), it was mentioned that inorganic nitrogen includes all the major inorganic nitrogen components such as nitrates (NO_3^-), nitrites (NO_2^-), ammonium ions (NH_4^+) and ammonia (NH_3).

Under aerobic conditions, nitrite is rapidly oxidised to nitrate by nitrifying bacteria, whereas nitrate is reduced to nitrite (then to molecular nitrogen) by denitrifying bacteria under anaerobic conditions (DWAF, 1996). However, since nitrate is the more stable of the two forms, the two most important forms of dissolved inorganic nitrogen found in natural waters are ammonium (the non-toxic form in which nitrogen is assimilated by most plants) and nitrates (DWAF, 1996). Nitrite (which is toxic even at low concentrations) is the inorganic intermediate, whereas nitrate is the end-product of the oxidation of ammonia and organic nitrogen (Davies and Day, 1998).

With the exception of ammonia (NH_3) and nitrates (NO_3^-), the concentrations of inorganic constituents of nitrogen measured in this study (ammonium-NH_4^+ and nitrites-NO_2^-) were found to be within the recommended levels. The high concentration of nitrates, observed at LGV, LPV, and WVV (figure 3.14), may result in the rapid growth of algae and other aquatic plants in these wetlands. However, the elevated concentrations of the highly toxic ammonia found at OOG (figure 3.11), may result in the death of most of the aquatic organisms found in this wetland. The effects of inorganic nitrogen on aquatic ecosystems are measured by assessing changes in the trophic status accompanied by the growth of algae and other aquatic plants (DWAF, 1996).

Natural sources of phosphorus include the weathering of rocks, followed by the leaching of phosphate salts into surface waters, as well as the decomposition of organic matter. As mentioned in section 3.3, phosphorus is one of the key elements necessary for the growth of plants and animals (Davies and Day, 1998).

It occurs in nature in forms such as orthophosphates, metaphosphates and organically bound phosphate (DWAF, 1996). Orthophosphate (PO_4), which is formed by the oxidation of phosphorus, is the most abundant dissolved inorganic form in aquatic environments (DWAF, 1996).

Phosphate stimulates the growth of plankton and aquatic plants which provide food for fish and other aquatic organisms (Davies and Day, 1998). This may cause an increase in the fish population and improve the overall water quality. However, elevated concentrations of phosphates will result in excessive plant growth (mostly algae) and decay, the reduction of oxygen levels, and an overall reduction in water quality, a state known as eutrophication (DWAF, 1996). The concentrations of phosphates in the studied wetlands were all below the recommended levels.

Although wetlands are expected to have high concentrations of nutrients, these levels should however, not be higher than permissible levels stipulated in table 3.1. Elevated concentrations on nutrients may result in changes in the trophic status of aquatic environments by stimulating the growth of algae and other aquatic plants (DWAF, 1996).

3.5 Conclusion

From studying the previous sections (sections 3.3 and 3.4), it can be seen that the environmental variables measured in this study were closely interrelated, and as a result, may influence other variables. This was seen in the case of DO saturation which is influenced by temperature as well as turbidity levels. It can therefore be concluded that all environmental variables measured in the study are important, as each variable has an influence on the next.

Based on the physico-chemical variables measured in the studied wetlands, it can be concluded that the water at Lotus River, Wildevoël Vlei and Zand Vlei is of poor quality.

The species composition of the diatoms found within all the studied wetlands will be discussed in the next chapter. Species found in each wetland will be used to derive diatom indices, and will also be correlated to the environmental variables measured in this chapter. The measured indices will in turn be used to infer water quality.

3.6 References

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CHAPTER FOUR

RESULTS AND DISCUSSION – APPLICATION AND TESTING OF DIATOM INDICES

4.1 Introduction

Diatoms have one of the shortest generation times of all biological indicators (Morales *et al.*, 2001, and U.S. EPA, 2002). They produce and respond rapidly to environmental change and provide early measures of both pollution and habitat restoration, thus they give short term reflections of water quality (Round, 1993).

This chapter discusses the importance of diatoms, by studying their abundance in the thirteen wetlands; their relationship to water quality variables, and by calculating diatom indices, as well as studying the correlation between these indices and the water quality variables discussed in chapter three.

4.2 Species composition

Since the value generated by a diatom index is the result of the mean of the water quality optima of the diatom taxa in a sample, weighted by the abundance of each taxon, it is therefore very important that diatom identification be as accurate as possible to increase the validity of the value generated by a particular index (Kriel, 2008). The identification of diatom taxa was made under a light microscope, and the scanning electron microscope was only used where diatoms could not be accurately identified using light microscopy.

A total of two hundred and twenty six diatom taxa from fifty seven genera were identified during the course of the study, and of these, forty eight were recorded as dominant (more than 5% of any given community). A list of the names of all the species encountered during the study is given in table 4.1, and a complete list (names and abbreviations) of species is found in Appendix C. The light and scanning electron micrographs of the 48 dominant species found in the studied wetlands are illustrated in Appendix E, and a list of the relative abundance of species occurring in each sample is given in Appendix C.

Table 4.1 The list of diatom species encountered in the study (dominant species are in bold).

Taxon
Achnanthes brevipes Agardh
Achnanthes coarctata (Brébisson) Grunow in Cl. & Grun.
Achnanthes linearioides Lange-Bertalot
Achnanthes oblongella Østrup Scandinavian
Achnanthes species
Achnanthes standeri Cholnoky
Achanthes subaffinis Cholnoky
Achnanthidium biasolettianum (Grunow in Cl. & Grun.) Lange-Bertalot
Achnanthidium eutrophilum (Lange-Bertalot)
Achnanthidium exiguum (Grunow) Czarnecki
Achnanthidium macrocephalum (Hust.) Round & Bukhtiyarova
Achnanthidium minutissimum (Kützing) Czarnecki
Achnanthidium rivulare Potapova & Ponader
Achnanthidium straubianum (Lange-Bertalot)
Adlafia species
Amphora coffeaeformis (Agardh) Kützing
Amphora 58alophi Krasske
Amphora pediculus (Kützing) Grunow
Amphora veneta Kützing
Anomoeoneis sphaerophora (Ehrenberg) Pfitzer
Aulacoseira ambigua (Grun.) Simonsen
Aulacoseira crassipunctata Krammer
Aulacoseira granulata var. angustissima (O.M.) Simonsen
Aulacoseira 58alophile (Ehrenberg) Simonsen
Aulacoseira subarctica (O. Müller) Haworth
Aulacoseira species
Brachysira brebissonii Ross in Hartley
Brachysira brebissonii fo.*thermalis* (Grunow in Van Heurck) Ross
Brachysira neoexilis Lange-Bertalot
Brachysira species.
Caloneis aequatorialis Hustedt
Caloneis molaris (Grunow) Krammer
Caloneis schumanniana (Grunow) Cleve
Caloneis species
Cocconeis engelbrenchti Cholnoky
Cocconeis placentula Ehrenberg
Cocconeis placentula var. euglypta (Ehrenberg) Grunow
Cocconeis placentula var. lineata (Ehrenberg) Grunow
Cosmioneis species.
Craticula ambigua (Ehrenberg) Mann
Craticula accomoda (Hustedt) Mann
Craticula acidoclinata Lange-Bertalot & Metzeltin
Craticula buderi (Hustedt) Lange-Bertalot
Craticula 58alophile (Grunow & Van Heurck) Mann
Craticula species
Craticula vixnegligenda Lange-Bertalot
Ctenophora pulchella (Ralfs & Kützing) Williams & Round
Cyclotella meneghiniana Kützin**g**

Taxon
Cyclotella ocellata Pantocsek
Cymbella kappii (Cholnoky)
Cymbella kolbei Hustedt
Cymbella simonsenii Krammer
Cymbella species
Denticula kuetzingii Grunow
Denticula subtilis Grunow
Denticula sundayensis Archibald
Diadesmis confervacea Kützing
Diadesmis contenta (Grunow & V. Heurck) Mann
Diatoma vulgaris Bory
Diploneis elliptica (Kützing) Cleve
Diploneis oblongella (Naegeli) Cleve-Euler
Diploneis puella (Schumann) Cleve
Diploneis species.
Diploneis subovalis Cleve
Discostella pseudostelligera (Hustedt) Houk & Klee
Encyonema minutum (Hilse in Rabh.) D.G. Mann
Encyonema neogracile Krammer
Encyonema species
Encyonopsis leei Krammer
Entomoneis paludosa (W. Smith) Reimer
Entomoneis pseudoduplex Osada & Kobayasi
Entomoneis species.
Eolimna minima (Grunow) Lange-Bertalot
Eolimna subminuscula (Manguin) Moser, Lange-Bertalot & Metzeltin
Epithemia species
Eunotia bilunaris (Ehr.) Mills
Eunotia flexuosa (Brébisson) Kützing
Eunotia formica Ehrenberg
Eunotia minor (Kützing) Grunow in Van Heurck
Eunotia pectinalis(Kütz.) var.undulata (Ralfs) Rabenhorst
Eunotia rhomboidea Hustedt
***Eunotia* species.**
Eunotia tridentula Ehrenberg
Fallacia pygmaea (Kützing) Stickle & Mann
Fallacia species
Entomoneis paludosa (W. Smith) Reimer
Fragilaria capucina Desmazieres
Fragilaria capucina var. rumpens (Kütz) Lange-Bert. teratological form
Fragilaria capucina Desmazieres var.vaucheriae (Kützing)Lange-Bertalot
Fragilaria exigua Grunow
Fragilaria germainii Reichardt & Lange-Bertalot
Fragilaria nanana Lange-Bertalot
***Fragilaria* species**
Fragilaria tenera (W. Smith) Lange-Bertalot
Fragilaria ulna (Nitzsch.) Lange-Bertalot
Fragilaria ulna var. acus (Kützing) Lange-Bertalot
Frustulia saxonica Rabenhorst
Frustulia vulgaris (Thwaites) De Toni

Taxon
Frustulia species
Gomphonema acuminatum Ehrenberg
Gomphonema affine Kutzing
Gomphonema affine gracile
Gomphonema capitatum Ehrenberg
Gomphonema gracile Ehrenberg sensu lato
Gomphonema gracile Ehrenberg sensu stricto
Gomphonema insigne Gregory
Gomphonema italicum Kützing
Gomphonema parvulum (Kützing) Kützing
Gomphonema parvulum var. *parvulius* Lange-Bertalot & Reichardt
Gomphonema parvulum var. *parvulum* f.*saprophilum* Lange-Bert. & Reichardt
Gomphonema pseudoaugur Lange-Bertalot
Gomphonema species
Gomphonema truncatum Ehr.
Gomphosphenia oahuensis (Hustedt) Lange-Bertalot
Hantzschia amphioxys (Ehrenberg) Grunow in Cleve & Grunow
Hantzschia distinctepunctata Hustedt in Schmidt *et al.*,
Hantzschia species.
Hippodonta capitata (Ehr.) Lange-Bert.Metzeltin & Witkowski
Hippodonta species
Lemnicola hungarica (Grunow) Round & Basson
Luticola goeppertiana (Bleisch in Rabenhorst) D.G. Mann
Luticola mutica (Kützing) D.G. Mann
Luticola species
Lyrella species.
Mastogloia smithii Thwaites
Mastogloia species.
Mayamaea species.
Melosira varians Agardh
Navicula cincta (Ehr.) Ralfs in Pritchard
Navicula cryptotenella Lange-Bertalot
Navicula gregaria Donkin
Navicula grevillei (Agardh) Heiberg
Navicula libonensis Schoeman
Navicula pusilla W.Smith
Navicula radiosa Kützing
Navicula recens (Lange-Bertalot) Lange-Bertalot
Navicula reichardtiana Lange-Bertalot
Navicula rhynchocephala Kützing
Navicula rostellata Kützing
Navicula species.
Navicula vandamii Schoeman & Archibald
Navicula variostriata Krasske
Navicula veneta Kützing
Navicula viridula (Kützing) Ehrenberg
Navicula zanoni Hustedt
Navicymbula pusilla Krammer
Navicula subrhynchocephala Hustedt
Navicula tenelloides Hustedt
Neidium species in Metzeltin & Lange Bertalot

Taxon
Nitzschia acicularis (Kützing) W.M. Smith
Nitzschia acidoclinata Lange-Bertalot
Nitzschia amphibia Grunow
Nitzschia archibaldii Lange-Bertalot
Nitzschia bacillum Hustedt
Nitzschia capitellata Hustedt in A. Schmidt *et al*.,
Nitzschia elegantula Grunow
Nitzschia etoshensis Cholnoky
Nitzschia filiformis (W.M.Smith) Van Heurck
Nitzschia fonticola Grunow
Nitzschia frustulum (Kützing) Grunow
Nitzschia hantzschiana Rabenhorst
Nitzschia heufleriana Grunow
Nitzschia liebetruthii Rabenhorst
Nitzschia linearis (Agardh) W.M. Smith
Nitzschia littorea Grunow in Van Heurck
Nitzschia microcephala Grunow in Cleve & Müller
Nitzschia nana Grunow in Van Heurck
Nitzschia obsidialis Hustedt
Nitzschia palea (Kützing) W. Smith
Nitzschia perspicua
Nitzschia pilum Hustedt
Nitzschia pura Hustedt
Nitzschia pusilla (Kützing) Grunow
Nitzschia radicula Hustedt
Nitzschia recta Hantzsch in Rabenhorst
Nitzschia sigma (Kützing) W.M. Smith
Nitzschia species
Nitzschia vitrea Norman
Nitzschia supralitorea Lange-Bertalot
Nitzschia umbonata (Ehrenberg) Lange-Bertalot
Petroneis humerosa (Brebisson & W.M. Smith) Stickle & Mann
Petroneis marina (Ralfs) D.G. Mann in Round *et al*.,
Pinnularia borealis Ehrenberg
Pinnularia divergens W.M. Smith
Pinnularia irrorata (Grunow) Hustedt
Pinnularia microstauron (Ehr.) Cleve var. rostrata Krammer
Pinnularia subbrevistriata Krammer
Pinnularia subcapitata f. *divergens* Hustedt
***Pinnularia* species**
Pinnularia viridiformis Krammer
***Placoneis* species**
Planothidium engelbrechtii (Cholnoky) Round & Bukhtiyarova
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot
Planothidium rostratum (Østrup) Round & Bukhtiyarova
Planothidium species
Pleurosigma salinarum (Grunow) Cleve & Grunow
Pseudostaurosira brevistriata (Grun.in Van Heurck) Williams & Round
Rhopalodia constricta (W. Smith) Krammer
Rhopalodia gibba (Ehr.) O. Müller
Rhopalodia gibberula (Ehrenberg) O.Müller

Taxon
Rhopalodia operculata (Agardh) Hakansson
Rhopalodia species
Sellaphora pupula (Kützing) Mereschkowksy
Sellaphora seminulum (Grunow) D.G. Mann
Sellaphora species
Sellaphora stroemii (Hustedt) Mann
Seminavis strigosa (Hustedt) Danieledis & Economou-Amilli
Stauroneis anceps Ehrenberg
Stauroneis marina Hustedt
Stauroneis phoenicenteron (Nitzsch) Ehrenberg
Stauroneis species
Staurosira elliptica (Schumann) Williams & Round
Surirella brebissonii Krammer & Lange-Bertalot
Surirella crumena Brebisson ex Kützing
Surirella species
Tabellaria flocculosa (Roth) Kützing
Tabularia fasciculata (Agardh) Williams & Round
Thalassiosira pseudonana Hasle & Heimdal
Tryblionella apiculata Gregory
Tryblionella calida (Grunow in Cleve & Grunow) D.G. Mann
Tryblionella debilis Arnott ex O'Meara
Tryblionella hungarica (Grunow) D.G. Mann
Tryblionella levidensis Wm. Smith
Tryblionella littoralis (Grunow in Cl. & Grun.) D.G. Mann

4.3 Water quality variables and dominant species

Individuals in a community have specific requirements or optimum conditions in which they can survive, and like in any other community, should there be a disturbance in the natural living conditions, the sensitive individuals or species would be the first ones to die, whereas the more resistant species would proliferate, and become dominant as there would be less competition for resources. Large differences in species composition can be found when studying variation within communities across different environmental conditions, however, these results often include certain consistency within the variation. (Lepš *et al.,* 2003). This might often be related to different but sometimes overlapping requirements of the individual species for certain environmental conditions such as certain pH, temperature, dissolved oxygen, and it can also be the result of the ability of certain species to compete for light, nutrients and other essential resources (Lepš *et al.,* 2003).

Diatom communities are not an exception; different species have different requirements, with some species showing more sensitivity to certain environmental variables than others. Thus since dominant species can be taken to be representative of their environment, this section looks at the relationship between water quality variables discussed in the previous chapter and the dominant species found in each wetland. To discover how these species respond to their environment, this study made use of an ordination technique called canonical correspondence analysis (CCA).

4.3.1 Canonical correspondence analysis (CCA)

Canonical correspondence analysis (CCA) is a multivariate gradient analysis technique, in which taxa are directly related to a set of environmental variables (de Almeida *et al.*, 2001). This technique detects the patterns of variation in community composition that can be explained best by the environmental variables (ter Braak, 1986). CCA allows obtaining a simultaneous representation of the sites, the objects, and the variables in two or three dimensions which are optimal for a variance criterion (ter Braak, 1986). It provides an integrated description of species-environment relationships by assuming a response model that is common to all species and the existence of single underlying environmental variables to which all species respond (ter Braak, 1986).

The figures below made use of CCA to show the relationship between the environmental variables and the dominant diatom species. Complete species names are given in Appendix C.

In the CCA diagrams below, the origin or centre (0,0) of the graph indicates the mean of the variable, whereas the arrows point in the direction of maximum change in the value of the associated variable (ter Braak *et al.,* 1995). Thus the length of the arrow is proportional to the maximum rate of change. The value of the variable does not change in the perpendicular direction (ter Braak *et al.,* 1995). In the opposite direction however, the arrow would point in the direction of minimum change of the associated vector (ter Braak *et al.,* 1995).

Tables 4.2 and 4.3 below give the summary of the water quality variables data used in the interpretations of the CCA diagrams in figure 4.1 and 4.2 respectively. These tables give the minimum and maximum levels of the measured variables which represent the lowest and highest values of the vectors on the CCA graphs. These values correspond to the levels preferred by the diatoms found within the studied wetlands.

Table 4.2 Summary of the environmental variable data used for the CCA presented in Figure 4.1.

	Mean	Standard Error	Median	Range	Standard Deviation	Minimum	Maximum
pH	8.081	0.043	8.100	5.100	0.719	5.400	10.500
Temp (˚C)	16.910	0.244	16.700	19.100	4.012	7.300	26.400
DO %	72.433	1.986	69.000	221.000	32.639	2.000	223.000
Turbidity (NTU)	51.894	9.672	20.000	2039.000	159.225	1.000	2040.000
EC (μS.cm^{-1})	302.093	31.080	107.500	2663.000	516.345	17.000	2680.000
NO3 (mg.L^{-1})	0.324	0.033	0.070	3.703	0.540	0.003	3.706
NH3 (mg.L^{-1})	0.206	0.028	0.060	3.787	0.457	0.003	3.790
PO4 (mg.L^{-1})	0.259	0.041	0.025	4.627	0.685	0.003	4.630

From table 4.2 above, the maximum levels of the measured variables are the values that would be represented by arrows on the CCA diagram in figure 4.1. As discussed above, the origin of the graph represents the mean of the variable, whereas the minimum value of a certain variable can be extrapolated from the graph in the opposite direction of that particular vector.
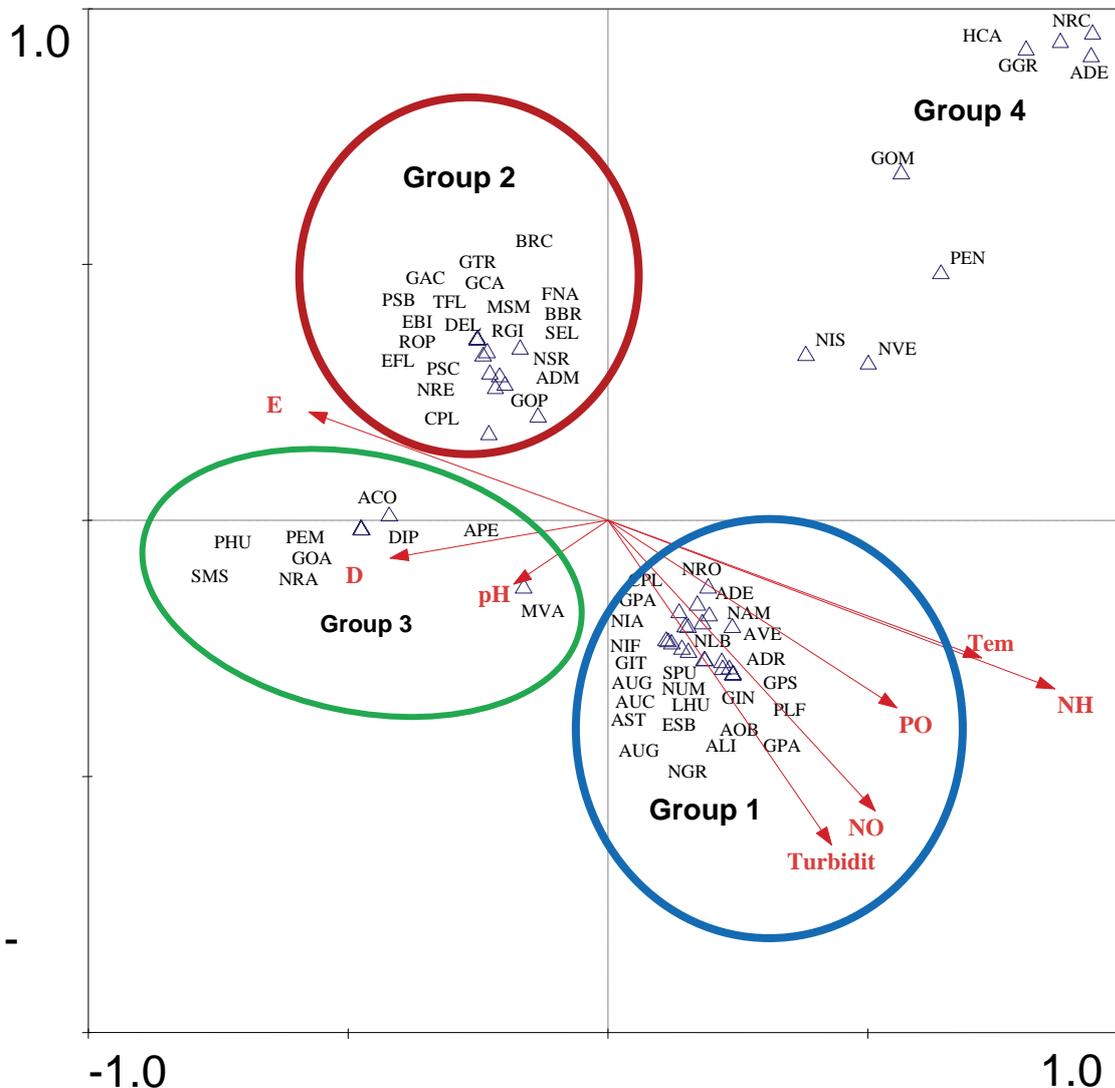
Figure 4.1 Canonical correspondence analysis biplot showing the relationship between the dominant diatom species and environmental variables measured in the wetlands whose water samples were collected over a period of three years. A summary of the environmental variable data used for the interpretation of this CCA is given in table 4.2 below. Full names with corresponding codes of species are listed in appendix C.

In figure 4.1 above it can be seen that the diatom species encountered in these wetlands can be divided into four groups, with the first group representing those diatoms that are associated with high levels of temperature, turbidity, and nutrients. The second group is represented by those diatoms associated with high levels of EC and low levels of temperature, turbidity, and nutrients. The third group of diatoms are those associated with increasing pH and DO, whereas the last group of diatoms are those that have a preference for low levels of pH and Dissolved oxygen saturation.

The first group of species are those with an affinity for high levels of temperature, turbidity and are also associated with elevated nutrient concentrations. These diatoms include *Achnanthes linearioides, Achnanthes oblongella, Achnanthidium exiguum, Achnanthidium rivulare, Amphora veneta, Aulacoseira crassipunctata, Aulacoseira granulata, Cocconeis placentula* var. *euglypta, Eolimna subminiscula, Gomphonema insigne, Gomphonema italicum, Gomphonema parvulum, Gomphonema parvulum* var. s*aprophilum, Lemnicola hungarica, Navicula gregaria, Navicula rostellata, Nitzschia amphibia, Nitzschia archibaldii, Nitzschia frustulum, Nitzschia liebertruthii, Nitzschia umbonata, Planothidium frequentissimum* and *Sellaphora pupula.*

The diatoms found in group 2 are those associated with high EC values. Since these diatoms occur on the opposite ends of the vectors of temperature, turbidity and nutrients, these species are therefore affiliated with low levels of these particular environmental variables than the species in group 1. Some of the species found in this group were *Achnanthidium minutissimum*, *Brachysira brebissoni*, *Brachysira* species, *Cocconeis placentula, Diploneis elliptica, Eunotia bilunaris, Eunotia flexuosa, Fragilaria nanana, Gomphonema acuminatum, Gomphonema capitatum, Gomphonema* species*, Gomphonema truncatum, Mastoglia smithii, Navicula subrhynchocephala, Nitzschia recta, Pinnularia subbrevistriata, Rhopalodia gibba, Rhopalodia operculata*, *Staurosira elliptica*, and *Tabellaria flocullosa*.

The diagram also shows that species such as *Amphora coffeaeformis, Amphora pediculus, Diploneis* species, *Gomphosphenia oahuensis, Melosira varians, Navicula radiosa, Petroneis humerosa, Petroneis marina,* and *Seminavis strigosa* which represent the third group on the diagram are those that are associated with increasing pH, and higher levels of dissolved oxygen.

The last group of diatoms (group 4) which can be seen on the diagram are those that are affiliated with low levels of pH and dissolved oxygen. These species include *Achnanthidium eutrophilum, Gomphonema* species, *Gomphonema gracile, Hippodonta capitata, Navicula reichardtiana, Navicula veneta*, and *Nitzschia* species. Furthermore, by looking at this figure it can be seen that the majority of the species found in these wetlands are those that are tolerant to pollution, and thus indicate poor water quality. Some of those tolerant species are *Amphora veneta, Cocconeis placentula, Eolimna subminiscula, Gomphonema parvulum, Gomphonema truncatum, Navicula veneta*, *Nitzschia amphibia, Nitzschia frustulum,* and *Sellaphora pupula* (Lange-Bertalot, 1979, Round, 1993, and Kelly *et al.*, 1995).

Table 4.3 Summary of the environmental variable data used for the CCA presented in Figure 4.2.

	Mean	Standard error	Median	Standard Deviation	Range	Minimum	Maximum
pH	7.653	0.173	7.650	1.352	5.970	4.210	10.180
Temp (°C)	18.051	0.598	17.500	4.668	18.300	10.900	29.200
DO %	48.021	6.737	9.490	52.615	164.370	1.630	166.000
Turbidity	4.370	0.810	2.500	6.328	44.800	0.700	45.500
EC (µS.cm⁻¹)	448.094	70.332	166.000	549.307	1825.610	2.390	1828.000
NO2 (mg.L⁻¹)	14.995	9.689	0.299	68.511	391.573	0.010	391.583
NO3 (mg.L⁻¹)	0.201	0.152	0.002	1.107	7.981	0.000	7.981
NH4 (mg.L⁻¹)	46.206	18.034	22.069	137.343	1053.847	0.230	1054.077
PO4 (mg.L⁻¹)	0.069	0.034	0.009	0.254	1.538	0.001	1.539

The maximum levels of the measured variables can also be seen in table 4.3 above; these are the values that are represented by arrows on the CCA diagram in figure 4.2 below. As previously discussed, the origin of the graph represents the mean of the variable, whereas the minimum value of a certain variable can be extrapolated from the graph in the opposite direction of that particular vector.

In figure 4.2 below, three distinct groups of diatoms can be seen, the first group of diatoms are those that require elevated levels of nitrites, whereas the second group of diatoms require increasing levels of temperature, nitrates, phosphates, dissolved oxygen, as well as ammonium ions, and the third group of diatoms are those that are associated with low pH, turbidity and electrical conductivity (EC).

Some of the species which form part of group 1 are species such as *Achnanthes coarctata, Cosmioneis* species, *Cymbella kappii, Eunotia bilunaris, Fragilaria capucina, Fragilaria exigua, Gomphonema parvulum, Hanzschia distintepuntata, Hanzschia* species, *Navicula cincta, Nitzschia archibaldii, Nitzschia* species, *Sellaphora pupula, Stauroneis anceps* and *Staurosira* species. Species such as *C. kappii, E. bilunaris*, *F.capucina,* and *N. archibaldii,* are *s*ome of those species that require low to moderate levels of electrolytes (Taylor *et al.,* 2007a). This group is also made up of species such as *G. Parvulum, N. cincta,* and *S. pupula* which are tolerant to moderate levels of pollution (Round, 1993).

The second group includes diatoms such as *Achnanthidium macrocephalum, Brachysira brebissoni*, *Diploneis oblongella, Diatoma vulgaris, Eunotia rhomboidea*, *Frustulia vulgaris*, *Lyrella* species, *Mastoglia smithii, Mastoglia* species, *Navicula pusilla*, *Navicula recens*, *Nitzschia acicularis, Nitzschia bacillum, Nitzschia filiformis, Nitzschia hantzschiana, Nitzschia heufleriana, Nitzschia obsidialis, Nitzschia pusilla, Nitzschia radicula, Nitzschia recta, Nitzschia sigma, Pinnularia borealis, Pinnularia subbrevistriata, Stauroneis marina, Stauroneis phoenicenteron, Stauroneis* species, *Tryblionella calida, Tryblionella levidensis* and *Tryblionella littoralis.*
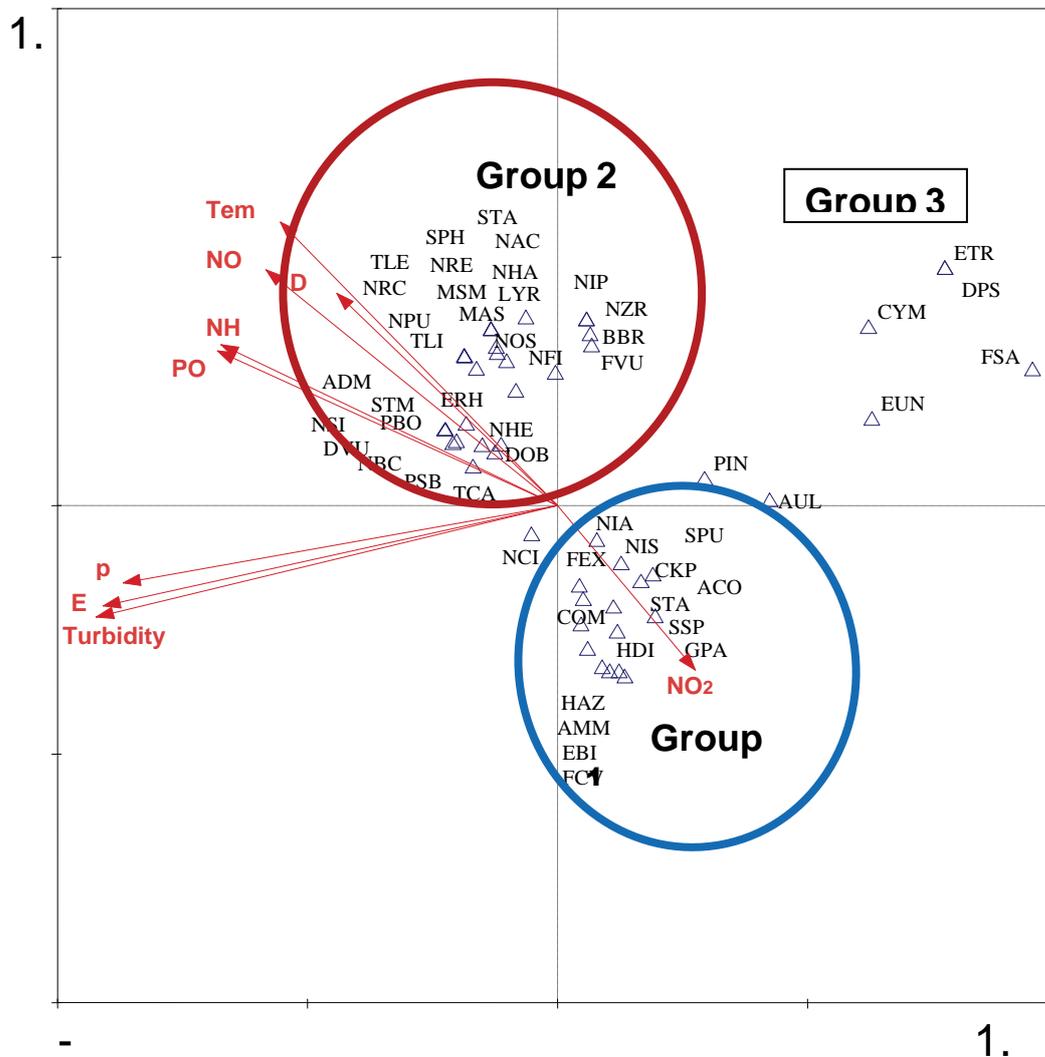
Figure 4.2 Canonical correspondence analysis biplot showing the relationship between the dominant diatom species and measured environmental variables measured in wetlands whose water samples were collected once-off. A summary of the environmental variable data used for this CCA is given in table 4.3.

The species found in group 3, were *Aulacoseira* species, *Cymbella* species, *Discostella pseudostelligera*, *Eunotia tridentula*, *Eunotia* species, *Fragilaria saxonica,* and *Pinnularia* species.

From this diagram it can be seen that variables such as temperature, dissolved oxygen, nitrates, ammonium ions and phosphates are the main drivers in these wetlands, as most of the diatoms are strongly affiliated with these variables. Most of the species in this group are those that are indicative of moderate pollution as well as moderate to electrolyte rich waters with species such as *Navicula recens, Nitzschia filiformis* and *Tryblionella levidensis* indicating critical to heavy levels of pollution (Taylor *et al.,* 2007a).

When observing the two diagrams (figure 4.1 and 4.2), it can be seen that the majority of species found in these wetlands are those that indicate poor water quality, represented by species such as, *Amphora veneta, Cocconeis placentula, Eolimna subminiscula, Cocconeis placentula, Gomphonema parvulum, Gomphonema truncatum, Mastoglia smithii, Navicula cincta, Navicula veneta, Nitzschia amphibia, Nitzschia frustulum, Nitzschia* species, and *Sellaphora pupula.* Some of the sensitive species found in the studied wetlands were *Achnanthidium minutissimum, Cocconeis placentula, Diatoma vulgaris, Gomphonema truncatum, Nitzschia frustulum, Nitzschia heufleriana* (Lange-Bertalot, 1979).

One of the species found dominating most sites is *Achnanthidium minutissimum* (group 2 on figure 4.1.). This species is found within less impacted waters, with low pH (Lange-Bertalot, 1979, Battarbee, 1997, Sabater, 2000, and Taylor *et al.,* 2007b). In other studies, however, this species has been found in nutrient rich waters, with a higher pH (Round, 1993) as well as in oligo-eutraphentic environments (van Dam, *et al.*, 1993), thus suggesting that *Achnanthidium minutissimum* has a wide tolerance range.

4.4 Application of diatom Indices

Once the sample has been counted in the correct manner (as discussed in chapter 2), the data can be entered into a computer data-base known as Omnidia (Lecointe *et al*., 1993) in which several diatom indices can be calculated using the sum of the water quality optima of all the species in the sample.

As it was mentioned in chapter 1, Omnidia calculates up to seventeen diatom indices. These include indices such as DES and SLA, which were developed for correlation with parameters related to organic pollution, and others such as CEE, GDI, LMI and SPI whose parameters are related to organic pollution, ionic strength, and eutrophication (Prygiel and Coste, 1993).

Based on the information in chapter 1, it is evident that the calculation of diatom indices is influenced by the number of species incorporated in the calculation. Since percentage composition plays such an important role in the calculation of diatom indices, it was deemed important to study the percentage composition of all the seventeen indices calculated in Omnidia.

For the purposes of this study, the reliability (or confidence level) of indices is evaluated based on the number of diatom species (percentage composition) included in the calculation, as well as the number of wetlands in which a particular index is represented by populations of 50% or more. Table 4.4 below, gives the percentage composition of the indices in different wetlands.

Table 4.4 The average percentage composition of the diatom indices calculated. Bold values represent a population of 50% or more.

	IDAP	EPI-D	BDI	SHE	SID	TID	WAT	SPI	SLA	DES	L&M	GDI	CEE	LOBO	IDP	DI-CH	TDI
AGU	34.55	**52.15**	**50.04**	**50.38**	**53.75**	**53.14**	11.33	**92.74**	48.76	13.91	28.62	**92.53**	**51.36**	25.74	26.19	34.01	**84.17**
DRI	32.36	**52.59**	**52.52**	42.06	45.65	48.19	12.13	**95.47**	47.26	17.84	28.26	**97.12**	48.37	21.12	31.09	34.66	**87.11**
GCV	**63.99**	**59.00**	**53.36**	**52.28**	**86.12**	**65.29**	38.18	**95.66**	**60.09**	**59.65**	**52.71**	**100.0**	**54.45**	**51.63**	12.36	**51.19**	**98.92**
KEN	12.94	21.04	21.04	24.54	25.42	26.42	7.73	**80.47**	20.39	10.30	18.04	**85.65**	25.62	14.24	8.48	18.19	**83.88**
LGV	**59.11**	**77.54**	**77.99**	**74.41**	**73.74**	**75.75**	7.26	**88.72**	**55.64**	32.29	46.15	**93.97**	**65.14**	40.11	35.75	**65.03**	**89.39**
LOT	27.45	38.72	44.13	38.69	39.95	41.49	4.91	**83.02**	39.13	14.90	26.68	**87.45**	45.75	16.08	13.55	28.50	**82.92**
LPV	**50.47**	**84.99**	**86.05**	**54.73**	34.28	**65.25**	34.75	**99.76**	**77.90**	25.18	45.15	**100.0**	**61.23**	**71.63**	**63.48**	**81.80**	**73.88**
MFU	31.98	**74.15**	**58.83**	**66.17**	**66.09**	**69.03**	15.93	**94.96**	**62.82**	28.55	39.35	**96.05**	**59.52**	31.81	39.68	**60.89**	**90.93**
OOG	**56.67**	**62.71**	**62.29**	**62.29**	**63.13**	**61.04**	5.63	**71.46**	**58.13**	4.17	2.92	**71.88**	**57.08**	4.79	3.33	**62.08**	**71.46**
RTV	12.88	33.17	34.87	32.93	32.69	31.96	3.89	**87.36**	12.88	5.10	27.34	**99.76**	34.87	6.68	24.54	30.74	**99.03**
WVV	**71.88**	**72.13**	**82.64**	**68.46**	**66.99**	**79.71**	17.85	**88.75**	**74.33**	**61.12**	**74.08**	**93.15**	**83.37**	**55.26**	**65.77**	**77.51**	**79.46**
ZDV	14.29	11.86	11.86	6.30	6.30	6.78	1.45	**99.76**	11.38	5.08	5.57	**100.0**	11.86	3.63	9.20	6.78	**99.03**
ZRV	**66.67**	**83.33**	**85.71**	**73.81**	**73.81**	**78.57**	16.67	**90.48**	**83.33**	30.95	38.10	**90.48**	**90.48**	35.71	30.95	**71.43**	**90.48**
Number	**6**	**9**	**9**	**8**	**8**	**8**	-	**13**	**7**	**2**	**2**	**13**	**8**	**3**	**2**	**7**	**13**

The last row of the table (number) represents the number of wetlands in which the different indices incorporated 50% of the diatom population or more. A complete table with the % of species used in the calculations of the indices is given in Appendix D.

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From this table, it can be seen that, of the seventeen indices calculated, only one index (WAT) incorporated less than 50% (with 38.18% being the highest) of the diatom population in all wetlands. It can also be seen from this table that the percentage composition of these indices fluctuated within the different wetlands, this is due to the fact that these wetlands had different diatom communities. It can also be seen from this table below, that only three indices incorporated most, if not all the diatom species found in each wetland. This study therefore, tested the applicability of these three indices in the selected wetlands.

The Generic Diatom Index (GDI), Specific Pollution Sensitivity index (SPI) as well as the Trophic Diatom Index (TDI), were applied in the studied wetlands, because they showed to be the most reliable indices as they had the highest percentage composition of diatom species in all the wetlands. The results of these three indices are given in figures 4.3 to 4.8 below. The interpretations of the results obtained from these indices were based on the index scores shown in table 4.5 and 4.6 below.

Table 4.5 The index scores indicating different classes of water quality based on the GDI and SPI scores (de la Rey *et al*., 2004).

Index score	Class
> 17	High Quality
15 to 17	Good Quality
12 to 15	Moderate Quality
9 to 12	Poor Quality
< 9	Bad Quality

This table shows the values given for the classification of water quality. These index scores range from zero to twenty, with an increasing score indicating good quality water (de la Rey *et al.,* 2004).
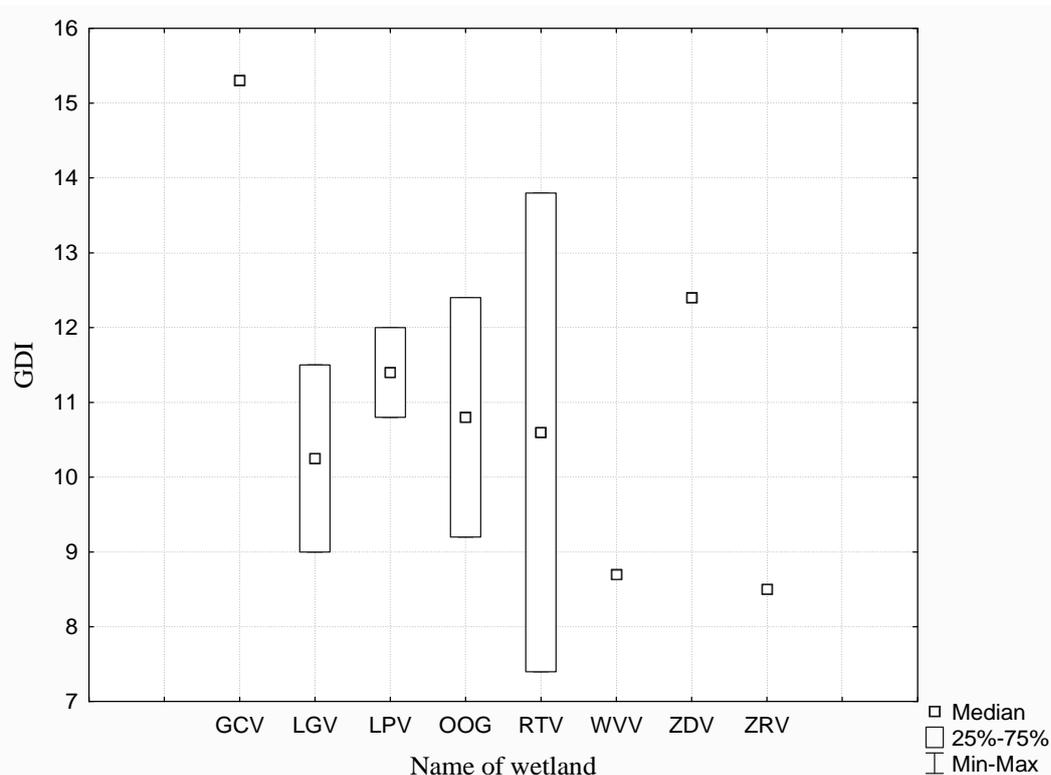
Figure 4.3 The median GDI values of the wetlands whose water samples were collected over three years.

It can be seen in figure 4.3; that the median GDI indicates poor water quality in most wetlands, with the median values at Glen Cairn Vlei and Zand Vlei showing slightly better quality than the rest of the wetlands.

The large variability in GDI scores in Rietvlei may be influenced by the presence of different species of diatoms encountered throughout the duration of the study. From the graph, it can also be seen that Zoar Vlei had the lowest median readings, suggesting lower water quality when these wetlands are compared with regard to their GDI median values.

From figure 4.4 below, it can be seen that the quality of water in the five wetlands was almost similar when comparing the median values. It can also be seen that the narrow variability in AGU and DRI suggests that the quality of water in these two wetlands might be uniform throughout the wetland, when compared to the large variability seen in Kenilworth and Lotus River, whose GDI ranges between 1 and 20 at Kenilworth and between 2 and 15 at Lotus River, suggesting that the water at some of the sites in these wetlands is of bad quality (table 4.5).
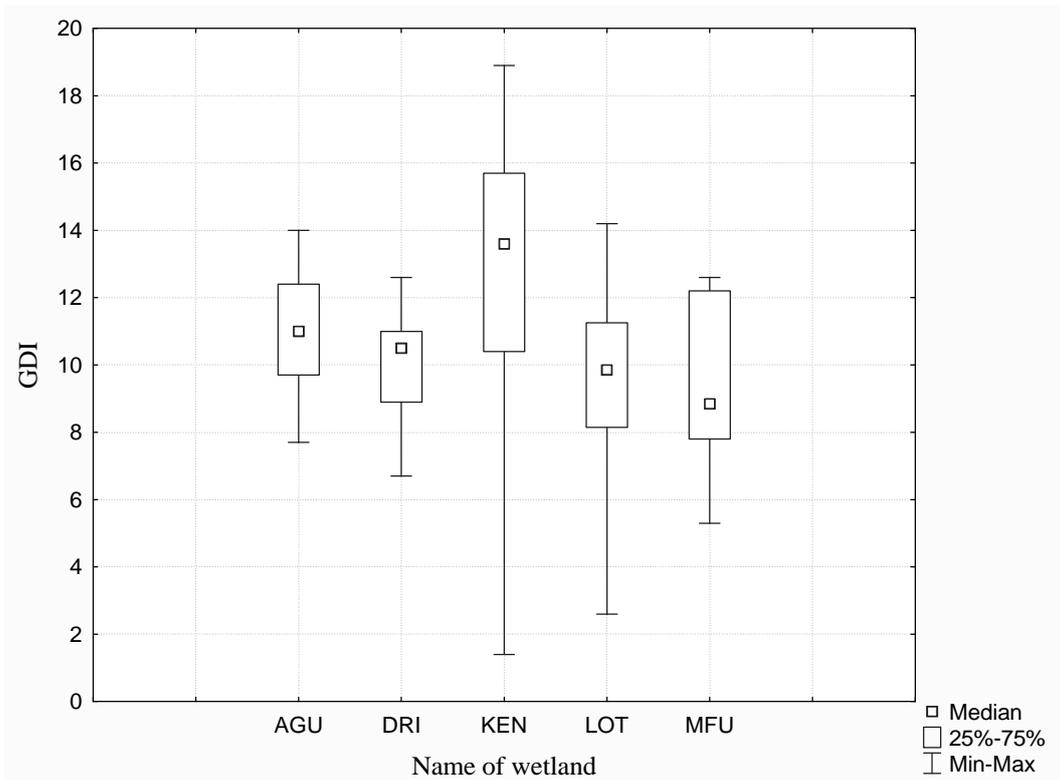
Figure 4.4 The median GDI values of the wetlands whose water samples were collected only once.

Figure 4.5 and 4.6 below; show the median SPI values in the different wetlands.
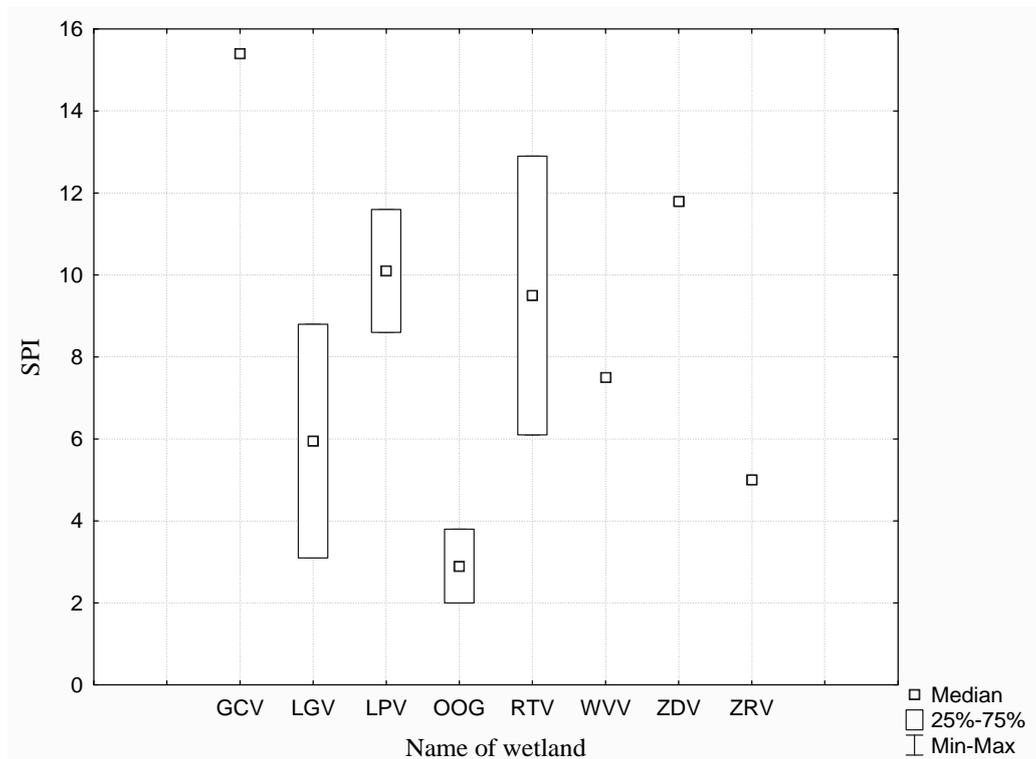
Figure 4.5 The median SPI values of the wetlands whose water samples were collected over three years.
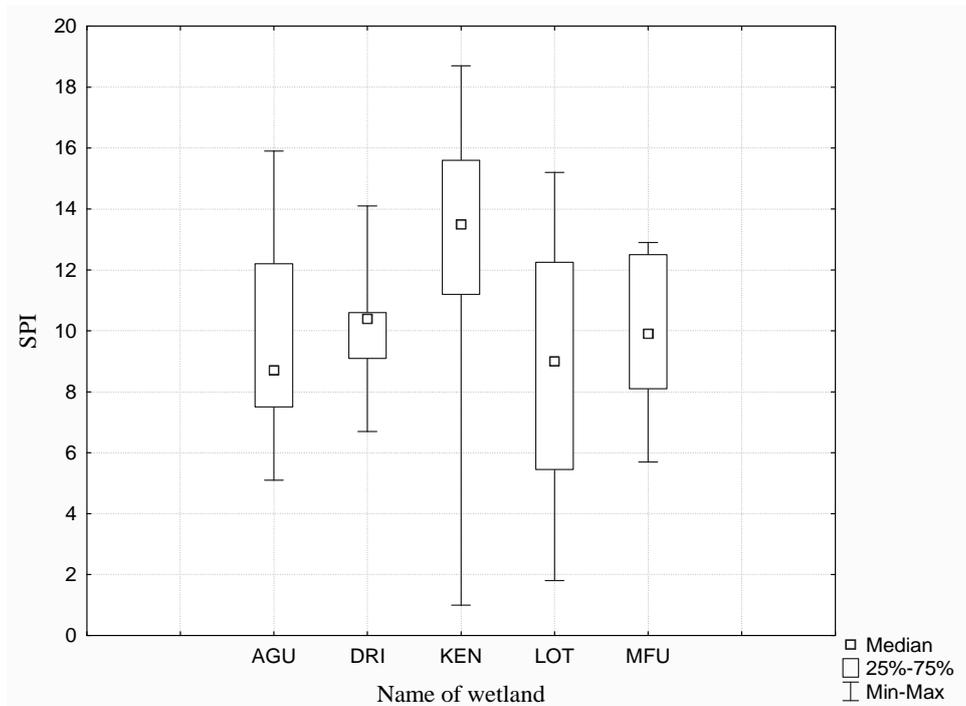
Figure 4.6 The median SPI values of the wetlands whose water samples were collected only once.

Based on the SPI values in figure 4.5, it can be seen that Glen Cairn Vlei had the highest median values when compared to the other wetlands, thus GCV has good water quality. It can also be seen from the graph that most of the wetlands (LGV, OOG, WVV and ZRV) have bad water quality as their SPI scores were lower than 9, whereas LPV, RTV and ZDV show poor to moderate water quality, with index scores ranging between 9 and 12 (table 4.5).

In figure 4.6, it can be seen that within each wetland, the sites studied have different water quality. Furthermore, it can be seen that the quality of water at Agulhas, Drift Sands and eMfuleni is slightly better when compared to some sites at Kenilworth and Lotus River. It can be seen that generally, the water at Kenilworth is of moderate to good quality as the 25%-75% quartile lies within the range of 11-16. The majority of the sites in Lotus River have bad to poor waters quality, as the 25-75% quartile lies within the range of 5 and 12.

Figure 4.7 and 4.8 below; show the median TDI values in the different wetlands.

TDI scores range from zero to a hundred, where a score of 0 indicates oligotrophic conditions and a score of 100 indicates eutrophication as it can be seen in table 4.6 below.

Table 4.6 TDI scores and their corresponding trophic status.

Index score	Trophic status
0 -20	Oligotrophic
21-40	Oligo-mesotrophic
41-60	Mesotrophic
61-80	Meso-eutrophic
Above 80	Eutrophic

The index scores and their corresponding trophic status (adjacent table), are based on the 1 to 5 scale of Kelly and Whitton (1995).

According to figure 4.7 below, GCV with a median value of 28.7, is the only wetland with low nutrient concentrations when compared to the other wetlands. It can also be seen from the graph that the TDI scores of the other wetlands range from 59 (minimum score at LPV) to 87 (maximum score at WVV).

Figure 4.8 below, shows the variability of TDI scores within the studied wetlands. It can be seen that KEN had the lowest scores ranging from 0 to 80, whereas TDI scores in the other wetlands ranged from 20 to a 100, with AGU showing the highest maximum value.
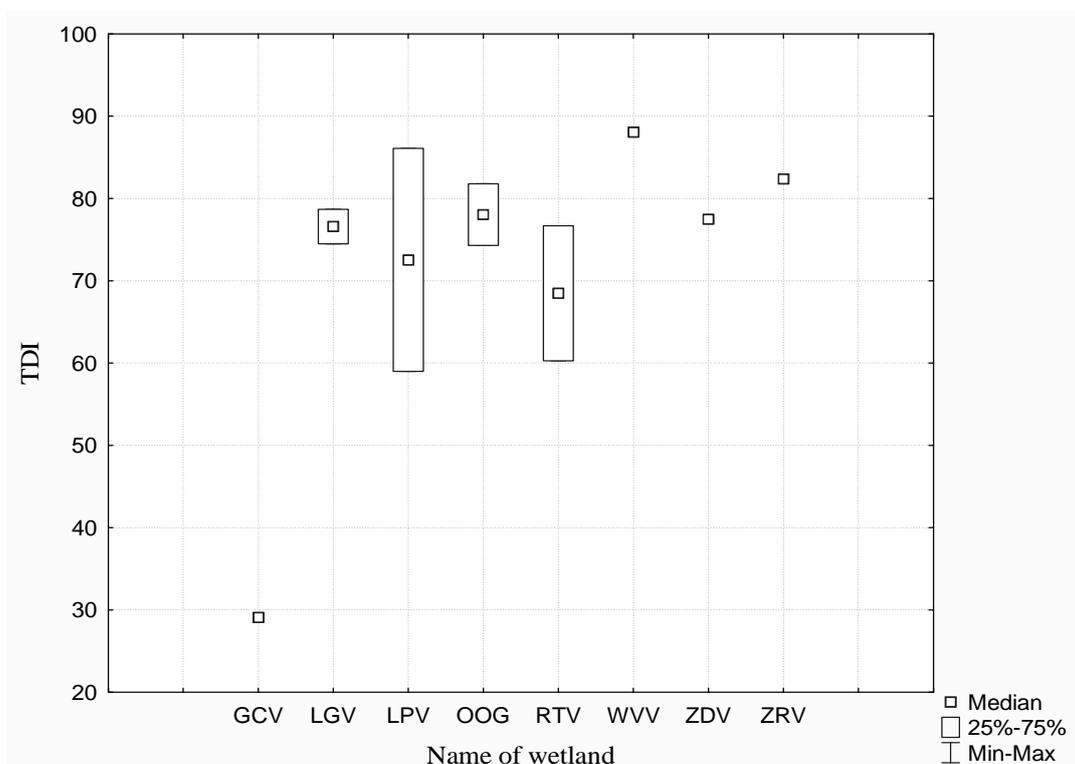
Figure 4.7 The median TDI values of the wetlands whose water samples were collected over three years.
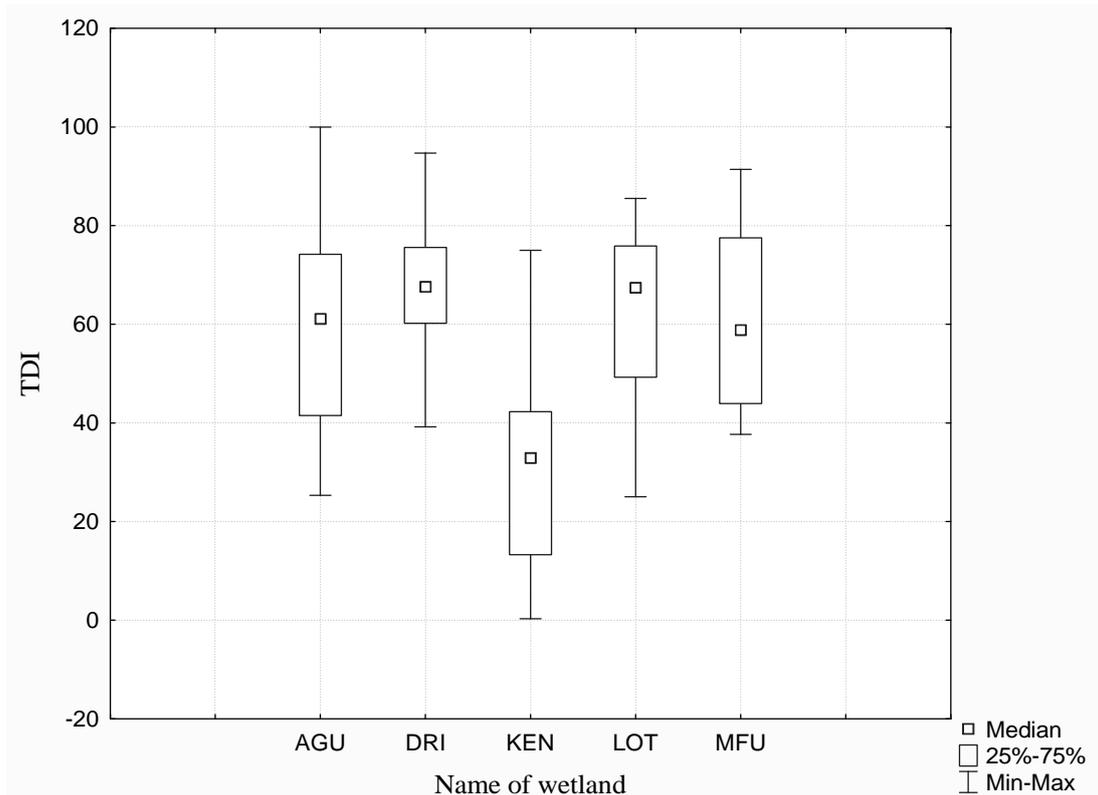
Figure 4.8 The median TDI values of the wetlands whose water samples were collected only once.

4.5 Correlation of diatom indices to environmental variables

Correlation is a technique which determines the strength of the relationship between variables; and a correlation coefficient is a number between -1 and +1 that measures both the strength and direction of the linear relationship between two variables (Rummel, 1976).

Correlation coefficient is a measure of the linear relation between two or more variables. The magnitude of the number represents the strength of the correlation, thus a correlation coefficient of zero represents no linear relationship, while a correlation coefficient of -1 or +1 represents a linear relationship, where the sign (+/-) of the correlation coefficient indicates the direction of the correlation (Rummel, 1976). Therefore, a positive (+) correlation coefficient means that as values on one variable increase, values on the other variable will also increase; and in a negative (-) correlation, when values on one variable increase, values on the other variable will decrease (Rummel, 1976).

In this study, Pearson's correlation of ≤0.05 was used to determine the efficacy with which GDI, SPI and GDI, indicate changes in the water quality with regard to the environmental variables discussed in Chapter 3.

This section is divided into two sub-sections; the first sub-section describes the correlation between environmental variables and index scores of samples collected concurrently from wetlands whose environmental variables were measured once-off, and the other sub-section examines the correlation between index scores and environmental variables of samples collected over a period of three years.

4.5.1 Diatoms collected concurrently from wetlands whose environmental variables were measured once-off

Diatom indices derived from diatom species composition were correlated to average values of environmental variables measured on the same day of sampling, as shown in table 4.7 below.

In the table, significant negative correlations can be seen between SPI and temperature, DO as well as with phosphates, whereas, significant positive correlations were identified in the association between GDI and nitrites (NO_2) as well as nitrates (NO_3). With TDI on the other hand, none of the correlations were significant.

Table 4.7 Pearson correlations coefficients between diatom indices and water quality data sampled concurrently to diatom sampling. Marked correlations are significant at $p < 0.05$

	SPI	GDI	TDI
Temp	-0.942356	-0.725342	0.515676
pH	-0.528678	-0.172268	0.868135
DO	-0.943516	-0.735134	0.511959
EC	0.252033	-0.113052	-0.285823
Turbidity	-0.157488	0.468063	0.034490
NO2	0.867152	0.887519	0.022218
NO3	0.673927	0.960301	-0.066988
NH4	0.864634	0.840620	0.210062
PO4	0.977968	0.869100	-0.054154

4.5.2 Diatoms collected from wetlands whose environmental variables were measured over a period of three years

The physico-chemical variables of these wetlands were measured every month for three years, and diatom samples were only collected once. In this sub-section, the average data collected over the three year period is correlated to the indices derived from data collected once. It was mentioned in Chapter 1 that living organism make better water quality indicators since they are continuously being exposed to their environment, thus their species composition is influenced by their environment, whereas physico-chemical monitoring is a snap-shot type of analysis, since measurements are influenced by factors taking place at the time of sampling.

This sub-section, determines the strength of the relationship between diatom indices derived from data of single sampling and water quality variables measured over a period of three years.

Table 4.8 to 4.11 show the correlation between diatom indices and environmental variables measured on the same day of sampling, correlations between diatom indices and average values of environmental variables collected a year prior to diatom sampling, during the year of sampling, as well as correlations with the average values of the data collected over the three year period.

From observing table 4.8, it can be seen that none of the correlation are significant. The correlation coefficients range between -0.03 (observed between SPI and DO %) and 0.63 (between TDI and Nitrates).

Table 4.8 Pearson correlations coefficients between diatom indices and average data of water quality variables sampled a year prior to diatom sampling. Marked correlations are significant at $p < 0.05$

	SPI	GDI	TDI
Temp	-0.453276	-0.331138	0.471994
pH	0.220151	-0.106882	0.202922
EC	0.205531	0.001066	0.127267
TSS	-0.277978	-0.526572	0.479753
DO	0.335385	0.277384	-0.024348
NO3	-0.033712	0.226181	0.133426
NH3	-0.579882	-0.362234	0.632676
PO4	-0.243240	-0.596593	0.312258

In table 4.9 below, diatom indices derived from diatom species composition were correlated to average values of environmental variables measured on the same day of sampling. This figure shows significant correlations between SPI and NH_3 and SPI and PO_4, as well as between GDI and DO %, whereas TDI shows no significant correlation to any of the variables.

Table 4.9 Pearson correlations coefficients between diatom indices and water quality data sampled on the day of diatom sampling. Marked correlations are significant at $p < 0.05$

	SPI	GDI	TDI
Temp	0.374136	0.104436	0.000813
pH	-0.117022	-0.453716	0.325433
EC	0.259866	0.191253	0.142625
TSS	0.080091	-0.410290	0.465724
DO	-0.635267	-0.851057	0.576540
NO3	-0.436536	-0.139566	0.111161
NH3	-0.882943	-0.701794	0.646154
PO4	-0.867924	-0.761175	0.566916

Table 4.10 Pearson correlations coefficients between diatom indices and average data of water quality variables sampled during the same year of diatom sampling. Marked correlations are significant at $p < 0.05$

	SPI	GDI	TDI
Temp	-0.369567	-0.370397	0.098828
pH	0.060305	-0.153307	0.291114
EC	0.170603	-0.006339	0.110500
TSS	0.176622	0.165521	0.371741
DO	0.405315	0.144573	0.021057
NO3	-0.103121	-0.039482	0.497301
NH3	-0.585290	-0.123848	0.413356
PO4	-0.372845	-0.709017	0.416163

From this table it can be seen that SPI had significant negative correlations to ammonia and phosphates, and GDI showed significant negative correlation to dissolved oxygen, whereas TDI showed no significant correlations to any of the measured environmental variables. With the exception of GDI which showed significant correlations to phosphates, table 4.10 above, shows that SPI and TDI had no significant correlation to all the environmental variables measured.

Table 4.11 Pearson correlations coefficients between diatom indices and the average water quality data collected over three years. Marked correlations are significant at $p < 0.05$

	SPI	GDI	TDI
Temp	-0.412480	-0.240420	0.300071
pH	0.145480	-0.082388	0.189714
EC	0.157088	-0.045006	0.139294
TSS	-0.464800	-0.615491	0.675347
DO	0.327141	0.056328	0.068604
NO3	0.009009	0.227207	0.215936
NH3	-0.774926	-0.471173	0.712553
PO4	-0.373977	-0.676185	0.403232

From table 4.11 above, it can be seen that both SPI and TDI showed significant correlation to ammonia concentrations However, SPI and ammonia had a negative correlation, whereas the correlation between TDI and ammonia was positive.

4.6 Discussion

Although species such as *Achnanthidium minutissimum,* and *Brachysira neoexilis*, were found amongst the dominant species in some of the samples, most of the dominant species found in the studied wetlands, are those species that are tolerant to some form of pollution, and are commonly found in eutrophic and highly polluted waters, thus indicating poor water quality. Some of the dominant species found in these wetlands were *Navicula veneta, Nitzschia amphibia, Nitzschia supralitorea, Nitzschia palea,* and *Nitzschia umbonata* which are indicative of eutrophic waters (Lange-Bertalot, 1979 and Round, 1993), and *Amphora veneta, Gomphonema parvulum, Nitzschia capitellata,* and *Sellaphora pupula,* which indicate polluted waters (Lange-Bertalot, 1979 and Round, 1993).

Diatom indices were designed for the management of aquatic resources, for example, a GDI or SPI score of 19 indicates high quality water, whereas a score of 5 would indicate bad water quality (de la Rey *et al*., 2004). The index scores in section 4.4 were derived from diatom species composition using Omnidia, where different indices were selected based on their relationship to general water quality and to different environmental variables. An index that may be useful for the purposes of providing initial indication of a polluted aquatic system is the GDI which is based on the identification of diatom taxa to genus level, and includes 174 taxa, thus making it the simplest index to use (Taylor, 2004). This index also uses every freshwater species from the database; as a result, it gives a realistic estimation of water quality (Prygiel and Coste, 1993).

Different species within a genus can have different requirements, as this index is based on the identification of diatoms to the genus level; it is more likely that it will indicate conditions preferred by the dominant genera.

As mentioned earlier in the chapter, although different individuals of a certain community may have different requirements, they also have some overlapping requirements, thus although the GDI is not specific, it can still be taken into account because it represents the preferred conditions of the dominant genera, however, it should not be used as the only index for water quality classification.

The Trophic Diatom Index was designed to monitor trophic status or eutrophication (Taylor, 2004). TDI scores range from 0 to a 100, with a score of 0 indicating low nutrient concentrations (oligotrophic waters) and a score of a 100 indicating eutrophication (high nutrient concentrations). As mentioned in chapter 1, the %PT estimates the reliability of TDI, with values above 20% indicating organic pollution as seen in table 4.12 below.

Table 4.12 The interpretation of the %PT scores (adapted from Kelly, 1998).

%PT	Interpretation
Below 20%	Site free from organic pollution
21-40%	There is some evidence of organic pollution
41-60%	Organic pollution likely to contribute significantly to eutrophication at the site
Above 61%	The site is heavily contaminated with organic pollution

A table with all the index scores as well as %PT can be found in Appendix D.

The Specific Pollution sensitivity Index (SPI) was designed to reflect general water quality (Coste in Cemagref, 1982). This index has the most extensive species base and the highest taxonomic resolution of all indices thus in some cases, this index may require taxa to be identified to the subspecies and form level (Taylor, 2004). SPI, therefore, provides a better reflection of water quality since it is based on the individual requirements of every single species (and subspecies) in a given sample.

An increase (beyond recommended levels) in the level or concentration of environmental variables is regarded as pollution, and it results in behavioural and physiological changes in the organisms living in that water. An increase in SPI and GDI indicates better water quality, whereas an increase in TDI indicates a decrease in water quality, thus, environmental variables are expected to show negative correlations between with GDI and SPI, whereas a positive correlation is expected between the measured environmental variables and TDI.

The above mentioned indices showed different correlations to environmental variables measured at different intervals. From studying table 4.6 through to table 4.10, it can be seen that although significant correlations were few, most of the high / strong correlations were observed in table 4.6. Table 4.6 measured the correlation of diatom indices to environmental variables measured on the same day as diatom sampling.

When studying the data in table 4.6 and that in table 4.8 (which also measured the correlation between diatom indices and environmental variables measured concurrently with diatom sampling), it can be seen that in both these tables, the indices showed strong correlations to more environmental variables than those in the other tables.

4.7 Conclusion

The preceding sections of this chapter show that over 95% of the diatom species encountered in this study are cosmopolitan, with a few species commonly found in brackish water. It was also shown in the chapter, that the structure of diatom communities is influenced by the relationship between diatoms and their environment, with dominant species having their optima close to the environmental variables measured at the different sites. Furthermore, diatom indices calculated from the relative abundance of diatoms encountered in the study, showed the applicability of different indices to water quality monitoring in South Africa.

4.8 References

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CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

The heterogeneous nature of wetlands makes it difficult for the identification of common indicator species (such as fish, and aquatic invertebrates) in these systems (Brazner *et al.*, 2007). This study therefore, demonstrated the necessity for a reliable indicator of water quality in South African wetlands. The bioassessment of wetlands is based on the premise that the biota (diatoms) will integrate the physico-chemical variables in the wetland and will reflect the overall wetland condition.

The environmental variables measured in the study were discussed in chapter 3, and in this chapter it was seen that these wetlands had good water quality in terms of pH and temperature as their values were well within acceptable/recommended levels. However, when combining all the measured variables, and looking at general water quality (since the environmental variables measured were closely interrelated), it can be seen that the water in the studied wetlands were of poor quality, with moderate pollution.

The results obtained in chapter 3 were confirmed in chapter 4, by the different diatom communities found within the studied wetlands. Some of the species found in the studied wetlands were *Amphora veneta, Cocconeis placentula, Eolimna subminiscula, Gomphonema parvulum, Gomphonema truncatum, Navicula veneta*, *Nitzschia amphibia, Nitzschia frustulum,* and *Sellaphora pupula* all of which are tolerant to pollution (Lange-Bertalot, 1979, Round, 1993, and Kelly *et al.,* 1995). However, one other species found dominating most of the sites was *Achnanthidium minutissimum* which is found within less impacted waters, with low pH (Lange-Bertalot, 1979, Battarbee, 1997, Sabater, 2000, and Taylor *et al.,* 2007). In other studies, however, this species has been found in nutrient rich waters, with a higher pH (Round, 1993) as well as in oligo-eutraphentic environments (van Dam, et al., 1993), thus suggesting that *Achnanthidium minutissimum* has a wide tolerance range.

The City of Cape Town has conducted several studies on wetlands found within the Cape Town metropolitan area; these studies were conducted on Wildevoël Vlei, Zand Vlei and other wetlands which were not included in this study. In 1998, these wetlands were classified according to their water quality and trophic status based on their physico-chemical variables. Zand Vlei was accorded class D (largely modified), due to the high trophic levels recorded in this wetland, whereas Wildevoël Vlei was accorded class E (seriously modified), due to the two episodes of toxic algal blooms which occurred during the summer months of 1998 (State of the environment). Table 5.1 below shows the different classes and their modification levels.

Table 5.1 The different classes of ecological classification (State of the environment).

Class	Modification
A	Natural, unmodified
B	Largely natural with few modifications
C	Moderately modified
D	Largely modified
E	Seriously modified
F	Critically modified (almost complete loss of biota and habitat

Now eight years later (diatom samples were collected in 2006) diatom species indicative of eutrophic waters were recorded as being dominant in these two wetlands. Some of the dominant species found in these wetlands include *Amphora veneta, Aulacoseira granulata, Cocconeis placentula var. lineata, Cyclotella meneghiniana, Gomphonema parvulum, Navicula veneta, Nitzschia amphibia, Planothidium engelbrechtii, Planothidium frequentissimum* and *Tabularia fasciculata.*

Although Wildevoël Vlei was the only wetland classified as being eutrophic in chapter 3, some of the dominant species found in Die Oog, Glen Cairn Vlei, Lange Vlei, Little Princess Vlei, Lotus River, Rietvlei, Zand Vlei, and Zoar Vlei were indicative of eutrophic conditions in these wetlands. This was confirmed by the results of a study conducted by the city of Cape Town. In their 2007/2008 state of the environment report, they classified Glen Cairn Vlei, Little Princess Vlei and Rietvlei as moderately eutrophic, Lange Vlei and Zand Vlei as eutrophic, and Die Oog, Lotus River, Wildevoël Vlei and Zoar Vlei were classified as hypertrophic.

Given the fluctuating nature of environmental variables, it is imperative that physico-chemical analysis be carried out regularly. As mentioned in chapter 1, it is difficult, if not impossible to measure all the different chemical variables that may be present in a system, furthermore, since this type of analysis represents a "snapshot" of the water quality at the time of sampling, physico-chemical analysis becomes time consuming and very expensive. Therefore, with the recent economic recession, cost effective methods of analysis (such as the use of diatom indicators) are necessary to augment water quality monitoring techniques in South African wetlands.

This study has demonstrated that most of the diatom species found in wetlands are the same as those commonly found in rivers and streams, thus important information such as optimum pH, temperature, electrolyte and nutrient requirements have already been identified. The study has also shown that most of the diatoms encountered in the studied wetlands, are included in the Omnidia database which is used for calculating the different diatom indices.

Since the diatoms encountered in the study are common to those found in rivers and streams, this implies that the indices currently used in rivers and streams can also be applied in wetlands, and this was demonstrated in chapter 4, where the application of all the indices was tested in the different wetlands.

This study has shown that the use of diatoms as indicators of water quality is relatively economical when compared to routine physico-chemical monitoring, this was demonstrated in that, when diatoms are used with greater intervals of time, they still provide similar, integrated reflections of water quality to that gained from regular physico-chemical monitoring (the diatoms species found in Wildevoël Vlei 2006, were reflective of eutrophic conditions measured in 1998), thus making diatom good indicators of past as well as current water quality.

Some of the species found in these wetlands were those commonly found in brackish water, and a few unknown species were also identified in the study. It is therefore recommended that further studies be carried out in other wetlands around the country to document the species abundance of diatoms commonly found in wetlands, as well as to observe if these unknown species are common to South African wetlands, and whether or not they are endemic.

The objective of the study was to provide a preliminary diatom-based index for South African wetlands, however, given the relatively small study area and the strong bias towards coastal wetlands it was deemed inadvisable to construct such an index. As a result, several indices are recommended for interim use until further research that more comprehensively covers wetlands in South Africa has been conducted. It is thus the recommendation of this study that more data is collected for comparison to other wetlands and that in the interim, indices such as GDI, SPI and TDI be applied for routine biomonitoring of these environments.

5.1 References

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APPENDICES

APPENDIX A:
Site Information.

Table 1 The site and sample information of the wetlands studied over three years

Wetland	Database code	Date of sampling	Substratu
Die oog	08-209	10th October 2006	Vegetatio
Die oog	08-210	10th October 2006	Vegetatio
Glencairn Vlei	08-211	17th October 2006	Vegetatio
Lange Vlei	08-219	10th October 2006	Vegetatio
Lange Vlei	08-220	10th October 2006	Vegetatio
Little Princess Vlei	08-217	10th October 2006	Vegetatio
Little Princess Vlei	08-218	10th October 2006	Vegetatio
Rietvlei pan	08-215	10th July 2007	Vegetatio
Rietvlei deep lake	08-216	10th July 2007	Vegetatio
Wildevoel Vlei	08-212	24th October 2006	Vegetatio
Zand Vlei	08-213	24th October 2006	Vegetatio
Zoar Vlei	08-214	10th July 2007	Vegetatio

Table 2 The site and sample information of wetlands sampled once off.

Wet-land	Site code	database code	Location		Date of sampling	Substratum
			Latitude	Longitude		
Agulhas	AGU-01	08-266	S: 34º 44' 25.8"	E: 19º 40' 45.9"	10th October 2007	Sediment
		08-292	S: 34º 44' 25.8"	E: 19º 40' 45.9"	10th October 2007	Vegetation
	AGU-04	08-275	S: 34º 44' 22.8"	E: 19º 43' 56.9"	10th October 2007	Sediment
	AGU-05	08-263	S: 33º 44' 16.1"	E: 19º 44' 12.7"	10th October 2007	Sediment
	AGU-07	08-267	S: 34º 41' 53.0"	E: 19º 43' 12.6"	11th October 2007	Sediment
		08-276	S: 34º 41' 53.0"	E: 19º 43' 12.6"	11th October 2007	Sediment
		08-286	S: 34º 41' 53.0"	E: 19º 43' 12.6"	11th October 2007	Sediment
	AGU-08	08-261	S: 34º 41' 49.2"	E: 19º 43' 14.4"	11th October 2007	Sediment
	AGU-11	08-274	S: 34º 43' 17.0"	E: 19º 45' 23.9"	11th October 2007	Sediment
	AGU-12	08-265	S: 34º 45' 09.5"	E: 19º 48' 06.1"	12th October 2007	Sediment
	AGU-13	08-264	S: 34º 38' 29.9"	E: 19º 49' 52.9"	13th October 2007	Sediment
	AGU-14	08-260	S: 34º 36' 09.1"	E: 19º 57' 04.9"	13th October 2007	Sediment
	AGU-15	08-262	S: 34º 35' 43.5"	E: 19º 57' 32.1"	13th October 2007	Sediment
	AGU-16	08-273	S: 34º 38' 32.2"	E: 19º 54' 19.2"	13th October 2007	Sediment
		08-294	S: 34º 38' 32.2"	E: 19º 54' 19.2"	13th October 2007	Vegetation
	AGU-17	08-259	S: 34º 40' 36.3"	E: 19º 54' 13.3"	13th October 2007	Sediment
	AGU-18	08-272	S: 34º 40' 56.7"	E: 19º 54' 02.0"	13th October 2007	Sediment
	AGU-19	08-258	S: 34º 40' 13.6"	E: 19º 53' 53.3"	14th October 2007	Sediment
	AGU-20	08-270	S: 34º 45' 01.9"	E: 19º 58' 45.7"	14th October 2007	Sediment
	AGU-21	08-257	S: 34º 45' 46.3"	E: 19º 54' 23.1"	14th October 2007	Sediment
	AGU-22	08-269	S: 34º 45' 48.8"	E: 19º 54' 06.3"	14th October 2007	Sediment
		08-291	S: 34º 45' 48.8"	E: 19º 54' 06.3"	14th October 2007	Vegetation
	AGU-23	08-271	S: 34º 42' 38.9"	E: 19º 55' 50.2"	14th October 2007	Sediment

riftsands	DRI01	08-268	S: 34º 00' 44.2"	E: 18º 39' 48.2"	04th October 2007	Sediment
		08-288	S: 34º 00' 44.2"	E: 18º 39' 48.2"	04th October 2007	Vegetation
	DRI02	08-281	S: 34º 00' 42.8"	E: 18º 39' 51.4"	04th October 2007	Sediment
		08-290	S: 34º 00' 42.8"	E: 18º 39' 51.4"	04th October 2007	Vegetation
	DRI03	08-279	S: 34º 00' 46.6"	E: 18º 39' 51.8"	04th October 2007	Sediment
		08-287	S: 34º 00' 46.6"	E: 18º 39' 51.8"	04th October 2007	Vegetation
	DRI04	08-280	S: 34º 00' 49.9"	E: 18º 39' 56.1"	04th October 2007	Sediment
		08-295	S: 34º 00' 49.9"	E: 18º 39' 56.1"	04th October 2007	Vegetation
	DRI05	08-283	S: 34º 00' 42.8"	E: 18º 40' 02.9"	04th October 2007	Sediment
	DRI06	08-285	S: 33º 59' 04.3"	E: 18º 39' 38.2"	05th October 2007	Sediment
	DRI07	08-282	S: 33º 59' 20.0"	E: 18º 39' 33.6"	05th October 2007	Sediment
eMfuleni	MFU01	08-277	S: 34º 00' 44.7"	E: 18º 40' 52.7"	01st October 2007	Sediment
		08-293	S: 34º 00' 44.7"	E: 18º 40' 52.7"	01st October 2007	Vegetation
	MFU02	08-284	S: 34º 00' 32.5"	E: 18º 40' 50.0"	01st October 2007	Sediment
		08-296	S: 34º 00' 32.5"	E: 18º 40' 50.0"	01st October 2007	Vegetation
	MFU03	08-278	S: 34º 00' 34.7"	E: 18º 40' 42.7"	01st October 2007	Sediment
		08-289	S: 34º 00' 34.7"	E: 18º 40' 42.7"	01st October 2007	Vegetation
Kenilworth	KEN01E	08-873	S: 33º 59' 922"	E: 18º 29' 159"	04th September 2008	Vegetation
	KEN01E	08-874	S: 33º 59' 922"	E: 18º 29' 159"	04th September 2008	Sediment
	KEN02E	08-875	S: 33º 59' 901"	E: 18º 29' 143"	01st September 2008	Vegetation
	KEN02E	08-876	S: 33º 59' 901"	E: 18º 29' 143"	01st September 2008	Sediment
	KEN03E	08-877	S: 33º 59' 908"	E: 18º 29' 238"	01st September 2008	Vegetation
	KEN03E	08-878	S: 33º 59' 908"	E: 18º 29' 238"	01st September 2008	Sediment
	KEN05E	08-879	S: 34º 00' 030"	E: 18º 29' 015"	01st September 2008	Vegetation
	KEN05E	08-880	S: 34º 00' 030"	E: 18º 29' 015"	01st September 2008	Sediment
	KEN06E	08-881	S: 33º 59' 912"	E: 18º 28' 907"	01st September 2008	Vegetation
	KEN06E	08-882	S: 33º 59' 912"	E: 18º 28' 907"	01st September 2008	Sediment
	KEN07E	08-883	S: 33 59' 867"	E: 18º 28' 928"	04th September 2008	Vegetation
	KEN07E	*08-113*	S: 33º 59' 867"	E: 18º 28' 928"	04th September 2008	Sediment
	KEN08E	08-884	S: 33º 59' 828"	E: 18º 28' 963"	04th September 2008	Vegetation
	KEN08E	08-885	S: 33º 59' 828"	E: 18º 28' 963"	04th September 2008	Sediment
	KEN09E	08-886	S: 33º 59' 769"	E: 18º 28' 923"	04th September 2008	Vegetation
	KEN09E	08-887	S: 33º 59' 769"	E: 18º 28' 923"	04th September 2008	Sediment
	KEN10E	08-888	S: 33º 59' 772"	E: 18º 29' 045"	04th September 2008	Vegetation
	KEN11E	08-889	S: 33º 59' 652"	E: 18º 29' 001"	04th September 2008	Vegetation
	KEN11E	08-890	S: 33º 59' 652"	E: 18º 29' 001"	04th September 2008	Sediment
	KEN12E	08-891	S: 33º 59' 669"	E: 18º 29' 093"	04th September 2008	Vegetation
	KEN12E	08-892	S: 33º 59' 669"	E: 18º 29' 093"	04th September 2008	Sediment
	KEN13E	08-893	S: 33º 59' 782"	E: 18º 29' 086"	04th September 2008	Vegetation
	KEN14E	08-894	S: 33º 59' 559"	E: 18º 29' 247"	04th September 2008	Vegetation
	KEN14E	08-895	S: 33º 59' 559"	E: 18º 29' .247"	04th September 2008	Sediment
	KEN17E	08-896	S: 34º 00' 229"	E: 18º 29' 237"	29th August 2008	Vegetation
	KEN17E	08-897	S: 34º 00' 229"	E: 18º 29' 237"	29th August 2008	Sediment
	KEN21E	08-898	S: 34º 00' 245"	E: 18º 29' 202"	29th August 2008	Vegetation
	KEN21E	08-899	S: 34º 00' 245"	E: 18º 29' 202"	29th August 2008	Sediment
	KEN23E	08-900	S: 34º 00' 678"	E: 18º 29' 424"	29th August 2008	Vegetation
	KEN25E	08-901	S: 34º 00' 593"	E: 18º 29' 344"	28th August 2008	Vegetation
	KEN25E	08-918	S: 34º 00' 593"	E: 18º 29' 344"	29th August 2008	Sediment
	KEN26E	08-902	S: 34º 00' 587"	E: 18º 29' 353"	29th August 2008	Vegetation
	KEN26E	08-903	S: 34º 00' 587"	E: 18º 29' 353"	29th August 2008	Sediment
	KEN27E	08-904	S: 34º 00' 210"	E: 18º 29' 281"	29th August 2008	Vegetation
	KEN28E	08-905	S: 34º 00' 014"	E: 18º 29' 130"	01st September 2008	Vegetation
	KEN29E	08-906	S: 34º 00' 072"	E: 18º 29' 267"	01st September 2008	Vegetation
	KEN29E	08-907	S: 34º 00' 072"	E: 18º 29' 267"	28th August 2008	Sediment
	KEN33E	08-908	S: 34º 00' 533"	E: 18º 29' 410"	29th August 2008	Vegetation
	KEN33E	08-909	S: 34º 00' 533"	E: 18º 29' 410"	29th August 2008	Sediment

Lotus River	LOT01E	08-853	S: 34º 03' 490"	E: 18º 30' 292"	02nd September 2008	Vegetation
	LOT01E	08-854	S: 34º 03' 490"	E: 18º 30' 292"	02nd September 2008	Sediment
	LOT02E	08-855	S: 34º 03' 478"	E: 18º 30' 229"	03rd September 2008	Vegetation
	LOT02E	08-856	S: 34º 03' 478"	E: 18º 30' 229"	03rd September 2008	Sediment
	LOT03E	08-857	S: 34º 03' 464"	E: 18º 30' 017"	10th September 2008	Vegetation
	LOT03E	08-858	S: 34º 03' 464"	E: 18º 30' 017"	10th September 2008	Sediment
	LOT04E	08-859	S: 34º 03' 233"	E: 18º 30' 298"	02nd September 2008	Vegetation
	LOT04E	08-860	S: 34º 03' 233"	E: 18º 30' 298"	02nd September 2008	Sediment
	LOT05E	08-861	S: 34º 02' 916"	E: 18º 30' 601"	02nd September 2008	Vegetation
	LOT05E	08-862	S: 34º 02' 916"	E: 18º 30' 601"	02nd September 2008	Sediment
	LOT06E	08-863	S: 34º 02' 308"	E: 18º 32' 086"	02nd September 2008	Vegetation
	LOT08E	08-864	S: 34º 01' 584"	E: 18º 32' 415"	02nd September 2008	Vegetation
	LOT08E	08-865	S: 34º 01' 584"	E: 18º 32' 415"	02nd September 2008	Sediment
	LOT09E	08-866	S: 34º 04' 042"	E: 18º 29' 733"	03rd September 2008	Vegetation
	LOT09E	08-867	S: 34º 04' 042"	E: 18º 29' 733"	03rd September 2008	Sediment
	LOT11E	08-868	S: 34º 04' 145"	E: 18º 29' 872"	03rd September 2008	Vegetation
	LOT11E	08-869	S: 34º 04' 145"	E: 18º 29' 872"	03rd September 2008	Sediment
	LOT13E	08-870	S: 34º 03' 626"	E: 18º 30' 226"	02nd September 2008	Vegetation
	LOT13E	08-871	S: 34º 03' 626"	E: 18º 30' 226"	02nd September 2008	Sediment
	LOT14E	08-872	S3: 4º 02' 893"	E: 18º 30' 977"	03rd September 2008	Vegetation

APPENDIX B:
Water Chemistry.

Table 1 values of the physico-chemical variables measured at the different wetlands.

Water Chemistry

Site Code	Temperature	pH	Conductivity	Turbidity	DO (mg\l)	DO (%)	NO3(ug\L)	NH3(ug\L)	PO4(ug\L)
Die Oog	18	7.82	124.4	29.17	5.608	58.858.8	0.245	0.621	0.143
Glencairn vlei	16	7.7	92.21	17.93	6.522	65.36	0.214	0.395	0.0114
Lange vlei	17	8.2	67.7	87.2	6.8	71.2	0.76	0.199	0.101
Little princess	16	7.5	50.68	27.69	5.562	57	0.427	0.206	0.0328
Rietvlei	17	8.6	450.90	28.128	8.892	94.38	0.0589	0.071	0.0160
Wildevoel vlei	17	8.61	148.28	82.185	8.473	88.47	0.4807	0.250	1.5127
Zand vlei	16	8.4	1292.16	36.837	8.671	92.37	0.2034	0.0792	0.0373
Zoar vlei	15	7.36	103.134	108.221	3.877	38.727	0.1091	0.1238	0.0935

Site Code	Temperature	pH	Conductivity	Turbidity	D O(mg\l)	DO(%)	NO2 (ug\L)	NO3 (ug\L)	NH4(ug\L)	PO4(ug\L)
AGU01	15	6.7	881	2.5	6.7	65	2.69	15.81	73.75	9.62
AGU04	23.7	8.2	2.8	0.9	8.6	102	0.03	8.88	40.99	9.42
AGU05	22.3	9.6	48.5	17	10.3	119	0	0	29.77	1.37
AGU07	18.6	8.1	826.3	6.3	9.6	105.7	0.26	1.6	23.4	11.2
AGU08	18.8	7.5	795	3	8.2	88	0	0	16.41	3.39
AGU11	22.4	8.9	1782	4	10.6	123	0.02	1.97	8.57	4.11
AGU12	24.9	7.7	3.01	1	9.7	113	0	0	0.23	6.36
AGU13	21.4	7.6	2.95	13	7.3	83	0.01	3.95	21.82	5.23
AGU14	23.4	9.3	7.9	8	9.1	91	0	0	14.13	1.83
AGU15	28.6	10.3	18.2	3	13.5	189	0	0	19.97	18.8
AGU16	28.3	9.9	8.9	3.5	10.7	143.5	0	0	11.39	1.25
AGU17	19.2	9.9	37	2	10	132	0	0	37.5	2.03
AGU18	27.6	8.6	1988	2	8.7	108	0.01	10.58	35.51	4.74
AGU19	24.8	10.2	22.2	2	7.4	92	0	0.58	25.51	3.57
AGU20	22.7	7.02	1285	1	5.4	62	0.12	1.22	8.86	1.27
AGU21	22.6	9.6	37.8	8	10.5	125	0.03	0.19	8.2	2.69
AGU22	22.4	9.3	11.2	5.5	11.5	131.5	0.01	0.08	3.28	1.37
AGU23	21	8.2	2.99	11	9.3	105	0	0	17.21	1.58
Average	**22.7**	**8.70**	**431.15**	**5.20**	**9.28**	**109.87**	**0.177**	**2.49**	**22.03**	**4.99**

Water Chemistry

Site Code	Temperature	pH	Conductivity	Turbidity	DO(mg/l)	DO(%)	NO2 (ug/L)	NO3 (ug/L)	NH4(ug\L)	PO4(ug\L)
KEN01E	14.5	7.46	294	2.9	6.80	65.7	0.01	0.01	11.16	6.37
KEN02E	11.0	6.92	236	3.9	6.63	59.9	2.60	9.62	23.18	7.64
KEN03E	11.4	5.93	125	0.7	4.92	44.8	0.12	1.11	18.88	8.92
KEN05E	11.9	7.02	211	4.6	5.81	53.1	0.03	0.89	19.74	39.49
KEN06E	13.6	7.22	338	2.4	7.08	68.6	0.01	0.05	17.17	2.55
KEN07E	13.1	5.16	165	1.8	4.51	42.5	2.16	7.11	24.03	6.37
KEN08E	13.7	4.31	128	1.8	6.59	62.6	0.57	2.48	22.32	1.27
KEN09E	16.4	7.18	448	1.9	5.57	55.1	0.03	0.95	14.59	0.00
KEN10E	14.9	4.83	170	1.3	3.79	35.8	1.20	10.45	35.19	8.92
KEN11E	15.2	6.68	222	1.1	5.47	53.6	0.58	0.75	18.03	0.00
KEN12E	17.8	4.69	173	2.1	6.34	63.2	1.03	5.72	26.61	11.47
KEN13E	15.6	4.22	156	2.8	5.18	50.2	0.98	8.09	33.48	7.64
KEN14E	19.4	7.03	466	5.2	7.41	81.3	0.02	0.30	18.88	6.37
KEN17E	19.0	7.99	108	2.5	9.35	100.2	0.01	0.17	21.46	12.74
KEN21E	17.7	7.45	292	3.0	6.19	65.0	1.52	19.96	15.45	8.92
KEN23E	14.1	6.85	323	3.3	4.20	40.2	2.94	71.22	21.46	30.58
KEN25E	15.6	6.74	152	2.8	6.44	64.7	0.28	13.66	29.18	310.85
KEN26E	15.9	6.73	117	1.5	7.78	77.9	0.11	1.98	16.31	25.48
KEN27E	15.6	7.56	533	1.5	1.87	18.3	0.72	0.71	18.03	54.78
KEN28E	10.9	6.25	219	7.0	4.80	43.3	0.16	1.35	0.00	0.00
KEN29E	12.1	6.64	130	1.7	6.30	57.9	2.06	3.03	18.88	16.56
KEN33E	15.6	6.33	152	3.8	4.30	42.0	2.20	20.54	24.89	33.12
Average	14.77	6.42	234.41	2.70	5.79	56.63	0.88	8.19	20.41	27.27

Water Chemistry

Site Code	Temperature	pH	Conductivity	Turbidity	DO (mg\l)	DO (%)	NO2 (ug\L)	NO3(ug\L)	NH4(ug\L)	PO4(ug\L)
LOT01E	13.4	7.62	1382.67	2.2	3.59	33.7	1.03	6.28	28.33	20.38
LOT02E	14.5	8.04	1245	1.8	6.22	59.9	1.05	5.39	23.18	10.19
LOT03E	13.1	7.41	1328.67	13.9	4.63	44.0	8.06	280.85	66.09	95.55
LOT04E	15.7	8.26	1333.67	3.7	8.58	85.8	2.31	131.46	181.97	30.58
LOT05E	12.0	7.56	832.333	2.1	3.78	35.6	1.05	1.03	33.48	73.89
LOT06E	13.4	7.65	1296.33	5.0	3.04	28.8	391.58	7980.98	1054.08	1538.96
LOT08E	13.0	8.23	1231.33	2.8	10.28	97.3	295.44	1246.98	75.54	1169.51
LOT09E	17.1	8.28	3.45667	2.5	7.20	73.3	0.27	1.09	23.18	14.01
LOT11E	16.4	8.46	2.81	2.2	7.11	71.7	0.02	1.50	17.17	10.19
LOT13E	14.3	7.76	5.16	1.4	3.18	30.8	0.84	4.41	30.90	22.93
LOT14E	13.1	7.32	564	2.2	1.34	12.5	22.28	737.29	49.79	151.60
Average	14.16	7.87	838.68	3.64	5.36	52.14	65.81	645.20	143.97	285.25

Water Chemistry

Site Code	Temperature	pH	Conductivity	Turbidity	Oxygen (mg\l)	Oxygen(%)	NO2 (ug\L)	NO3 (ug\L)	NH4(ug\L)	PO4(ug\L)
DRI01	19	8.6	3.8	1.5	9	99	1.05	4.66	23.2	5.13
DRI02	19.3	8.1	1736.5	2	5.4	59.5	1.31	1.85	34.33	14.1
DRI03	22.5	9.01	4.5	1.5	9.6	112.5	0	0.56	20.06	2.81
DRI04	23.3	8.6	6.5	2	9	108.5	0.12	1.06	18.36	20.69
DRI05	23	8.3	3.5	7	6	71	0.23	2.1	96.93	13.71
DRI06	19	8.3	3.8	1	1.4	17	0.24	1.58	15.3	7.91
DRI07	21.1	8.02	2.4	6	1.9	20	0.32	1.96	27.18	6.97
Average	**21.0**	**8.42**	**251.57**	**3**	**6.043**	**69.64**	**0.467**	**1.97**	**33.62**	**10.18**

Water Chemistry

Site Code	Temperature	pH	Conductivity	Turbidity	Oxygen (mg\l)	Oxygen(%)	NO2 (ug\L)	NO3 (ug\L)	NH4(ug\L)	PO4(ug\L)
eMFU01	20	7.7	1155	1	3.1	34.5	0.02	0.02	42.98	10.75
eMFU02	23	8.2	877.5	2	8.1	95	0	0.04	5.61	1.69
eMFU03	22.1	8.3	1437.5	2	7.5	85.5	0.02	0.03	10.64	0
Average	**21.7**	**8.067**	**1156.67**	**1.67**	**6.23**	**71.67**	**0.013**	**0.03**	**19.74**	**4.15**

APPENDIX C:
Species abundance.

Dominant Species List

Table 1.1 .A complete list of species encountered in the study.

Code	Species name
ABRE	*Achnanthes brevipes* Agardh.
ACOA	*Achnanthes coarctata* (Brebisson) Grunow in Cl. & Grun.
ALIO	*Achnanthes linearioides* Lange-Bertalot.
AOBG	*Achnanthes oblongella* Øestrup.
ACHS	*Achnanthes* species.
ACS1	*Achnanthes standeri.*
ACS2	*Achanthes subaffinis.*
ADBI	*Achnanthidium biasolettianum* (Grunow in Cl. & Grun.) Lange-Bertalot.
ADEU	*Achnanthidium eutrophilum* (Lange-Bertalot)
ADEG	*Achnanthidium exiguum* (Grunow) Czarnecki.
ADMA	*Achnanthidium macrocephalum* (Hust.)Round & Bukhtiyarova.
ADMI	*Achnanthidium minutissimum* (Kützing) Czarnecki.
ADRI	*Achnanthidium rivulare* Potapova &Ponader.
ADSB	*Achnanthidium straubianum* (Lange-Bertalot).
ADSP	*Adlafia* species
ACOF	*Amphora coffeaeformis* (Agardh) Kützing.
APED	*Amphora pediculus* (Kützing) Grunow.
AVEN	*Amphora veneta* Kützing.
ASPH	*Anomoeoneis sphaerophora* (Ehrenberg) Pfitzer.
AAMB	*Aulacoseira ambigua* (Grun.) Simonsen.
AUCS	*Aulacoseira crassipunctata* Krammer.
AUGA	*Aulacoseira granulata* (Ehr.) Simonsen var.angustissima (O.M.) Simonsen.
AUGR	*Aulacoseira granulata* (Ehrenberg) Simonsen.
AUSU	*Aulacoseira subarctica* (O.Müller) Haworth.
BBTH	*Brachysira brebissonii fo.thermalis* (Grunow in Van Heurck) Ross.
BBRE	*Brachysira brebissonii* Ross.
BNEO	*Brachysira neoexilis* Lange-Bertalot.
BRCS	*Brachysira* species.
CAQT	*Caloneis aequatorialis* Hustedt.
CMOL	*Caloneis molaris* (Grunow) Krammer.
CSHU	*Caloneis schumanniana* (Grunow) Cleve.
CALS	*Caloneis* species.
COCS	*Cocconeis engelbrenchti* Cholnoky.
CPLA	*Cocconeis placentula* Ehrenberg.
CPLE	*Cocconeis placentula var. euglypta* (Ehrenberg) Grunow.
CPLI	*Cocconeis placentula var. lineata* (Ehrenberg) Grunow.
COMS	*Cosmioneis* species.
CAMB	*Craticula ambigua* (Ehrenberg) Mann.
CRAC	*Craticula accomoda* (Hustedt) Mann.
CACD	*Craticula acidoclinata* Lange-Bertalot & Metzeltin.
CRBU	*Craticula buderi* (Hustedt) Lange-Bertalot.
CHAL	*Craticula halophila* (Grunow ex Van Heurck) Mann.
CRTS	*Craticula* species
CVIX	*Craticula vixnegligenda* Lange-Bertalot.
CTPU	*Ctenophora pulchella* (Ralfs & Kützing) Williams & Round.
CMEN	*Cyclotella meneghiniana* Kützing.
COCE	*Cyclotella ocellata* Pantocsek

Code	Species name
CKPP	*Cymbella kappii* (Cholnoky)
CKOL	*Cymbella kolbei* Hustedt.
CSMO	*Cymbella simonsenii* Krammer
CYMS	*Cymbella* species
DKUE	*Denticula kuetzingii* Grunow.
DSUB	*Denticula subtilis* Grunow.
DSUN	*Denticula sundayensis* Archibald.
DCOF	*Diadesmis confervacea* Kützing.
DCOT	*Diadesmis contenta* (Grunow & V. Heurck) Mann.
DVUL	*Diatoma vulgaris* Bory
DELL	*Diploneis elliptica* (Kützing) Cleve.
DOBL	*Diploneis oblongella* (Naegeli) Cleve-Euler.
DPUE	*Diploneis puella* (Schumann) Cleve.
DIPS	*Diploneis* species.
DSBO	*Diploneis subovalis* Cleve.
DPST	*Discostella pseudostelligera* (Hustedt) Houk & Klee
ENMI	*Encyonema minutum* (Hilse in Rabh.) D.G. Mann.
ENNG	*Encyonema neogracile* Krammer.
ENSP	*Encyonema* species.
ENLE	*Encyonopsis leei* Krammer
EPAL	*Entomoneis paludosa* (W. Smith) Reimer.
EPDU	*Entomoneis pseudoduplex* Osada & Kobayasi.
ETOS	*Entomoneis* species.
EOMI	*Eolimna minima* (Grunow) Lange-Bertalot.
ESBM	*Eolimna subminuscula* (Manguin) Moser, Lange-Bertalot & Metzeltin.
EPIS	*Epithemia* species.
EBIL	*Eunotia bilunaris* (Ehr.) Mills.
EFLE	*Eunotia flexuosa* (Brébisson) Kützing.
EFOR	*Eunotia formica* Ehrenberg.
EMIN	*Eunotia minor* (Kützing) Grunow in Van Heurck
EPUN	*Eunotia pectinalis* (Kütz.)Rabenhorst var.undulata (Ralfs) Rabenhorst.
ERHO	*Eunotia rhomboidea* Hustedt.
EUNS	*Eunotia* species.
ETRI	*Eunotia tridentula* Ehrenberg
FPYG	*Fallacia pygmaea (*Kützing) Stickle & Mann
FALS	*Fallacia* species.
FCAP	*Fragilaria capucina* Desmazieres.
FRUT	*Fragilaria capucina var. rumpens* (Kütz)Lange-Bert.ex Bukht.fo. teratogene.
FCVA	*Fragilaria capucina* Desmazieres var. vaucheriae (Kützing)Lange-Bertalot
FEXI	*Fragilaria exigua* Grunow.
FGER	*Fragilaria germainii* Reichardt & Lange-Bertalot.
FNAN	*Fragilaria nanana* Lange-Bertalot.
FRAS	*Fragilaria* species.
FTEN	*Fragilaria tenera* (W. Smith) Lange-Bertalot.
FULN	*Fragilaria ulna* (Nitzsch.) Lange-Bertalot

Code	Species name
FUAT	*Fragilaria ulna* (Kützing) Lange-Bertalot.
FSAX	*Frustulia saxonica Rabenhorst.*
FVUL	*Frustulia vulgaris* (Thwaites) De Toni.
FRSP	*Frustulia* species
GACU	*Gomphonema acuminatum* Ehrenberg.
GAFF	*Gomphonema affine* Kützing.
GOM1	*Gomphonema affine gracile.*
GCAP	*Gomphonema capitatum* Ehrenberg.
GGRA	*Gomphonema gracile* Ehrenberg.
GGSS	*Gomphonema gracile* sensu stricto
GINS	*Gomphonema insigne* Gregory.
GITA	*Gomphonema italicum* Kützing.
GPAR	*Gomphonema parvulum* (Kützing)
GPPA	*Gomphonema parvulum* var. parvulius Lange-Bertalot & Reichardt.
GPAS	*Gomphonema parvulum* var. parvulum f. saprophilum Lange-Bert.& Reichardt.
GPSA	*Gomphonema pseudoaugur* Lange-Bertalot.
GOMS	*Gomphonema* species.
GTRU	*Gomphonema truncatum* Ehr.
GOAH	*Gomphosphenia oahuensis* (Hustedt) Lange-Bertalot.
HAMP	*Hantzschia amphioxys* (Ehrenberg) Grunow in Cleve & Grunow
HDIS	*Hantzschia distinctepunctata* Hustedt in Schmidt *et al.*,
HAZS	*Hantzschia* species.
HCAP	*Hippodonta capitata* (Ehr.) Lange-Bert.Metzeltin & Witkowski.
HIPS	*Hippodonta* species.
LHUN	*Lemnicola hungarica* (Grunow) Round & Basson.
LGOE	*Luticola goeppertiana* (Bleisch in Rabenhorst) D.G. Mann.
LMUT	*Luticola mutica* (Kützing) D.G. Mann.
LUSP	*Luticola* species.
LYRE	*Lyrella* species.
MSMI	*Mastogloia smithii* Thwaites.
MASP	*Mastogloia* species.
MAYA	*Mayamaea* species.
MVAR	*Melosira varians* Agardh.
NCIN	*Navicula cincta* (Ehr.) Ralfs in Pritchard.
NCTE	*Navicula cryptotenella* Lange-Bertalot.
NGRE	*Navicula gregaria* Donkin.
NGVL	*Navicula grevillei* (Agardh) Heiberg.
NLIB	*Navicula libonensis* Schoeman.
NPUS	*Navicula pusilla* W. Smith.
NRAD	*Navicula radiosa* Kützing.
NRCS	*Navicula recens* (Lange-Bertalot).
NRCH	*Navicula reichardtiana* Lange-Bertalot.
NRHY	*Navicula rhynchocephala* Kützing.
NROS	*Navicula rostellata* Kützing.

Code	Species name
NASP	*Navicula* species.
NSRH	*Navicula* subrhynchocephala Hustedt.
NTEN	*Navicula tenelloides* Hustedt.
NVDA	*Navicula vandamii* Schoeman & Archibald.
NVAR	*Navicula variostriata* Krasske.
NVEN	*Navicula veneta* Kützing.
NVIR	*Navicula viridula* (Kützing) Ehrenberg.
NZAN	*Navicula zanoni* Hustedt.
NCPU	*Navicymbula pusilla* Krammer.
NESP	*Neidium* species
NACI	*Nitzschia acicularis* (Kützing) W.M. Smith.
NACD	*Nitzschia acidoclinata* Lange-Bertalot.
NAMP	*Nitzschia amphibia* Grunow.
NIAR	*Nitzschia archibaldii* Lange-Bertalot.
NBCL	*Nitzschia bacillum* Hustedt.
NCPL	*Nitzschia capitellata* Hustedt in A.Schmidt & al.
NELE	*Nitzschia elegantula* Grunow.
NETO	*Nitzschia etoshensis* Cholnoky.
NFIL	*Nitzschia filiformis* (W.M. Smith) Van Heurck.
NFON	*Nitzschia fonticola* Grunow.
NIFR	*Nitzschia frustulum* (Kützing) Grunow.
NHAN	*Nitzschia hantzschiana* Rabenhorst.
NHEU	*Nitzschia heufleriana* Grunow.
NLBT	*Nitzschia liebetruthii* Rabenhorst.
NLIN	*Nitzschia linearis* (Agardh) W.M. Smith.
NLTT	*Nitzschia littorea* Grunow in Van Heurck.
NMIC	*Nitzschia microcephala* Grunow in Cleve & Müller.
NNAN	*Nitzschia nana* Grunow in Van Heurck.
NOSD	*Nitzschia obsidialis* Hustedt.
NPAL	*Nitzschia palea* (Kützing) W. Smith.
NPRP	*Nitzschia perspicua.*
NZPI	*Nitzschia pilum* Hustedt.
NIPR	*Nitzschia pura* Hustedt.
NIPU	*Nitzschia pusilla* (Kützing) Grunow.
NZRA	*Nitzschia radicula* Hustedt.
NREC	*Nitzschia recta* Hantzsch in Rabenhorst.
NSIG	*Nitzschia sigma* (Kützing) W.M. Smith.
NIS1	*Nitzschia* species.
NZSU	*Nitzschia supralitorea* Lange-Bertalot.
NUMB	*Nitzschia umbonata* (Ehrenberg) Lange-Bertalot.
NIVI	*Nitzschia vitrea* Norman.
PHUM	*Petroneis humerosa* (Brebisson & Wm. Smith) Stickle & Mann.
PEMA	*Petroneis marina* (Ralfs)D.G. Mann in Round *et al.*,

Code	Species name
PBOR	*Pinnularia borealis* Ehrenberg.
PDIV	*Pinnularia divergens* W.M.Smith.
PIRR	*Pinnularia irrorata* (Grunow) Hustedt.
PMRO	*Pinnularia microstauron* (Ehr.) Cleve var. rostrata Krammer.
PIN1	*Pinnularia* species *1*
PIN2	*Pinnularia* species 2.
PSBV	*Pinnularia subbrevistriata* Krammer.
PSCD	*Pinnularia subcapitata f. divergens* Hustedt.
PVIF	*Pinnularia viridiformis* Krammer.
PLAS	*Placoneis* species.
PLEN	*Planothidium engelbrechtii* (Cholnoky) Round & Bukhtiyarova.
PLFR	*Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot.
PRST	*Planothidium rostratum* (Østrup) Round & Bukhtiyarova.
PLTD	*Planothidium* species Round & Bukhtiyarova.
PSAL	*Pleurosigma salinarum (*Grunow) Cleve & Grunow.
PSBR	*Pseudostaurosira brevistriata* (Grun.in Van Heurck) Williams & Round.
RCON	*Rhopalodia constricta* (W. Smith) Krammer.
RGIB	*Rhopalodia gibba* (Ehr.) O. Müller
RGBL	*Rhopalodia gibberula (*Ehrenberg) O.Müller.
ROPE	*Rhopalodia operculata* (Agardh) Hakansson.
RHOS	*Rhopalodia* species.
SPUP	*Sellaphora pupula* (Kützing) Mereschkowksy.
SSEM	*Sellaphora seminulum* (Grunow) D.G. Mann.
SELS	*Sellaphora* species.
SSTM	*Sellaphora stroemii* (Hustedt) Mann.
SMST	*Seminavis strigosa* (Hustedt) Danieledis & Economou-Amilli.
STAN	*Stauroneis anceps* Ehrenberg
STMA	*Stauroneis marina* Hustedt.
SPHO	*Stauroneis phoenicenteron* (Nitzsch) Ehrenberg.
STAS	*Stauroneis* species.
SELI	*Staurosira elliptica* (Schumann) Williams & Round.
SSPE	*Staurosira* species
SANG	*Surirella angusta* Kützing.
SBRE	*Surirella brebissonii* Krammer & Lange-Bertalot.
SCRU	*Surirella crumena* Brebisson & Kützing.
SOVI	*Surirella ovalis* Brebisson.
SURS	*Surirella* species.
TFLO	*Tabellaria flocculosa* (Roth) Kützing.
TFAS	*Tabularia fasciculata* (Agardh) Williams et Round.
TPSN	*Thalassiosira pseudonana* Hasle & Heimdal.
TAPI	*Tryblionella apiculata* Gregory.
TCAL	*Tryblionella calida (*Grunow in Cleve & Grunow) D.G. Mann.
TDEB	*Tryblionella debilis* Arnott ex O'Meara
THUN	*Tryblionella hungarica* (Grunow) D.G. Mann.
TLEV	*Tryblionella levidensis* Wm. Smith.
TLIT	*Tryblionella littoralis* (Grunow in Cl. & Grun.) D.G. Mann.

Relative Abundance.

Table 1.2. Relative abundance of diatom species in the different wetlands.

	AGU	DRI	MFU	KEN	LOT	OOG	GCV	LGV	LPV	RTV	WVV	ZDV	ZRV
ABRE	0	0	16.6667	0	0	0	0	0	0	0	0	0	0
ACOA	0	0	0	2.17258	0.87209	0	0	0	0	0	0	0	0
ALIO	0.86957	0	0	0.78947	0	0	0	25	0	0	0	0	0
AOBG	0.20067	0.27972	0	1.45749	0.15385	0	0	14.6154	2.30769	0	0	0	0
ACHS	0.67633	5.45455	2.96296	0	0.22222	0	0	0	1.11111	0	0	0	0
ASTE	0	0	0	1.89145	0.78125	0	0	6.25	0	0	0	0	0
CALS	0	0.70922	0	2.27697	0.10638	0	0	0	0	1.77305	0	0	0
ADBI	0	0	0	0	0	0	0	0	50	0	0	0	0
ADEU	2.17391	0	0	0	0	25	0	0	0	0	0	0	0
ADEG	0.31969	0.66845	0.4902	0.81269	0.14706	1.4706	4.41176	11.0294	5.88235	0	7.35294	0	0
ADMA	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
ADMI	1.25	0.42208	2.09821	0.80827	0.36607	0.0446	14.1071	0	0.08929	0.75893	0	0	0.08929
ADRI	1.52456	0	0	0	0.06494	1.2987	0	17.5325	1.2987	0	23.3766	0	0
ADSP	0	0	0	2.63158	0	0	0	0	0	0	0	0	0
ACOF	1.61302	4.87506	0.87128	0	0.05902	0	0	0	0	0.25295	0	2.36088	0
AMMO	0	0	0	0	5	0	0	0	0	0	0	0	0
APED	0	0	0	0	0	0	0	0	0	0	0	100	0
AVEN	2.07238	0.96154	0.43498	0.50969	0.78984	0.0687	0	1.2706	0.1717	0.20604	0.48077	0	0.06868
ASPH	0.08361	6.64336	2.88462	0	0.38462	0	0	0	0	0	0	0	0
AAMB	0	0	0	0	0	0	0	0	0	0	100	0	0
AUCS	0	0.6402	0	0	0	0	0	7.04225	39.4366	0	0	0	0
AUGA	0	4.54545	0	0	0	0	0	25	0	0	0	0	0
AUGR	0	0.61875	0	0	0	0	0	5.75916	40.8377	0	0	0	0
AULS	0	0	0	1.05263	3	0	0	0	0	0	0	0	0
AUSU	0	0	0	0	0	0	0	0	0	0	0	0	0
BBRE	2.84487	0	0	0.09747	0	0	30.8642	0	0	0	0	0	0
BBTH	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
BNEO	4.27655	0	0	0.04314	0	0	0	0	0	0	0	0	0

	AGU	DRI	MFU	KEN	LOT	OOG	GCV	LGV	LPV	RTV	WVV	ZDV	ZRV
BRCS	0.27174	0	0	0.32895	0	0	81.25	0	0	0	0	0	0
CAQT	2.89855	0	0	0	0	0	0	0	0	16.6667	0	0	0
CMOL	1.87643	0.19139	0.52632	0.94183	0.78947	0	0	0	0	0	0	0	0
CPLA	0	0	0	0	0.35714	0	50	0	0	0	14.2857	21.4286	7.14286
CSHU	0	0	16.6667	0	0	0	0	0	0	0	0	0	0
CALS	1.43759	0.07331	0.53763	1.59168	0.12097	0	0	0	0	0	0	0	0
COCE	0	0	0	2.63158	0	0	0	0	0	0	0	0	0
CPLE	0.22297	5.82751	0	0	0	0	0	0	5.12821	0	20.5128	0	0
CPLI	0.02651	1.74612	0	0	0	0	0	1.98171	37.8049	0	0	0.60976	0
COMS	0.33445	2.44755	0	0	3.26923	0	0	0	0	0	0	0	0
CKPP	0	0	0	1.40759	2.32558	0	0	0	0	0	0	0	0
CAMB	0	6.52681	0.64103	0.57355	0	0	0	1.28205	0	0	0	0	0
CRAC	0	9.09091	0	0	0	0	0	0	0	0	0	0	0
CACD	2.28833	0	7.89474	0	0	0	0	0	0	0	0	0	0
CRBU	1.21079	0.69045	10.7595	0	0	0	0	0	0	0	0	0	0
CHAL	0.19324	0	7.31481	0.39474	1.83333	0	0	0	0	0	0	0	0
CRTS	0.62112	4.22078	0	0.37594	1.25	0	0	0	0	0	0	0	0
CVIX	2.37154	4.13223	0	0	0	0	0	0	0	0	0	0	0
CTPU	0	1.18577	0	0	0	0	8.69565	0	17.3913	21.7391	0	0	0
CMEN	0.03732	2.02887	3.07582	0.06777	1.43777	0.4292	0	0	1.71674	1.07296	20.6009	0	0
CKOL	0	0	0	0	0	0	0	0	50	0	0	0	0
CSMO	0	0	0	0	5	0	0	0	0	0	0	0	0
CYMS	0	0	0	2.63158	0	0	0	0	0	0	0	0	0
DKUE	0	1.00708	14.7705	0	0.01497	0	0	0	0	0	0	0	0
DSUB	0	0	16.6667	0	0	0	0	0	0	0	0	0	0
DSUN	0	0	0	1.57895	0	0	0	0	0	0	40	0	0
DCOF	0	0	0	0	0	0	0	0	0	0	100	0	0
DCOT	0	0	0	0	0	0	0	0	0	50	0	0	0

	AGU	DRI	MFU	KEN	LOT	OOG	GCV	LGV	LPV	RTV	WVV	ZDV	ZRV
DVUL	2.17391	0	0	0	0	0	0	0	0	25	0	0	0
DELL	0.73633	0	0	1.97368	0.12097	0	4.83871	0	0	0.40323	0	0	0
DOBL	2.7592	0.87413	4.48718	0	0	0	0	0	0	0	0	0	0
DPUE	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
DIPS	2.41546	0	1.85185	0	1.11111	0	0	0	0	0	0	11.1111	0
DPST	0	0	0	2.63158	0	0	0	0	0	0	0	0	0
DSBO	0	0	0	0	0	0	0	0	0	0	100	0	0
ENMI	0	7.27273	0	0.52632	0	0	0	0	0	0	0	0	0
ENNG	0	0	0	0	0	0	0	0	0	0	0	0	0
ENSP	0.17391	0.36364	0	0.21053	2.8	0	0	0	0	14	0	0	0
ENLE	0	0	0	2.63158	0	0	0	0	0	0	0	0	0
EPAL	0	9.09091	0	0	0	0	0	0	0	0	0	0	0
EPDU	0	0	0	0	0	0	0	0	0	0	0	0	0
ETOS	0.36232	0	6.94444	0	2.29167	0	0	0	0	2.08333	0	0	0
EBIL	0	0	0	0.05371	4.89796	0	0	0	0	0	0	0	0
EOMI	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
ESBM	0	0	0	0	0	0	0	37.5	3.125	0	18.75	0	0
EPIS	3.56152	1.16054	0.88652	0	0	0	0	0	0	0	0	0	0
EBIL	0	0	0	0	0	0	100	0	0	0	0	0	0
EFLE	0.86957	0	0	0	0	0	70	0	5	0	0	0	0
EFOR	0	9.09091	0	0	0	0	0	0	0	0	0	0	0
EMIN	0.26756	0.27972	0.51282	2.30769	0	0	0	0	0	0	0	0	0
EPUN	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
ERHO	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
EUNS	0.02179	0	0.04177	2.5953	0.03133	0	0	0	0	0	0	0	0
ETRI	0	0	0	2.63158	0	0	0	0	0	0	0	0	0
FPYG	4.07609	0	0	0	0.3125	0	0	0	0	0	0	0	0
FALS	0	9.09091	0	0	0	0	0	0	0	0	0	0	0
FCAP	0.13587	0.28409	0	1.06908	1.875	0	0	0	3.125	4.6875	0	0	0

	AGU	DRI	MFU	KEN	LOT	OOG	GCV	LGV	LPV	RTV	WVV	ZDV	ZRV
FCVA	0	0	0	0	5	0	0	0	0	0	0	0	0
FRUT	0	0	0	0	0	0	0	0	50	0	0	0	0
FEXI	0	3.34928	0	1.06187	0.96491	0	0	0	0	1.75439	0	0	0
FGER	0	0	0	0	0	0	0	0	0	50	0	0	0
FNAN	0	0	0	0	0	0	100	0	0	0	0	0	0
FRAS	0.00778	5.02157	1.62638	0.09659	1.55774	0	0	0	0	0	0	0	0
FTEN	0.47186	6.48344	2.58398	0.0204	0.07752	0	0	0	0	0	0	0	0
FULN	0	0.64935	0	0	0	0	0	0	46.4286	0	0	0	0
FUAC	0	6.20843	5.28455	0	0	0	0	0	0	0	0	0	0
FSAX	0	0	0	2.63158	0	0	0	0	0	0	0	0	0
FRSP	0	0	0	2.63158	0	0	0	0	0	0	0	0	0
FVUL	4.01338	0	0	0	0.38462	0	0	0	0	0	0	0	0
GACU	0	0	0	0	0	0	100	0	0	0	0	0	0
GAFF	0	0	0	0	2.63158	0	31.5789	0	0	0	5.26316	0	10.5263
GOPS	0.26894	0	0.17182	1.9669	0.61856	0	4.12371	0	0	0	1.54639	0	0
GCAP	0	0	0	0	0	0	100	0	0	0	0	0	0
GGRA	1.214	2.5974	1.19048	0.59809	0.48701	1.9481	0	0	0	0	0	0	0
GGSC	0	0	0	0.98684	3.125	0	0	0	0	0	0	0	0
GITA	0	1.51515	0	0	0	0	0	8.33333	33.3333	0	0	0	0
GPAR	0.05145	0.26896	0	0.07007	1.4645	0.4438	0.88757	8.57988	4.5858	0	34.9112	0	0.88757
GPPA	0	0	16.6667	0	0	0	0	0	0	0	0	0	0
GPAS	0	0	0	0	0	0	0	50	0	0	0	0	0
GPSA	0	0	0	0	0	0	0	50	0	0	0	0	0
GOMS	0.91054	0.38077	0.69808	1.28134	0.78534	2.0942	0	0	0	1.04712	0	0	0
GTRU	0	0	0	0	0	0	70	0	0	15	0	0	0
GOAH	0	0	0	0	0	0	0	0	0	0	0	100	0
HAMP	0.15684	2.27728	7.51503	0.31642	0.61122	0	0	1.002	0	0	0	0	0
HDIS	0	4.54545	0	1.05263	0.5	0	0	0	0	0	0	0	0
HAZS	0.01575	1.18577	0	0.67696	3.04348	0	0	0	0	0	0	0	0

	AGU	DRI	MFU	KEN	LOT	OOG	GCV	LGV	LPV	RTV	WVV	ZDV	ZRV
HCAP	0	1.51515	0	0	0	41.667	0	0	0	0	0	0	0
HIPS	0.23407	0	0	1.11922	0	0	0	0	0	16.4872	0	19.1117	0
LHUN	0	0.30303	0	0	0.16667	0	0	41.6667	0	0	10	0	0
LGOE	0.27174	0	0	0.16447	4.375	0	0	0	0	0	0	0	0
LMUT	0.79777	4.58716	3.66972	0	0.04587	0	0	4.12844	0	0	0	0	0
LUSP	0.22883	0.95694	0	2.21607	0	0	0	0	0	0	0	0	0
LYRE	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
MSMI	1.24224	0	0	0	0	0	71.4286	0	0	0	0	0	0
MASP	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
MAYA	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
MVAR	0	1.51515	0	0	0	0	0	0	22.9167	0	0	37.5	0
NCIN	1.39566	2.68078	1.37831	0.22335	1.0827	0	0	0	0	0	0	0	0
NCTE	1.50502	0	0	0	0	0	0	0	0	32.6923	0	0	0
NGRE	0	0	0	0	0	0	0	50	0	0	0	0	0
NGVL	0	5.09978	7.31707	0	0	0	0	0	0	0	0	0	0
NLIB	0	0	0	0	0	0	0	0	0	0	0	0	0
NPUS	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
NRAD	0	0	0	0	0	0	0	0	0	0	0	100	0
NRCS	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
NRCH	0	0	0	0	0	50	0	0	25	0	0	0	0
NRHY	0	4.54545	0	0	0	0	0	0	0	0	0	0	0
NROS	0	0	0	0	0	0	0	31.8182	18.1818	0	0	0	0
NASP	0.803	0.14683	0.11704	0.49527	1.70997	0.1404	0	0.70225	0.17556	4.74017	0.49157	14.1854	0
NTEN	0.70652	0	13.9583	0	0	0	0	0	0	0	0	0	0
NVDA	4.04449	0	0	0	0	0	0	3.48837	0	0	0	0	0
NVAR	0	0	16.6667	0	0	0	0	0	0	0	0	0	0
NVEN	1.40476	1.06991	1.22132	0.0487	1.28608	4.7187	0.07402	3.62694	1.11029	0.46262	0.48113	0.11103	0.51813
NVIR	0	0	0	0	0	0	0	0	50	0	0	0	0
NZAN	4.34783	0	0	0	0	0	0	0	0	0	0	0	0

	AGU	DRI	MFU	KEN	LOT	OOG	GCV	LGV	LPV	RTV	WVV	ZDV	ZRV
NCPU	2.50547	2.55146	0.78616	0	0.47956	0	0	0	0	0	0	0	0
NESP	0	0	0	2.63158	0	0	0	0	0	0	0	0	0
NACI	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
NACD	0	0	0	0	0	0	0	0	0	0	0	0	0
NAMP	0.40486	3.27567	3.50877	0.23439	0.18219	0.4049	0	0	4.25101	0	11.7409	0	0
NIAR	0.90857	0.38791	0.98468	1.08545	1.02298	0	0.21882	0.32823	0.76586	0	4.48578	0	0.32823
NBCL	3.71156	1.33038	0	0	0	0	0	0	0	0	0	0	0
NCPL	2.17391	1.61964	1.53257	0	1.14943	0	0	0	0	0	0	0	0
NELE	0	2.73973	10.9589	0	0	0	0	2.05479	0	0	0	0	0
NETO	0.42418	0	0	0	4.5122	0	0	0	0	0	0	0	0
NFIL	2.41546	2.0202	0	0	0	0	0	0	0	0	22.2222	0	0
NFON	0	0	0	0	5	0	0	0	0	0	0	0	0
NIFR	0.98373	2.46318	2.83985	0	0	0.1397	0.55866	6.70391	1.25698	0	14.2458	1.95531	0.27933
NHAN	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
NHEU	4.11491	0	0.89286	0	0	0	0	0	0	0	0	0	0
NLBT	0.84186	1.582	1.75654	0.05805	2.45098	0	0	0.73529	0	0	0	0	0
NLIN	2.17391	1.51515	5.55556	0	0	0	0	0	0	0	0	0	0
NLTT	0	0	0	0	0	0	0	0	0	0	0	0	0
NMIC	1.68067	5.57678	0	0	0	0	0	0	0	0	0	0	0
NNAN	2.06412	0	6.56566	0.34556	0	0	0	0	0	0	0	0	0
NOSD	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
NPAL	3.04878	1.55211	1.06707	0	0.12195	0	0	0.15244	1.06707	0	0	0	1.52439
NPRP	1.24224	0	0	0	3.57143	0	0	0	0	0	0	0	0
NZPI	0	0	0	0	0	0	0	0	0	0	0	0	0
NIPR	0	4.27807	0	0	0	0	0	0	26.4706	0	0	0	0
NIPU	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
NZRA	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
NREC	1.52174	0	0	0	0	0	55	0	5	0	0	0	0
NIS1	0.3269	0.08078	0	1.17099	2.16336	0.7519	0.61517	0.17088	0.68353	0	0	0	0

	AGU	DRI	MFU	KEN	LOT	OOG	GCV	LGV	LPV	RTV	WVV	ZDV	ZRV
NZSU	0.3566	0.78864	2.68139	0.11622	1.07256	0	0	6.30915	0	14.1167	0	0.31546	0
NUMB	1.64651	5.00269	0.19724	0.03114	0.05917	0	0	0.59172	1.18343	0	0	0	0
NIVI	1.67224	0	0	1.01215	1.15385	0	0	0	0	0	0	0	0
PHUM	0	0	0	0	0	0	0	0	0	0	0	100	0
PEMA	0	0	0	0	0	0	0	0	0	0	0	100	0
PBOR	3.59168	0.79051	1.44928	0	0	0	0	0	0	0	0	0	0
PDIV	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
PIRR	1.00334	6.75991	0	0	0	0	0	0	1.28205	0	0	0	0
PMRO	2.12089	4.65632	0	0	0	0	0	0	0	0	0	0	0
PSBV	2.48447	0.64935	2.38095	0	0	0	21.4286	0	0	0	0	0	0
PSCD	0.6865	0.95694	6.14035	0	0	0	21.0526	0	0	7.89474	0	0	0
PINS	0.27822	0.12818	0.81345	2.1064	0.36334	0	0	0	0	0	0	0	0
PVIF	2.82609	1.81818	1.66667	0.13158	0	0	0	0	0	0	0	0	0
PENG	0.73844	0.45578	0.6348	0.99924	1.37777	2.6234	0	1.04936	0	0.03887	1.08822	0	0.15546
PLFR	0.03716	2.05905	0.07123	0	0.10684	0	0	23.0769	13.8889	0	0	0	0
PTRO	0	4.79339	0	0	0	0.9091	0	6.36364	4.54545	11.8182	0	0	0
PSAL	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
PLTD	0	0	0	0	0	0	0	0	0	50	0	0	0
PSBR	0	0	0	0	3.33333	0	0	0	0	16.6667	0	0	0
PSBV	0	0	0	2.47678	0.29412	0	0	0	0	0	0	0	0
PSCA	0	0	0	2.13816	0.9375	0	0	0	0	0	0	0	0
PLAS	0	0	0	1.53551	2.08253	0	0	0	0	0	0	0	0
RGIB	0.72464	0	0	0	0	0	83.3333	0	0	0	0	0	0
RCON	4.2349	0	0	0	0.12987	0	0	0	0	0	0	0	0
RGBL	3.26957	0.67273	2.3	0	0.18	0	0	0	0	0	0	0	0
ROPE	1.17057	0	0	0.20243	3.07692	0	3.84615	0	0	0	0	0	0
SPUP	0.31269	0.49813	5.70776	0.57678	1.35274	0	0	0.85616	1.19863	0	0	0	0
SSEM	2.95652	0	0	0.21053	0	0	0	2	0	10	0	0	0
SELS	1.77134	1.3468	0	0.77973	0.74074	0	0	0	0	0	0	0	0

	AGU	DRI	MFU	KEN	LOT	OOG	GCV	LGV	LPV	RTV	WVV	ZDV	ZRV
SSPE	0	0	0	1.21457	2.69231	0	0	0	0	0	0	0	0
SSTM	0.61022	3.34928	3.21637	0.78486	0	0	0	0	0	0	0	0	0
STAN	0	0	0	1.98108	1.23596	0	0	0	0	0	0	0	0
STMA	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
SPHO	4.13043	0	0	0.13158	0	0	0	0	0	0	0	0	0
SELI	0.24845	0.32468	0	0	0.03571	2.1429	72.1429	0	0	5	3.57143	0	0
STAS	3.80435	0	0	0.32895	0	0	0	0	0	0	0	0	0
SANG	0	0.09091	0	0	4.85	0	0	0	0	0	0	0	2
SBRE	2.17391	0	0	0	0	0	0	25	0	0	0	0	0
SCRU	0	0	0	0	0	0	0	0	0	0	0	0	0
SOVI	0.62112	0	0	0	0	0	0	42.8571	0	0	0	0	0
SURI	1.94903	0	0	0	0	0	0	27.5862	0	0	0	0	0
TFLO	1.26227	0	1.6129	1.52801	0	0	3.22581	0	0	0	0	0	0
TFAS	0.14553	5.17307	1.60391	0	0.1046	0	0	0.20921	4.39331	2.51046	2.92887	8.78661	2.09205
TPSN	0	9.09091	0	0	0	0	0	0	0	0	0	0	0
TAPI	3.91304	0.14663	0	0	0.41935	0	0	0	0	0	0	0	0
TCAL	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
TDEB	0	0	0	2.63158	0	0	0	0	0	0	0	0	0
THUN	1.24884	0	0	0	3.56383	0	0	0	0	0	0	0	0
TLEV	4.34783	0	0	0	0	0	0	0	0	0	0	0	0
TLIT	4.34783	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX D:
Index Scores.

Table 1.1 List of index scores of the different wetlands

Name	GDI	SPI	TDI	%PT
Die oog	9.2	3.8	81.8	2.1
Die oog	12.4	2	74.3	0.3
Glencairn Vlei	15.3	15.4	29.1	6.3
Lange Vlei	11.5	8.8	78.7	24.3
Lange Vlei	9	3.1	74.5	21.8
Little Princess	10.8	8.6	86.1	11.4
Little Princess	12	11.6	59	7.2
Rietvlei	7.4	6.1	60.3	44.3
Rietvlei	13.8	12.9	76.7	0
Wildevoel Vlei	8.7	7.5	88.1	52.1
Zand Vlei	12.4	11.8	77.5	2.2
Zoar Vlei	8.5	5	82.4	28.6

Table 1.2 The   index scores of the sites at Agulhas wetland.

Site code	GDI	SPI	TDI	%PT
	13.5	14.6	62.7	25.4
AGU-01	12.2	11.7	34.7	8.3
AGU-04	11	8.4	74.2	10.2
AGU-05	14	14.5	64.3	20.5
	12.4	12.8	40.5	24.1
	9.3	6.7	56.7	28.9
AGU-07	12	9.3	42.8	11.8
AGU-08	12.8	12.2	26	5.4
AGU-11	9.2	10.6	41.7	34.7
AGU-12	9.6	8	81.2	24.1
AGU-13	7.9	8.7	61.1	15.4
AGU-14	11.2	7.5	81.6	0.2
AGU-15	10.7	10.4	41.5	21.7
	10	6.5	61.5	20.1
AGU-16	7.7	5.1	100	21.1
AGU-17	10.9	7	81	0.7
AGU-18	10.3	10.3	58.4	15
AGU-19	11.5	7.8	62.5	0
AGU-20	9.7	8.4	43	31
AGU-21	11	5.1	85	0
	13.6	14.4	33.5	18.4
AGU-22	13.4	15.9	25.3	18.7
AGU-23	10.7	8	70.8	20.2

Table 1.3 The index scores of the sites at Drift Sands wetland.

Site code	GDI	SPI	TDI	%PT
DRI01	10.7	10.4	73.8	13.4
	10.5	10.1	66.2	10.9
DRI02	10.6	10.1	56	11.4
	12.6	14.1	39.2	1.2
DRI03	10.1	10.5	75.6	22.7
	9	9.1	71.8	21.4
DRI04	12.5	12.5	67.6	4.2
	6.7	6.9	94.7	15.1
DRI05	11	10.5	60.2	13.3
DRI06	8.9	10.6	63.3	11.8
DRI07	7.8	6.7	76.8	14.7

Table 1.4 The index scores of the sites at eMfuleni wetland.

Site code	GDI	SPI	TDI	%PT
	5.3	5.7	91.4	4.6
MFU01	8.3	10.3	47.8	25.7
	12.2	12.5	43.9	5.6
MFU02	12.6	12.9	37.7	10.8
	9.4	8.1	77.5	25.9
MFU03	7.8	9.5	69.9	22

Table 1.5 The index scores of the sites at Kenilworth wetland.

Site code	GDI	SPI	TDI	%PT
KEN01E	9.3	11.2	60.4	4.4
KEN02E	10.9	12.4	49.4	18.2
	8.5	12.2	41.9	21.2
KEN03E	6.2	15.4	41.7	50
	12.4	13.4	18.2	11.6
KEN05E	15.2	13.4	19	0
	15.7	15.6	12.1	1.1
KEN06E	16.9	13.3	50	0
	10.2	8.4	72.8	2.8
KEN07E	12	11.7	16.1	0
	16	16.2	31.3	0
KEN08E	14	10.3	32.9	4
	15.5	15.5	11.1	0
KEN09E	15.6	14.8	25	0
	12.7	15	37.2	11.8
KEN10E	10.4	11.5	25	20
KEN11E	18	17.6	18.1	0
	14.6	13	21.9	6.9

KEN12E	17	16.4	4.5	1.9
	17.9	18.3	1.1	1.3
KEN13E	18.9	19.7	0.3	0
	15.9	13.9	9	4.4
KEN14E	6.3	5.2	41.1	1.2
	16.5	15	13.3	0
KEN17E	12.6	11.1	71.8	0.2
	15.3	14.7	32.8	0
KEN21E	13.6	13.6	65.9	0
KEN23E	11.1	10.3	45.2	3.8
	12.2	12.4	41.2	0
KEN25E	14.8	17.2	9.4	14.3
	8.9	7.3	41.7	0
KEN26E	16.6	17.9	5	7.7
KEN27E	4	1.7	69.7	0
KEN28E	15.5	15.5	10.8	4
	5	3.4	40.4	0
KEN29E	10.4	15.9	42.3	35.7
	1.4	1	75	0
KEN33E	13.6	15.9	39.6	11.9

Table 1.6 The index scores of the sites at Lotus River.

Site code	GDI	SPI	TDI	%PT
	9.9	11.1	47.5	9.2
LOT01E	6.2	6.9	77	24.9
	13.4	15.2	25	0
LOT02E	11.1	9.8	51	4.4
	14.2	8.2	51.3	1.9
LOT03E	10.6	5.1	68.7	6.8
	11.4	14	36.3	7.5
LOT04E	7.4	7.5	62.8	32.6
	2.6	1.8	76.1	1
LOT05E	3.3	3.8	81.3	0
LOT06E	7.6	4.2	69.2	1.5
	9	2.9	85.5	3.8
LOT08E	10.4	7.6	79.6	6.2
	12.6	14.2	66.1	12.7
LOT09E	9.7	10.3	75.6	11.5
	10.7	11.4	69	13
LOT11E	8.7	5.8	71.9	11.6
	9.8	12.6	41.7	0
LOT13E	12.5	11.9	46.9	16.4
LOT14E	9.4	14	61.9	55.1

Species Contribution to Index Calculations

Table 1.6 The percentage of species used from total count for calculation of the different indices.

Name	IDAP	EPI-D	IBD	SHE	SID	TID	WAT	IPS	SLA	DES	L&M	IDG	CEE	LOBO	IDP	DI-CH	TDI
Oog	33.33	41.67	33.33	41.67	41.67	33.33	16.67	83.33	33.33	16.67	16.67	91.67	33.33	25.00	16.67	41.67	91.67
Oog	42.86	64.29	57.14	57.14	57.14	50.00	28.57	92.86	57.14	35.71	28.57	92.86	50.00	42.86	28.57	50.00	85.71
Gcv	24.14	57.72	41.38	48.28	62.07	58.62	17.24	89.66	58.62	44.83	37.93	100.0	48.28	27.59	24.14	37.93	96.55
Wvv	54.17	54.17	58.33	50.00	45.83	54.17	29.17	91.67	66.67	25.00	37.50	95.83	62.50	50.00	33.33	41.67	83.33
Zdv	50.00	56.25	56.25	43.75	43.75	50.00	18.75	93.75	50.00	31.25	37.50	100.0	56.25	25.00	43.75	50.00	87.50
Zrv	66.67	83.33	83.33	75.00	75.00	83.33	25.00	91.67	83.33	50.00	58.33	91.67	91.67	58.33	50.00	67.67	91.67
Rtv	30.77	46.15	50.00	50.00	46.15	50.00	19.23	84.62	42.31	26.92	26.92	96.15	50.00	26.92	26.92	38.46	88.46
Rtv	57.14	42.86	42.86	28.57	28.57	28.57	-	100.0	42.86	-	-	100.0	42.86	12.29	28.57	14.29	100.0
Lpv	39.29	60.71	60.71	57.14	46.43	57.14	25.00	96.43	60.71	35.71	46.43	100.0	60.71	46.43	35.71	46.43	85.71
Lpv	52.17	73.91	78.26	65.22	60.87	69.57	21.74	100	78.26	56.52	56.52	100.0	69.57	47.83	47.83	65.22	91.30
Lgv	38.71	48.39	61.29	61.29	45.16	51.61	25.81	90.32	61.29	25.81	48.39	96.77	61.29	45.16	32.26	38.71	83.87
Lgv	30.00	70.00	60.00	45.00	50.00	50.00	15.00	85.00	55.00	30.00	30.00	95.00	65.00	35.00	40.00	35.00	95.00
AGU	66.67	66.67	66.67	66.67	100.00	66.67	-	100.0	66.67	-	33.33	100.0	66.67	33.33	33.33	-	100.0
AGU	100.0	50.00	50.00	50.00	50.00	50.00	50.00	100.0	50.00	50.00	50.00	100.0	50.00	50.00	50.00	50.00	100.0
AGU	30.00	45.00	45.00	35.00	45.00	15.00	15.00	90.00	45.00	15.00	35.00	100.0	45.00	20.00	20.00	25.00	95.00
AGU	38.46	46.15	46.15	46.15	46.15	46.15	15.38	92.31	46.15	15.38	30.77	92.31	46.15	15.38	23.08	23.08	84.62
AGU	13.04	47.83	43.48	43.48	47.83	52.17	17.39	95.65	43.48	26.09	39.13	95.65	47.83	30.43	21.74	26.09	95.65
AGU	53.85	61.54	53.85	46.15	61.54	61.54	7.69	100.0	69.23	23.08	53.85	100.0	53.85	23.08	23.08	38.46	100.0
AGU	25.00	35.00	40.00	30.00	35.00	40.00	5.00	85.00	45.00	20.00	30.00	95.00	40.00	20.00	20.00	25.00	85.00
AGU	21.74	43.48	47.83	43.48	47.83	47.83	8.70	91.30	47.83	17.39	34.78	95.65	56.52	13.04	26.09	30.43	82.61
AGU	30.00	50.00	50.00	60.00	60.00	60.00	5.00	95.00	50.00	15.00	40.00	95.00	50.00	20.00	30.00	45.00	90.00
AGU	35.00	50.00	45.00	40.00	50.00	45.00	5.00	90.00	40.00	5.00	35.00	95.00	45.00	15.00	25.00	25.00	90.00
AGU	23.08	50.00	42.31	46.15	46.15	46.15	11.54	92.31	42.31	7.69	38.46	96.15	42.31	15.38	26.92	38.46	92.31
DRI	50.00	62.50	68.75	62.50	56.25	59.38	18.75	96.88	65.63	40.63	50.00	96.88	62.50	43.75	37.50	50.00	87.50
AGU	15.63	62.50	56.25	37.50	53.13	59.38	12.50	90.63	59.38	18.75	43.75	93.75	59.38	28.13	37.50	40.63	90.63
AGU	27.59	55.17	58.62	51.72	58.62	58.62	17.24	93.10	48.28	20.69	34.48	96.55	51.72	24.14	24.14	41.38	93.10
AGU	30.00	45.00	45.00	55.00	50.00	50.00	15.00	95.00	40.00	15.00	30.00	95.00	45.00	25.00	25.00	35.00	90.00
AGU	21.43	53.57	50.00	53.57	60.71	57.14	17.80	96.43	60.71	21.43	42.86	96.43	50.00	28.57	32.14	39.29	96.43
AGU	25.81	45.16	48.39	38.71	45.16	48.39	12.90	90.32	41.94	12.90	20.03	93.55	54.84	19.35	19.35	19.35	87.10
AGU	21.21	54.55	51.52	42.42	57.58	57.58	21.21	90.91	54.55	15.15	33.33	96.97	48.48	24.24	24.24	33.33	93.94
AGU	23.08	50.00	53.85	46.15	57.69	53.85	11.54	96.15	46.15	11.54	30.77	96.15	53.85	26.92	30.77	38.46	88.46

AGU	41.67	66.67	66.67	66.67	66.67	66.67	41.67	**91.67**	58.33	41.67	16.67	**91.67**	66.67	50.00	25.00	50.00	**91.67**
MFU	30.43	69.57	52.17	52.17	60.87	56.52	260.9	**95.65**	56.52	30.43	30.43	**95.65**	56.52	47.83	21.74	47.83	**95.65**
MFU	42.86	71.43	57.14	57.14	57.14	64.29	21.43	**100.0**	50.00	35.71	21.43	**100.0**	57.14	35.71	35.71	50.00	**78.57**
DRI	33.33	61.90	47.62	47.62	52.38	57.14	14.29	**95.24**	47.62	28.57	19.05	**100.0**	52.38	28.57	28.57	38.10	**85.71**
DRI	30.77	61.54	53.85	53.85	50.00	50.00	12.23	**96.15**	50.00	30.77	26.92	**96.15**	50.00	38.46	34.62	42.31	**88.46**
DRI	30.77	57.69	57.69	46.15	53.85	57.69	15.38	**92.31**	50.00	34.62	19.23	**96.15**	58.85	23.08	30.77	38.46	**84.62**
DRI	32.00	60.00	56.00	56.00	56.00	64.00	20.00	**88.00**	56.00	40.00	20.00	**96.00**	60.00	32.00	28.00	40.00	**92.00**
DRI	34.43	60.87	56.52	56.52	47.83	56.52	21.74	**91.30**	60.87	43.48	21.74	**95.65**	60.87	34.78	30.43	43.48	**86.90**
MFU	29.63	62.96	48.15	59.26	55.56	55.56	22.22	**92.59**	62.96	33.33	22.22	**96.30**	59.26	29.63	33.33	40.74	**92.59**
DRI	17.86	46.43	39.29	39.29	46.43	46.43	10.71	**82.14**	42.86	21.43	14.29	**100.0**	39.29	21.43	21.43	32.14	**92.86**
AGU	27.27	63.64	51.52	54.55	57.58	57.58	24.24	**93.94**	60.61	36.36	24.24	**96.97**	60.61	42.42	27.27	36.36	**96.97**
DRI	30.0	50.00	55.00	50.00	60.00	60.00	15.00	**95.00**	60.00	35.00	15.00	**95.00**	50.00	20.00	25.00	35.00	**90.00**
DRI	36.36	59.09	54.55	40.91	54.55	54.55	22.73	**95.45**	59.09	31.82	22.73	**95.45**	59.09	36.36	36.36	36.36	**86.36**
MFU	17.24	65.52	51.72	48.28	58.62	62.07	20.69	**93.10**	62.07	41.38	20.69	**96.55**	55.17	34.48	31.03	37.93	**93.10**
DRI	29.41	58.88	58.88	52.94	52.94	58.82	17.65	**94.12**	64.71	35.29	29.41	**94.12**	64.71	35.29	35.29	41.18	**82.35**
AGU	21.05	42.11	42.11	36.84	42.11	31.58	5.26	**89.47**	42.37	31.58	21.05	**94.74**	47.37	21.05	26.32	21.05	**89.47**
AGU	-	33.33	33.33	22.22	33.33	22.22	11.11	**88.89**	22.22	11.11	22.22	**88.89**	22.22	22.22	22.22	44.44	**88.89**
MFU	29.17	66.67	50.00	45.83	45.83	50.00	25.00	**95.83**	54.17	37.50	33.33	**95.83**	54.17	37.50	37.50	45.83	**91.67**
AGU	33.33	-	33.33	33.33	-	-	33.33	**66.67**	33.33	-	-	**66.67**	33.33	33.33	-	-	**66.67**
DRI	29.41	52.94	35.29	35.29	47.06	52.94	23.53	**94.12**	58.82	35.29	29.41	**94.12**	58.82	29.41	29.41	35.29	**82.35**
MFU	31.82	54.55	50.00	50.00	45.45	54.55	22.73	**90.91**	54.55	40.91	22.73	**95.45**	59.09	22.73	31.82	40.91	**86.36**
LOT	13.33	26.67	20.00	26.67	20.00	20.00	13.33	**66.67**	20.00	13.33	13.33	**93.33**	26.67	13.33	-	13.33	**73.33**
LOT	26.09	43.48	47.83	52.17	52.17	52.17	26.09	**82.61**	39.13	30.43	17.39	**95.65**	52.17	26.09	13.04	34.78	**91.30**
LOT	-	-	-	-	-	-	-	**50.00**	-	-	-	**50.00**	-	-	-	-	**50.00**
LOT	22.22	27.78	27.78	38.89	38.89	38.89	11.11	**88.89**	27.78	27.78	11.11	**94.44**	38.89	11.11	5.56	22.22	**94.44**
LOT	33.33	40.00	40.00	40.00	40.00	40.00	6.67	**86.67**	33.33	20.00	13.33	**93.33**	40.00	13.33	6.67	40.00	**86.67**
LOT	18.52	29.63	25.93	25.93	33.33	37.04	3.70	**85.19**	33.33	25.93	7.41	**96.30**	37.04	7.41	7.41	14.81	**92.59**
LOT	26.67	40.00	33.33	40.00	40.00	46.67	6.67	**86.67**	46.67	40.00	33.33	**93.33**	53.33	26.67	13.33	40.00	**86.67**
LOT	29.17	41.67	41.67	41.67	41.67	45.83	-	**87.50**	41.67	37.50	8.33	**95.83**	54.17	8.33	16.67	29.17	**91.67**
LOT	30.00	40.00	40.00	40.00	40.00	40.00	-	**70.00**	40.00	20.00	20.00	**90.00**	30.00	20.00	30.00	40.00	**70.00**
LOT	27.27	36.36	36.36	27.27	27.27	36.36	9.09	**72.73**	36.36	18.18	-	**90.91**	36.36	-	-	18.18	**81.82**
LOT	50.00	62.50	37.50	37.50	43.75	56.25	12.50	**87.50**	50.00	31.25	31.25	**93.75**	50.00	37.50	25.00	43.75	**81.25**
LOT	52.94	58.82	47.06	41.18	41.18	52.94	23.53	**88.24**	58.82	29.41	48.18	**94.12**	58.82	41.18	29.41	47.00	**82.35**

LOT	**86.67**	40.00	26.67	40.00	60.00	**93.33**	46.67	26.67	53.33	**93.33**	13.33	66.67	60.00	60.00	73.33	66.67	40.00
LOT	**88.89**	22.22	22.22	33.33	38.89	**100.0**	16.67	22.22	38.89	**88.89**	11.11	27.78	22.22	33.33	33.33	38.89	22.22
LOT	**88.00**	28.00	20.00	16.00	48.00	**96.00**	32.00	12.00	44.00	**84.00**	8.00	48.00	44.00	40.00	40.00	44.00	24.00
LOT	**83.33**	29.17	29.17	20.83	45.83	**100.0**	25.00	16.67	37.50	**95.83**	8.33	37.50	37.50	45.83	41.67	41.67	16.67
LOT	**85.19**	33.33	29.63	29.63	51.85	**96.60**	33.33	25.93	48.15	**92.59**	14.81	48.15	44.44	44.44	44.44	48.15	29.63
LOT	**75.00**	-	-	-	-	**75.00**	-	-	-	**75.00**	-	-	-	-	-	-	-
LOT	**90.48**	38.10	19.05	28.57	47.62	**47.62**	95.24	38.10	47.62	**80.95**	14.29	42.86	42.86	33.33	42.86	42.86	19.05
LOT	**85.00**	55.00	20.00	40.00	65.00	**95.00**	50.00	40.00	50.00	**85.00**	10.00	65.00	55.00	50.00	55.00	55.00	20.00
LOT	**90.48**	28.57	14.29	19.05	47.62	**95.24**	23.81	14.29	33.33	**80.95**	9.52	52.38	42.86	47.62	33.33	38.10	23.81
KEN	**82.35**	35.29	5.88	23.53	41.18	**94.12**	35.29	29.41	35.29	**82.35**	11.76	35.29	29.41	29.41	35.29	29.41	11.76
KEN	**96.15**	30.77	7.69	26.92	53.85	**96.15**	26.92	23.08	46.15	**92.31**	23.08	42.31	42.31	46.15	38.46	42.31	23.08
KEN	**100.0**	100.0	50.00	50.00	100.0	**100.0**	50.00	50.00	50.00	**100.0**	-	100.0	50.00	100.0	100.0	50.00	50.00
KEN	**71.43**	35.71	21.43	28.57	50.00	**92.86**	28.57	14.29	35.71	**78.57**	21.43	42.86	35.71	42.86	28.57	42.86	14.29
KEN	**80.00**	20.00	-	20.00	-	**80.00**	-	20.00	-	**80.00**	-	20.00	20.00	-	20.00	20.00	-
KEN	**92.86**	14.29	14.29	21.43	21.43	**92.86**	14.29	14.29	14.29	**78.57**	14.29	21.43	21.43	21.43	14.29	21.43	14.29
KEN	**33.33**	-	-	-	-	**66.67**	-	-	-	**33.33**	-	-	-	-	-	-	-
KEN	**93.33**	20.00	6.67	13.33	36.67	**96.67**	23.33	10.00	26.67	**90.00**	13.33	40.00	36.67	33.33	20.00	36.67	13.33
KEN	**85.71**	14.29	-	14.29	14.29	**100.0**	14.29	14.29	14.29	**85.71**	14.29	14.29	14.29	14.29	14.29	14.29	14.29
KEN	**90.91**	27.27	27.27	36.36	45.45	**100.0**	27.27	36.36	36.36	**81.82**	9.09	45.45	27.27	36.36	45.45	45.45	27.27
KEN	**100.0**	16.67	16.67	16.67	16.67	**100.0**	16.67	16.67	16.67	**100.0**	16.67	16.67	16.67	16.67	16.67	16.67	16.16
KEN	**75.00**	12.50	12.50	12.50	12.50	**87.50**	12.50	12.50	12.50	**75.00**	12.50	12.50	12.50	12.50	12.50	12.50	12.50
KEN	**92.31**	23.08	15.38	19.23	34.62	**96.15**	23.08	11.54	26.92	**84.62**	19.23	38.46	34.62	34.62	26.92	38.46	15.38
KEN	**100.0**	25.00	-	25.00	50.00	**100.0**	50.00	25.00	25.00	**100.0**	-	50.00	50.00	25.00	50.00	25.00	-
KEN	**75.00**	33.33	16.67	41.67	33.33	**91.67**	33.33	50.00	41.67	**83.33**	25.00	41.67	41.67	16.67	41.67	41.67	16.67
KEN	**87.50**	18.75	-	12.50	37.50	**93.75**	37.50	25.00	25.00	**87.50**	12.50	31.25	25.00	25.00	31.25	25.00	6.25
KEN	**88.89**	22.22	-	11.11	33.33	**100.0**	22.22	22.22	33.33	**88.33**	11.11	33.33	33.33	33.33	33.33	22.22	22.22
KEN	**60.00**	20.00	10.00	30.00	20.00	**100.0**	30.00	10.00	30.00	**90.00**	20.00	20.00	10.00	10.00	30.00	10.00	20.00
KEN	**71.43**	14.29	-	14.29	28.57	**85.57**	14.29	28.57	14.29	**71.43**	14.29	28.57	28.57	41.29	14.29	28.57	14.29
KEN	**90.00**	40.00	20.00	30.00	50.00	**100.0**	30.00	20.00	40.00	**90.00**	10.00	50.00	50.00	50.00	50.00	40.00	40.00
KEN	**94.12**	29.41	29.41	23.53	35.29	**94.12**	35.29	17.65	35.29	**88.24**	5.88	41.18	41.18	41.18	35.29	41.18	17.65
KEN	**83.33**	33.33	16.67	50.00	50.00	**83.33**	33.33	33.33	50.00	**83.33**	33.33	50.00	50.00	50.00	50.00	50.00	50.00
KEN	**85.71**	14.29	14.29	21.43	50.00	**92.86**	21.43	28.57	50.00	**85.71**	14.29	35.71	35.71	28.57	50.00	42.86	21.43
KEN	**88.89**	27.78	11.11	16.67	33.33	**94.44**	16.67	11.11	33.33	**88.89**	11.11	38.89	38.89	44.44	33.33	38.89	27.78

KEN	22.73	31.82	27.27	27.27	31.82	36.36	4.55	**86.36**	31.82	9.09	18.18	**95.45**	36.36	13.64	4.55	13.64	**90.91**
KEN	22.22	27.78	33.33	38.89	33.33	33.33	11.11	**77.78**	27.78	16.67	27.78	**94.44**	38.89	22.22	22.22	22.22	**88.89**
KEN	15.38	30.77	30.77	30.77	23.08	23.08	15.38	**92.31**	30.77	30.77	15.38	**92.31**	30.77	30.77	15.38	30.77	**92.31**
KEN	50.00	50.00	50.00	50.00	50.00	50.00	50.00	**100.0**	50.00	50.00	50.00	**100.0**	50.00	50.00	50.00	50.00	**100.0**
KEN	-	20.00	30.00	10.00	20.00	30.00	-	**80.00**	20.00	10.00	10.00	**90.00**	20.00	-	10.00	20.00	**70.00**
KEN	33.33	33.33	33.33	16.67	16.67	33.33	33.33	**50.00**	33.33	33.33	33.33	**83.33**	33.33	33.33	16.67	33.33	**33.33**
KEN	10.00	30.00	35.00	35.00	35.00	40.00	10.00	**85.00**	35.00	35.00	20.00	**95.00**	40.00	25.00	25.00	30.00	**85.00**
KEN	-	-	-	-	-	-	-	**100.0**	-	-	-	**100.0**	-	-	-	-	**100.0**
KEN	15.00	30.00	25.00	30.00	30.00	30.00	15.00	**80.00**	25.00	15.00	30.00	**95.00**	30.00	20.00	10.00	25.00	**85.00**
KEN	-	-	-	-	-	-	-	**33.33**	-	-	-	**66.67**	-	-	-	-	**33.33**
KEN	6.67	13.33	20.00	26.67	26.67	20.00	-	**80.00**	13.33	13.33	13.33	**93.33**	26.67	13.33	13.33	20.00	**80.00**
KEN	14.29	14.29	28.57	28.57	28.57	28.57	14.29	**71.43**	14.29	14.29	28.57	**85.71**	28.57	14.29	-	28.57	**71.43**
KEN	33.33	33.33	33.33	33.33	33.33	33.33	33.33	**66.67**	33.33	33.33	33.33	**66.67**	33.33	33.33	-	33.33	**66.67**

APPENDIX E: Plates.

Commonly Occuring Dominant Species.

Araphideae.

Fragilaria

Figure 001-008: *Fragilaria* species unknown

Fig. 001-005: LM micrographs.

Fig. 006-008: SEM micrographs.

The SEM micrographs show both the interior and exterior valve view, with figure 007 showing the structure of the areolae.

001   002   003   004   005

006   007   008

10.0 μm

Plate 01

123
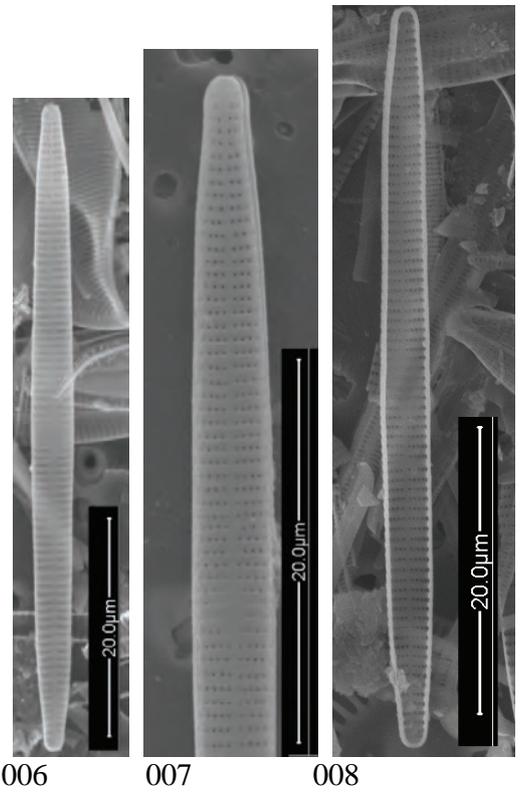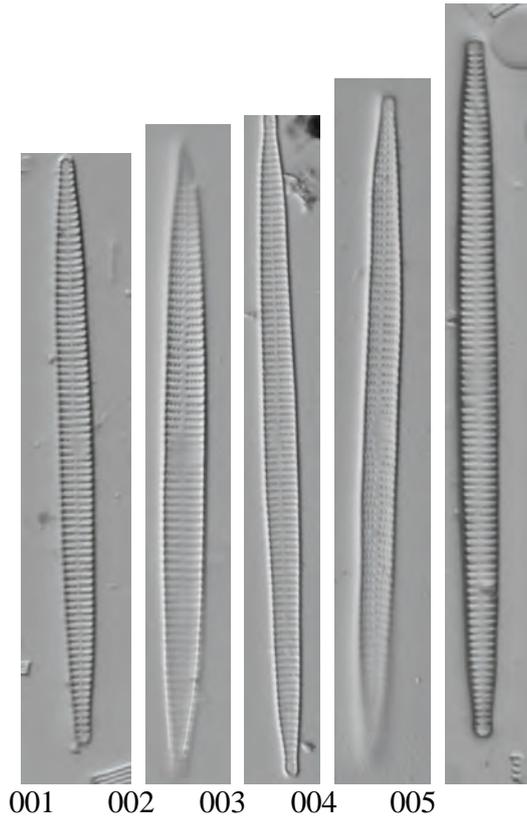
Monoraphideae.

Achnanthidium

Figure 009-011: *Achnanthidium minutissimum* (Kützing) Czarnecki.

Fig. 009-011: LM micrographs, figure 011 shows the girdle view of *A. minutissimum.*

Figure 012: SEM micrograph.

The SEM micrograph shows the RLV of *A. minutissimus.*

Planothidium

Figure 012-014: *Planothidium engelbrechtii* (Cholnoky) Round & Bukhtiyarova.

Fig. 012 & 013: LM micrographs.

Figure 014: SEM micrograph.

009　010　011

012　013　014
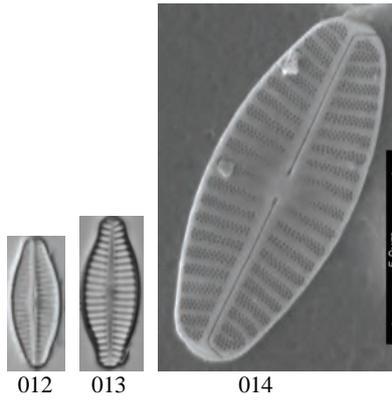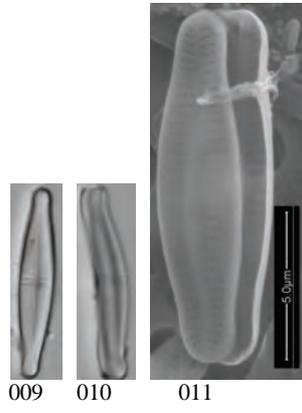
10.0 μm

Plate 02

Biraphideae.

Amphora coffeaeformis

Figure 015-017: *Amphora coffeaeformis* (Agardh) Kützing.

Fig. 016 - 016: LM micrographs.
Figure 017: SEM micrograph.

Amphora veneta

Figure 018-020: *Amphora veneta* Kützing.

Fig. 018 & 019: LM micrographs.
Fig. 20: SEM micrograph.

Hantzschia

Figure 021-023: *Hantzschia abundans* Lange-Bertalot

Fig. 021 & 021: LM micrographs.
Fig. 023: SEM micrograph.

The SEM micrograph shows interior of the valve.

015   016   017

018   019   020

021   022   023
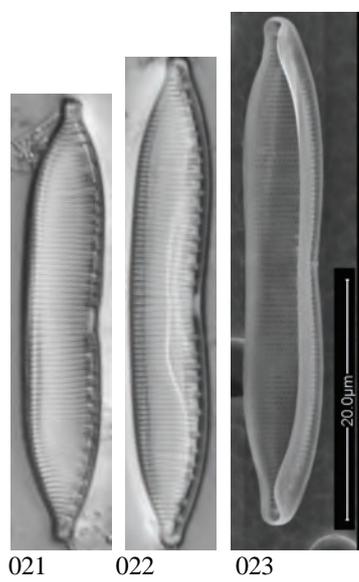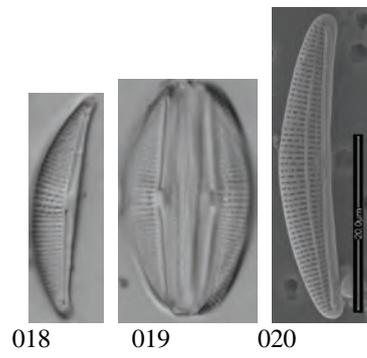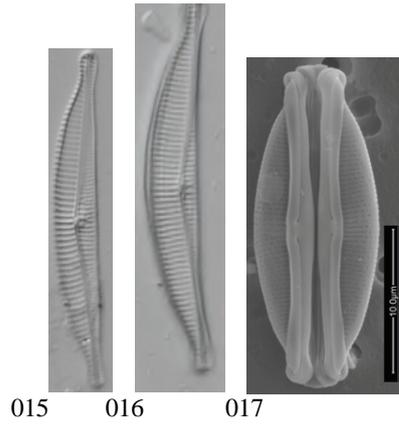
10.0 μm

Plate 03

Hippodonta

Figure 024-027: *Hippodonta* (?) species.

Fig. 024 - 026: LM micrographs.
Figure 027: SEM micrograph.

Navicula

Figure 028-031: *Navicula cincta* (Ehr.) Ralfs in Pritchard.

Fig. 028 - 030: LM micrographs.
Figure 031: SEM micrograph.

The SEM micrographs show both the interior and exterior valve views.

Figure 032-035: *Navicula* species

Fig. 032 - 034: LM micrographs.
Figure 035: SEM micrograph.

Figure 036-038: *Navicula veneta* Kützing.

Fig. 036 (?) - 037: LM micrographs.
Figure 038: SEM micrograph.

Navicymbula

Figure 039-043: *Navicymbula pusilla* Krammer

Fig. 039 - 041: LM micrographs.
Fig. 042 - 043: SEM micrograph.

024   025   026   027

028   029   030   031

032   033   034   035

036   037   038

039   040   041   042   043
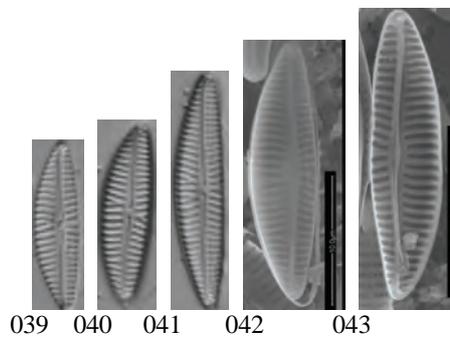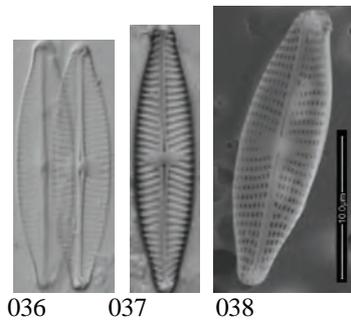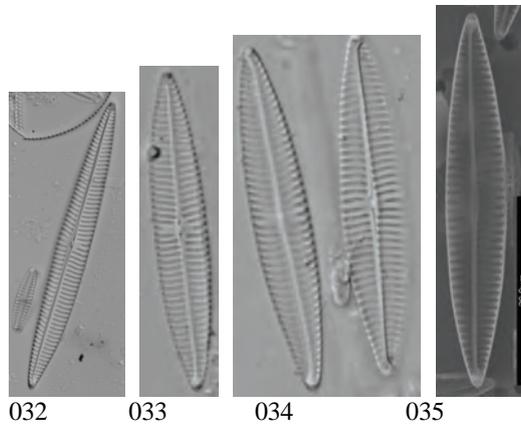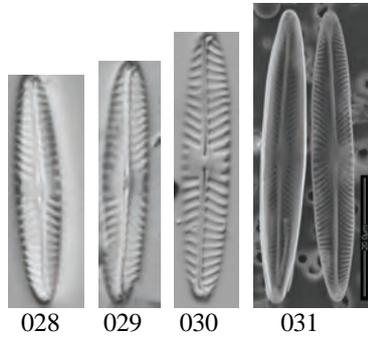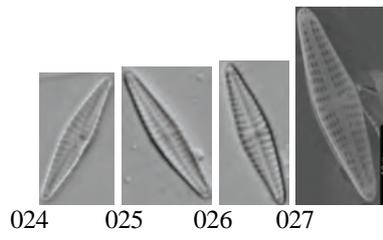
10.0 μm

Plate 04

Nitzschia

Figure 044-047: *Nitzschia* species

Fig. 044 – 046: LM micrographs.
Figure 047: SEM micrograph.

Figure 048-050: *Nitzschia archibaldii* Lange-Bertalot
Fig. 048- 050: LM micrographs.

Figure 051-054: *Nitzschia supralitorea* Lange-Bertalot

Fig. 051 - 053: LM micrographs.
Figure 054: SEM micrograph.

Pinnularia

Figure 055-058: *Pinnularia* species 1.
Fig. 055 - 056: LM micrographs.
Fig. 057 - 058: SEM micrograph.

The SEM micrographs show exterior valve views.

044   045   046   047

048   049   050

051   052   053   054

055 056   057   058

10.0 μm

131
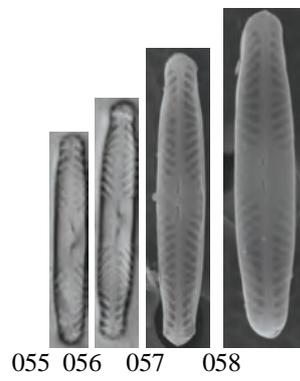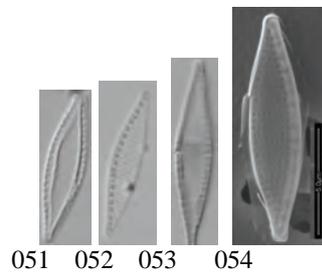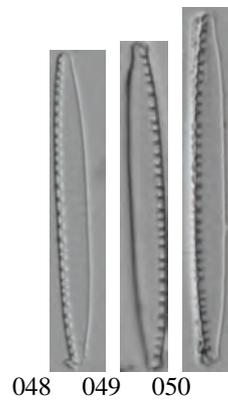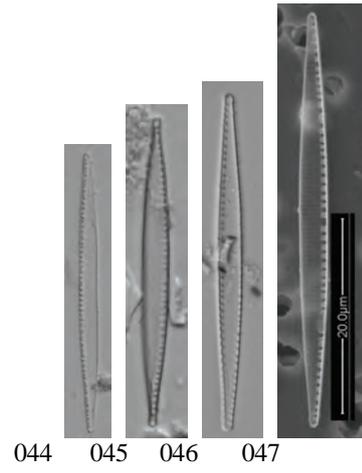
Pinnularia

Figure 059-061: *Pinnularia* species 2.

Fig. 059 - 061: LM micrographs.

Placoneis

Figure 062-065: *Placoneis* species.

Fig. 062 - 064: LM micrographs.
Figure 065: SEM micrograph.

Rhopalodia

Figure 066-069: *Rhopalodia gibberula* (Ehrenberg) O.Müller.

Fig. 066 - 067: LM micrographs.
Fig. 068 - 069: SEM micrograph.

059   060   061

062   063   064   065

066   067   068   069
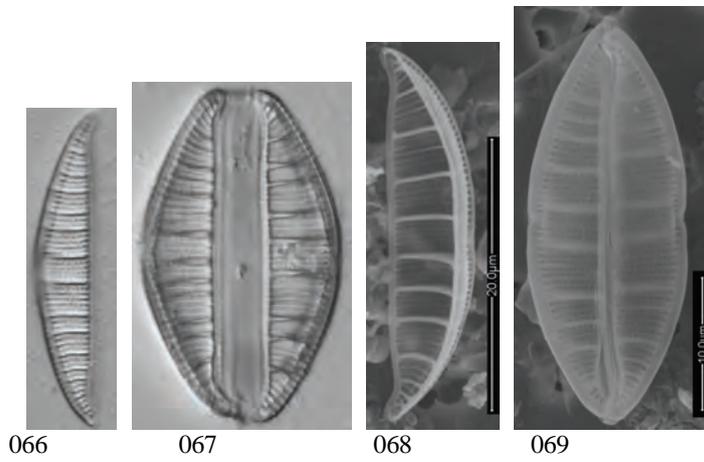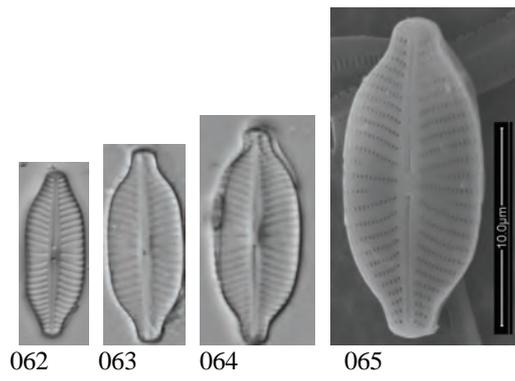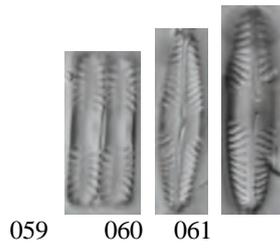
10.0 µm

Plate 06

Raphidioideae

Eunotia

Figure 070 - 073: *Eunotia* species 1 aff. *bilunaris*

Fig. 070 - 071: LM micrographs.
Fig. 077 - 073: SEM micrograph

Figure 074 - 075: *Eunotia* species 2

Fig. 074: LM micrographs.
Fig. 075: SEM micrograph

070   071   072   073   074   075
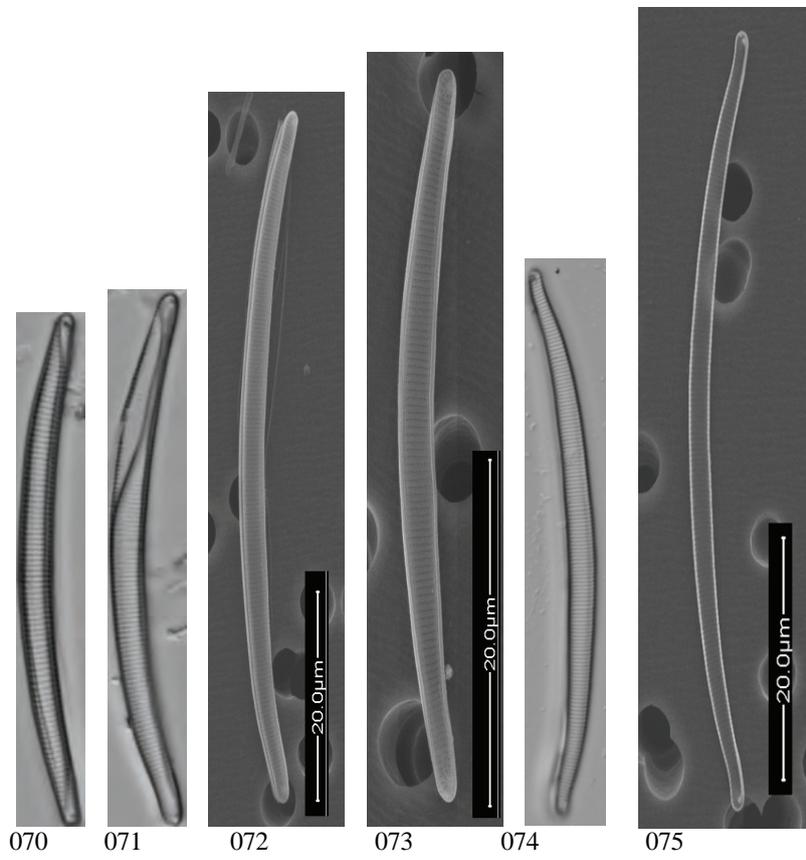
10.0 µm

Plate 07

Dominant species

Araphideae

Tabellaria

Figure 076-080: *Tabellaria flocculosa* (Roth) Kützing.

Fig. 076-079: LM micrographs.
Fig. 080: SEM micrograph of internal septum.

Figure 079 shows a single cell of *T. flocullosa* in girdle view.

076 077 078

079 080
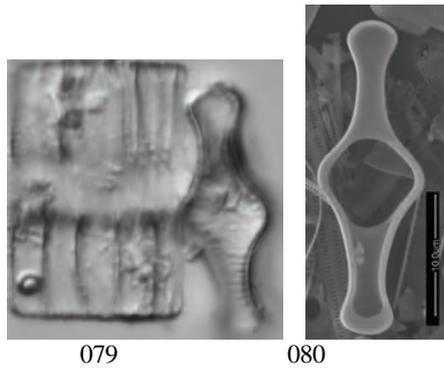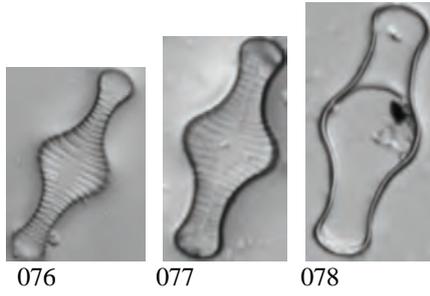
10.0 μm

Plate 08

Monoraphideae

Cocconeis

Figure 081-084: *Cocconeis placentula* var. *lineata* (Ehrenberg) Grunow.

Fig. 081-082: LM micrographs.

Fig. 083-084: SEM micrographs.

081 082
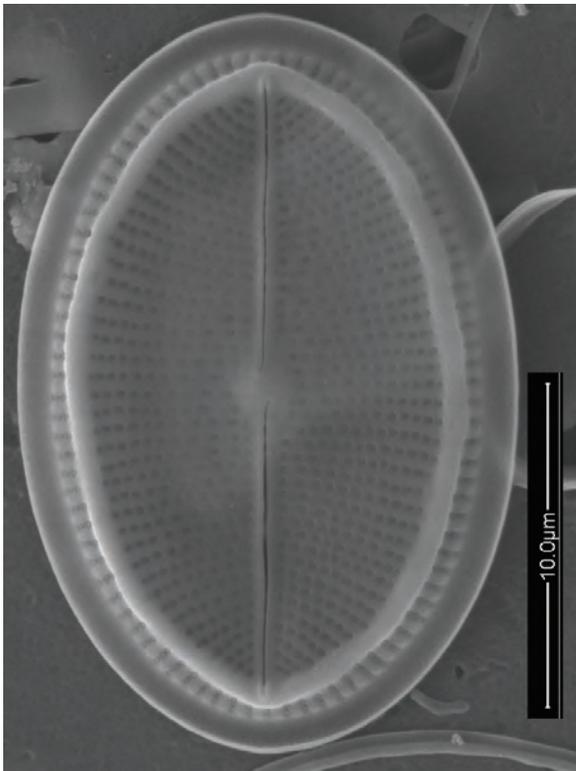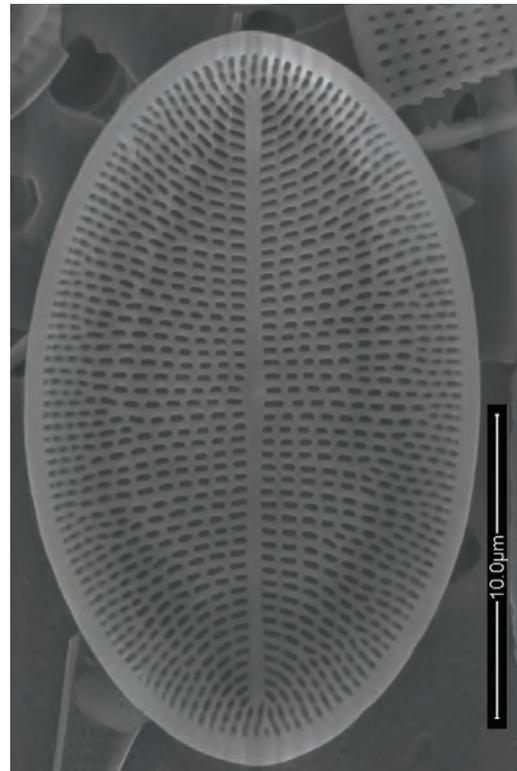
083 084

10.0 μm

Plate 09

Biraphideae

Denticula

Figure 084-087: *Denticula kuetzingii* Grunow

Fig. 084-085: LM micrographs.
Fig. 086-087: SEM micrographs.

Gomphonema

Figure 088-091: *Gomphonema parvulum* (Kützing).

Fig. 088-091: LM micrographs.

Nitzschia

Figure 092-094: *Nitzschia frustulum* (Kützing) Grunow.

Fig. 092-093: LM micrographs.

Fig. 094: SEM micrograph.

Figure 095: *Nitzschia palea* (Kützing) W. Smith.

Fig. 095: LM micrograph.

084   085   086   087

088   089   090   091

92   93   94

95
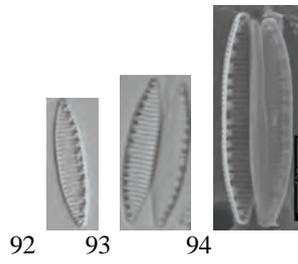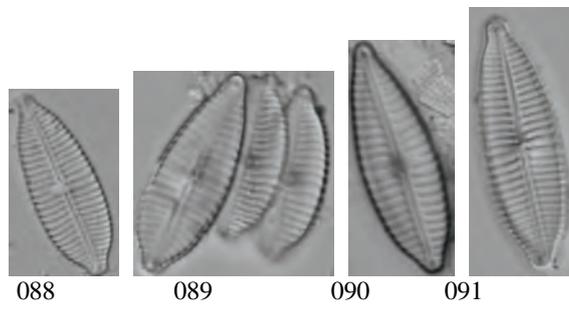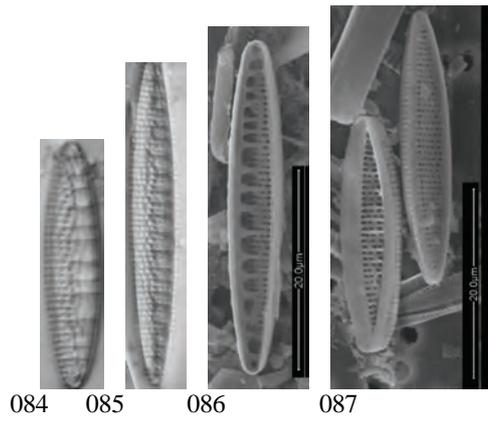
10.0 μm

Plate 10

Tryblionella

Figure 096-100: *Tryblionella apiculata* (Gregory).

Fig. 096-098: LM micrographs.
Fig. 099-100: SEM micrographs.

Plate 11

096   097   098   099   100
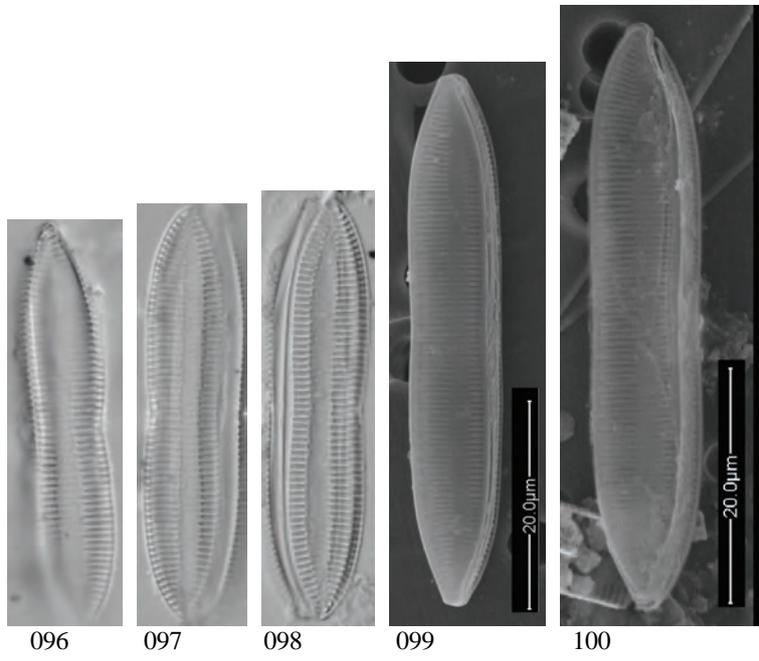
10.0 μm

Sub-dominant Species.

Centric Diatoms.

Aulacoseira

Figure 101-105: *Aulacoseira granulata* (Ehrenberg) Simonsen

Fig. 101-104: LM micrographs.
Figure 105: SEM micrograph.

Figure 106-109: *Aulacoseira ambigua* (Grunow) Simonsen

Fig. 106-107: LM micrographs.
Fig. 108-109: SEM micrographs.

Cyclotella

Figure 110-113: *Cyclotella meneghiniana* Kützing

Fig. 110-111: LM micrographs.

Fig. 112-113: SEM micrographs, with figure 126 showing the interior opening of the valve mantle and valve face fultoportulae.

101   102   103   104   105

106   107   108   109

110   111   112   113

10.0 μm

Plate 12

149
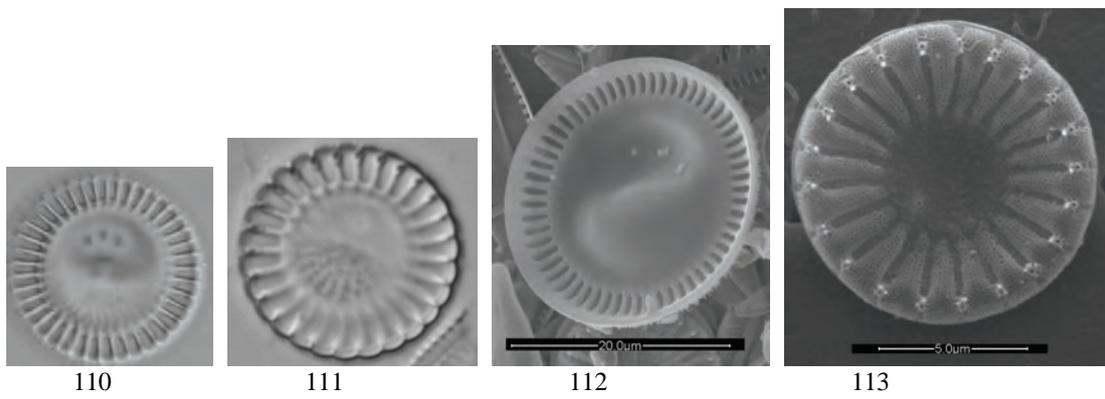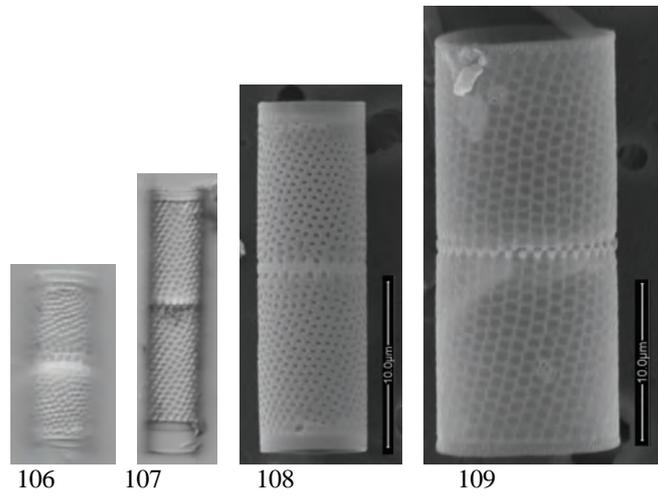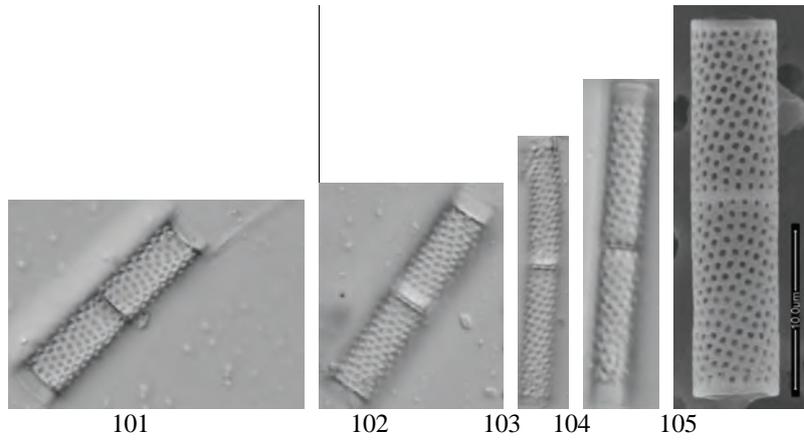
# Araphideae

Fragilaria

Figure 114-117: *Fragilaria germainii* Reichardt & Lange-Bertalot

Fig. 114-115: LM micrograph of *F. germainii*
Fig. 116-117: SEM micrographs of *F. germainii*.

Staurosira

Figure 118-121: *Staurosira elliptica* (Schumann) Williams & Round.

Fig. 118-119: LM micrographs.
Fig. 120-121: SEM micrographs.

Tabularia

Figure 122-125: *Tabularia fasciculata* (Agardh) Williams *&* Round.

Fig. 122-123: SEM micrographs.
Fig. 124-125: LM micrographs.

114 115 116 117

118 119 120 121

122 123 124 125
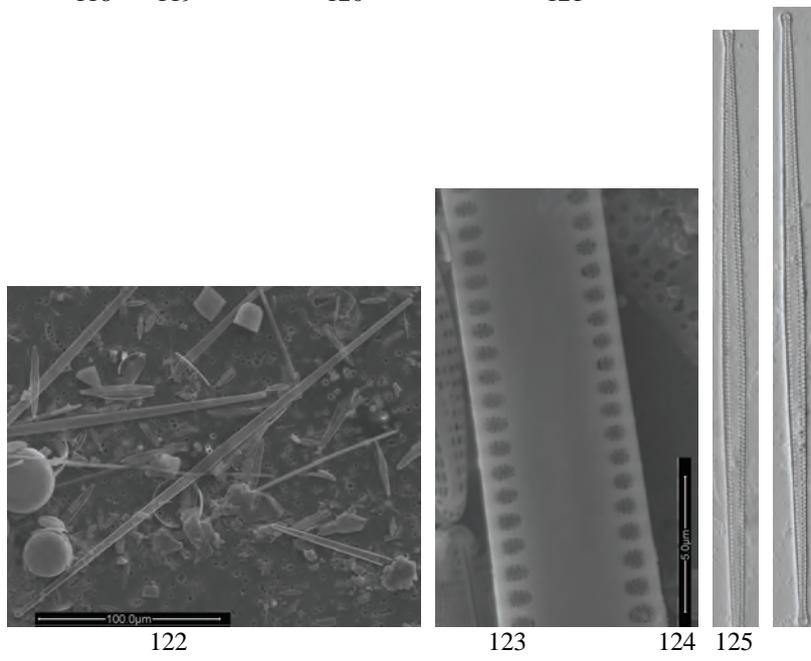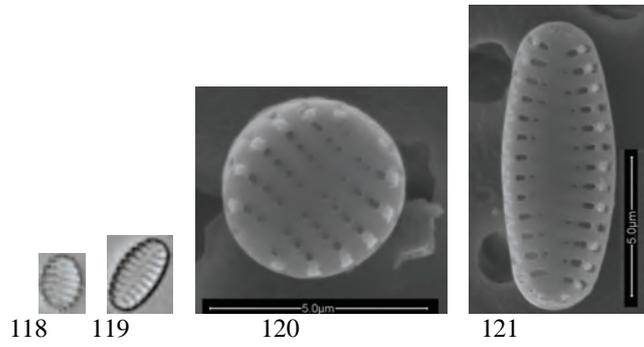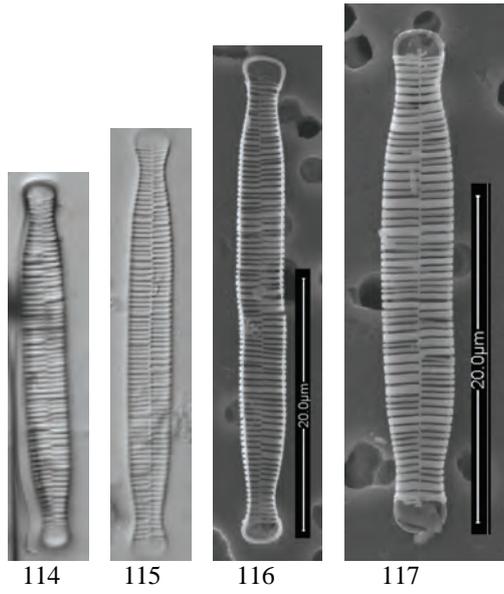
10.0 μm

Plate 13

Monoraphideae

Planothidium

Figure 126-128: *Planothidium* species (?)

Fig. 126: LM micrographs.
Fig. 127-128: SEM micrographs.

Cocconeis

Figure 129-131: *Cocconeis engelbrechtii* Cholnoky

 Figure 129: LM micrograph.
 Fig. 130-131: SEM micrographs.

Planothidium

Figure 132-134: *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot

Fig. 132-133: LM micrographs.
Fig. 134: SEM micrographs.

126   127   128

129   130   131

132   133   134
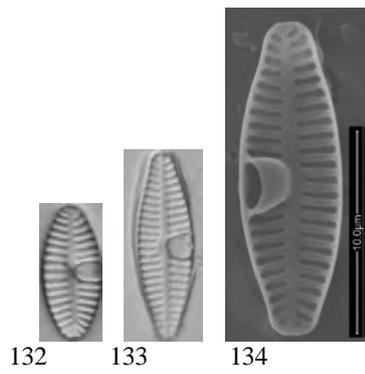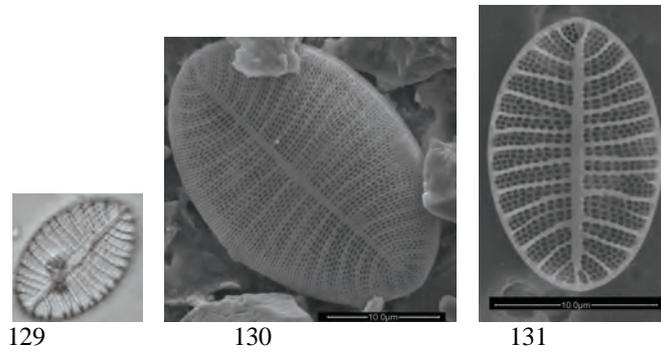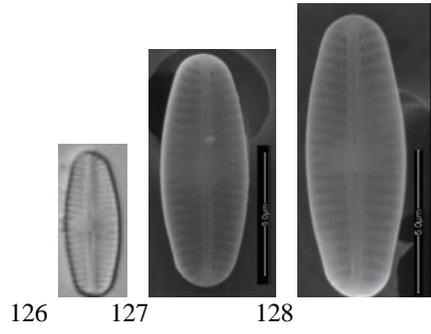
10.0 μm

Plate 14

Biraphideae

Brachysira

Figure 135-138: *Brachysira neoexilis* Lange-Bertalot.

Fig. 135-136: LM micrographs.
Fig. 137-138: SEM micrographs.

Craticula

Figure 139-141: *Craticula halophila* (Grunow ex Van Heurck) Mann.

Fig. 139: LM micrographs.
Fig. 140-141: SEM micrographs.

Cymbella

Figure 142-144: *Cymbella kappii* (Cholnoky) Cholnoky.

Fig. 142-144: LM micrographs.

Diploneis

Figure 145-148: *Diploneis elliptica* (Kützing) Cleve.

Fig. 145-146: LM micrographs.
Fig. 147-148: SEM micrographs.

135 136 137 138

139 140 141

142 143 144

145 146 147 148
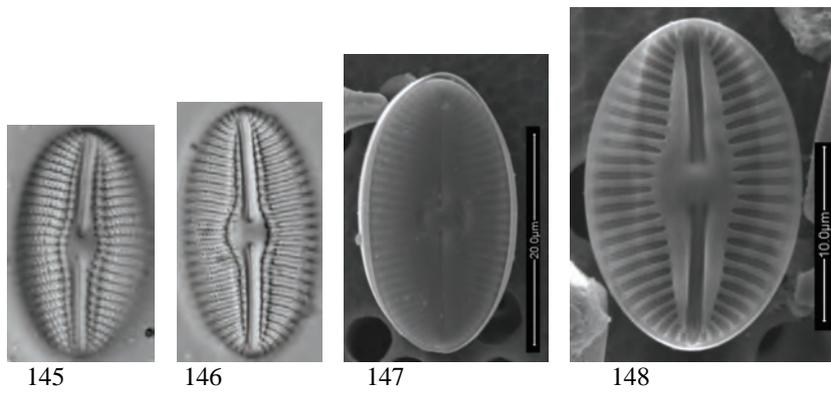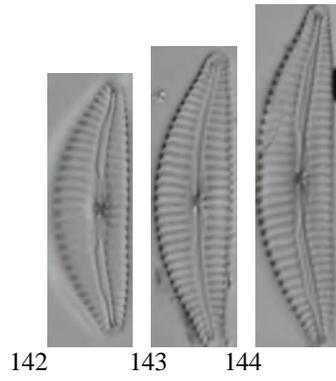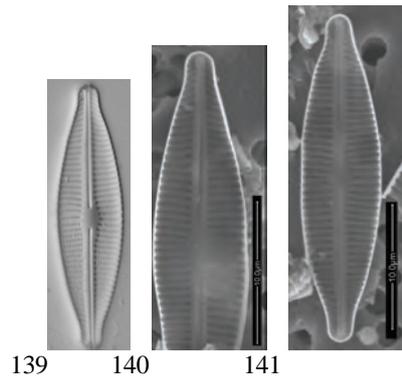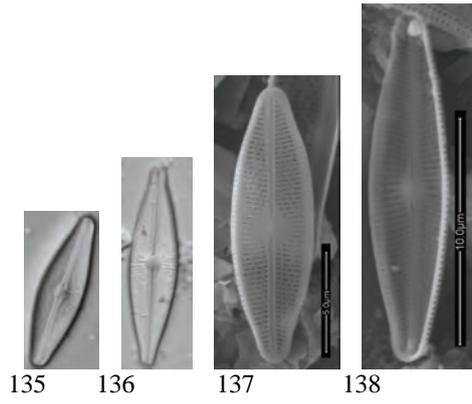
10.0 μm

Plate 15

Hantzschia

Figure 149-150: *Hantzschia* species.

Fig. 149: LM micrographs.
Figure 150: SEM micrograph.

Luticola

Figure 151-152: *Luticola mutica* (Kützing) D.G. Mann

Fig. 151: LM micrograph.
Figure 152: SEM micrographs.

Navicula
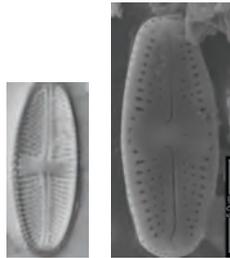
Figure 153-157: *Navicula subrhynchocephala* Hustedt.

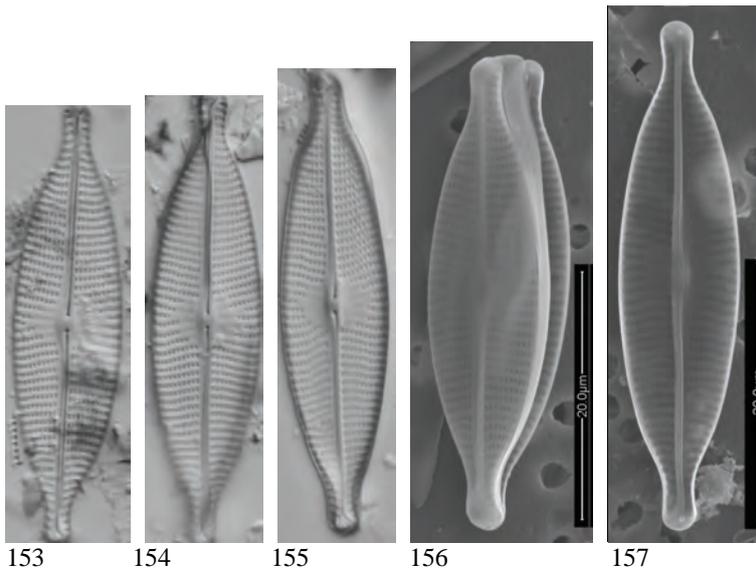Fig. 153-155: LM micrographs.
Fig. 156-157: SEM micrographs.
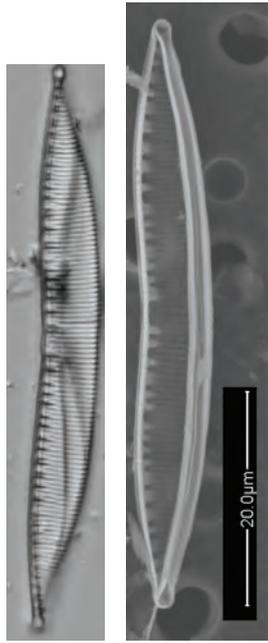
149   150

151   152

153   154   155   156   157

10.0 μm

Plate 16

157

Nitzschia

Figure 158-159: *Nitzschia amphibia* Grunow.

Fig. 158: LM micrograph.
Fig. 159: SEM micrograph.
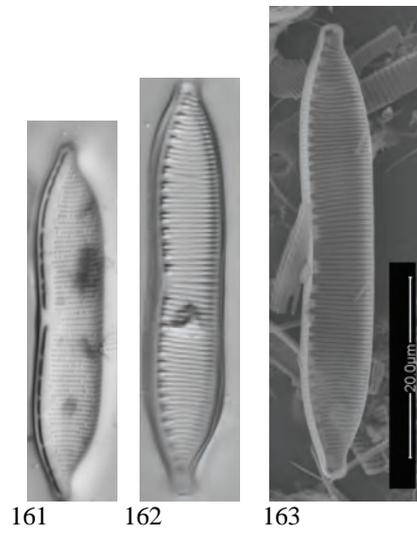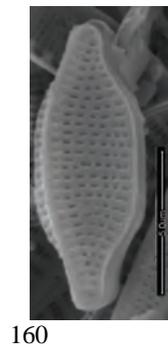

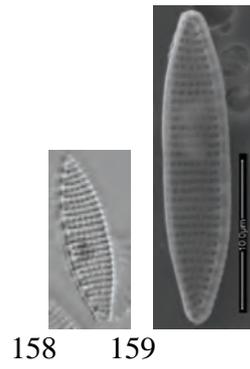**Figure 160:** *Nitzschia microcephala* Hustedt.

Fig. 160: SEM micrograph.

Hantzschia

Figure 161-163: *Hantzschia amphioxys* (Ehr.) Grunow sensu lato

Fig. 161-162: LM micrographs.
Fig. 163: SEM micrograph.

158   159

160

161   162   163

10.0 μm

Plate 17

Sellaphora

Figure 164-167: *Sellaphora pupula* (Kützing) Mereschkowksy sensu lato
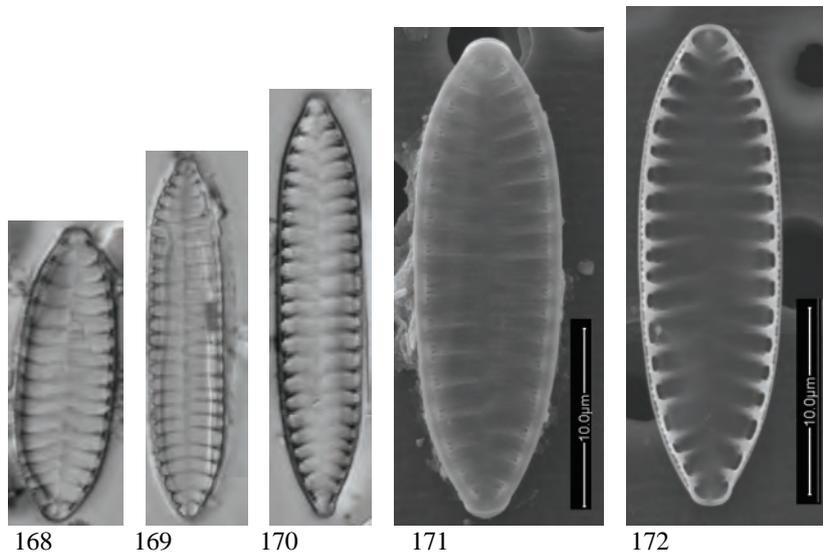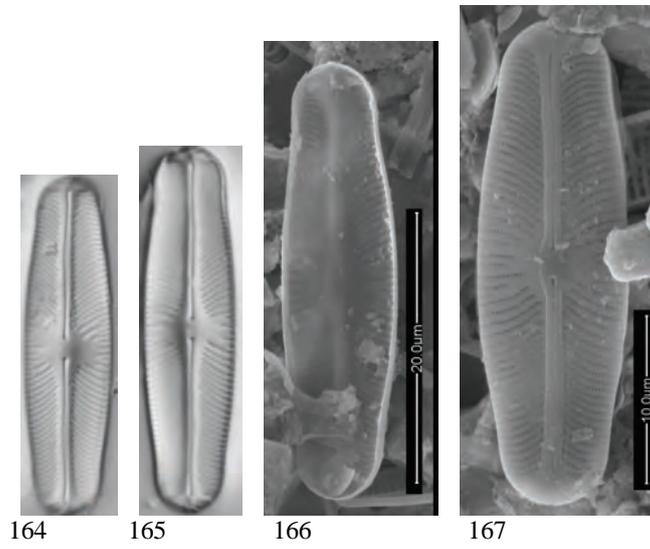
Fig. 164-165: LM micrographs.
Fig. 166-167: SEM micrographs.

Surirella

Figure 168-172: *Surirella angusta* Kutzing.

Fig. 168-170: LM micrographs.
Fig. 171-172: SEM micrographs

164   165   166   167
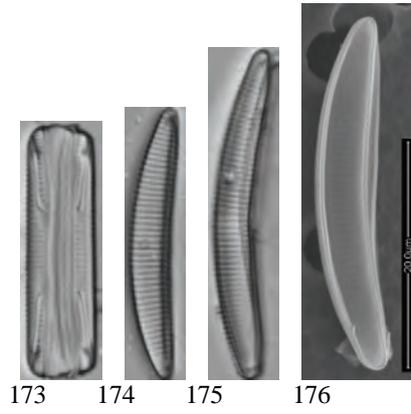
168   169   170   171   172

10.0 μm

Plate 18

Raphidioideae

Eunotia

Figure 173-176: *Eunotia bilunaris* (Ehr.) Mills

Fig. 173-174: LM micrographs, figure 173 shows the girdle view of *E. bilunari.*
Fig. 175-176: SEM micrographs.

173 174 175 176

10.0 μm

Plate 19

APPENDIX F:

Conference Contributions

The use of Diatoms for analysing water quality in wetlands, a South African perspective.

Malebo Matlala, Jonathan Taylor and Leon van Rensburg .

School of Environmental Sciences and Development, North West University,

Potchefstroom Campus.

Wetlands are an important water resource worldwide; they reduce floodwater velocity and therefore lessen the damage caused by floods, particularly erosion. Wetlands act as filters by trapping sediments, nutrients, and pollutants thereby improving the quality of the water.

Diatoms, which constitute approximately 40% of any algal community, are unicellular, occasionally filamentous algae of microscopic size, characterized by having a cell wall composed of silica. Diatoms are found throughout all aquatic habitats and communities of these organisms change in reaction to different environmental conditions such as eutrophication, salinisation and changes in pH. For this reason, the use of diatoms for bioassessment provides a valuable tool for inferring water quality.

Water quality is generally defined in terms of the chemical, physical and biological characteristics of water. In this project, these parameters will be determined and related to the structure of the diatom communities at particular sites. Samples will be collected around the country from both un-impacted and degraded wetlands of a lentic or lotic nature.

The main aim of the project is to formulate a diatom index for South African wetlands. This index is to be based on species commonly occurring in the examined wetlands, and the index will yield information on trophic status, levels of organic pollution, oxygen saturation, salinity and pH.

Keywords: *Diatoms, water quality, bioassessment, eutrophication, wetlands, diatom index.*

THE USE OF DIATOMS TO INDICATE THE QUALITY OF WATER IN WETLANDS, A SOUTH AFRICAN PERSPECTIVE

Malebo Matlala, Jonathan C. Taylor and Leon van Rensburg

School of Environmental Sciences and Development, North–West University, Potchefstroom Campus,

Potchefstroom 2520.

ABSTRACT

Wetlands have diverse ecological attributes and provide important ecosystem services such as water storage, biogeochemical cycling and maintenance of biodiversity and biotic productivity. Wetlands must be monitored in order to manage these resources in a sustainable manner so as to preserve ecological integrity. The use of diatoms for bioassessment provides a valuable tool for inferring water quality, based on the fact that diatoms are found throughout all aquatic habitats and their communities change in response to different environmental conditions such as eutrophication, organic enrichment, salinisation and changes in pH. A project, funded by the Water Research Commission, has been undertaken to examine diatom communities from these environments with the ultimate aim of formulating a diatom index for South African wetlands. This index is to be based on species commonly occurring in the examined wetlands, and the index will yield information on trophic status, levels of organic pollution, oxygen saturation, salinity and pH.

INTRODUCTION

In a semi- arid country with an average rainfall of 452 mm per year (1), increasing population numbers and an increasing evaporation rates, water in South Africa is a threatened resource. The national water act, number 36 of 1998, states that a water resource must be protected, conserved, managed and controlled in an equitable and sustainable manner for the benefit of all mankind. Thus to implement this act, our water resources must be monitored on a regular basis to maintain sustainable use and ensure their conservation (2).

The general quality of a resource can only be assessed by regular monitoring. Water quality is a term used to describe the physical, chemical, biological as well as the aesthetic properties of water (3). The physical properties of water include temperature and turbidity, and the chemical properties include the presence and concentration of dissolved salts, pH, and metals, biological criteria assess the response of living organisms to their non-living environment. The aesthetic properties of water will not be discussed in this paper. The quality of water in South Africa has most often been monitored using chemical and physical analyses, however these analyses reflect the general quality of the resource only at the time of sampling and, furthermore, these types of analyses are costly and time consuming.

These factors led therefore to the introduction of extensive biomonitoring programmes. Biomonitoring is a process whereby the ecological condition of a resource is studied by examining how the organisms living in a particular environment interact with their surroundings (4).

Wetlands are an important water resource worldwide; they reduce floodwater velocity and therefore lessen the damage caused by floods, particularly erosion. They act as filters by trapping sediments and nutrients, and in addition they also trap pollutants such as heavy metals and pesticides, thereby improving the quality of the water. Wetlands have diverse ecological attributes and provide important ecosystem services such as water storage, biogeochemical cycling and the maintenance of biodiversity and biotic productivity (6). The National water act states (2) that a wetland is that land which is transitional between a terrestrial and an aquatic system, where the water table is usually at or near the surface or where the land is periodically covered with shallow water and usually inhabited by hydrophytic vegetation.

Some of the biomonitoring techniques used in South African aquatic systems are SASS5, and MIRAI – macro-invertebrate response assessment indices, VEGRAI - riparian vegetation Response Assessment Index, IHAS -habitat integrity, FRAI – fish response assessment index, HAI- hydrology driver assessment index and GAI-geomorphology driver assessment index; these monitoring systems form part of the National Biomonitoring Programme for Aquatic Ecosystems (5). These biomonitoring techniques are mostly practiced in riverine systems where organisms such as fish and macro-invertebrates can be readily found and be used for FRAI and SASS5 respectively; however these biomonitoring techniques are usually not used in aquatic systems such as wetlands, where the environment is rather different. Many wetlands do not have a channelled bottom and the water may be dispersed in several discrete areas within the wetland. In addition water is usually shallow and may not be deep enough to support fish and other large organisms. In such an environment, it is difficult if not impossible to use techniques such as FRAI and SASS5. Therefore to study the water quality in South African wetlands, another monitoring technique is proposed.

Algae (including diatoms) and other micro-organisms attached to submerged surfaces occur in most shallow aquatic habitats where there is penetration of sufficient light. In most wetlands, these aggregations of algae known as periphyton grow attached to submerged substrata such as sediment, woody and herbaceous plants and rocky substrata. Because of their high dispersal rates, rapid growth rate and their direct response to environmental changes, algae provide the first indication of changes and are thus one of the most widely used indicators of biological integrity and physico-chemical conditions in aquatic ecosystems.

This present study proposes the use of diatoms to indicate water quality in wetlands. Diatoms, which constitute approximately 40% of any algal community, are unicellular, occasionally filamentous algae belonging to the group Bacillariophyceae, which are characterized by having a cell wall composed of silica (7).

These microscopic organisms are found throughout most aquatic, sub-aerial and terrestrial habitats. Their communities react rapidly and specifically to changes in environmental conditions such as eutrophication, organic enrichment, salinisation and changes in pH (8). Diatom community structures can be used to study current water quality as well as historical conditions (9; 10). These organisms are very easy to sample and permanent records can be made from each sample collected. They differ from fish and macro-invertebrates in that, in general, they do not need any specialised food, habitat, depth or velocity of water (11), and they occur anywhere where there is water. For these reasons, the use of diatoms for bioassessment in wetlands may provide a valuable tool for inferring water quality.

As micro-organisms they lack dispersal barriers (12), and may be transported by wind, aerosols, by wading birds and may even survive passage through insect's digestive tracts. Many hundreds of thousands of cells may be produced within a few square centimetres of a wetland environment and this adds to the ease with which they are dispersed. The implication of this is that in an area with more or less homogenous water quality will have more or less homogenous diatom communities, i.e. even if discrete pools within a wetland are spatially dispersed but still share similar water quality, a single diatom sample from any one of the pools should provide an adequate reflection of the water quality in the wetland as a whole. It should also be borne in mind, as stated above, that diatoms may inhabit all ecosystems from aquatic, to moist sub-aerial to terrestrial. Thus, they may be used as indicators even in dry wetlands.

As diatoms cell walls are composed mostly of silica they can remain preserved for thousands of years. These preserved cell walls or frustules when removed in a core from the sediment may be used to trace the history of a wetland. The persistence of diatoms in sediments, even when wetlands are dry, may provide a year round approach for assessing the ecological integrity of wetlands when other organisms are not present. Furthermore, their rapid growth rates enable experimental manipulation of environmental conditions to determine cause-effect relationships between diatomic response and specific environmental stressors (10).

The ultimate aim of the project, of which this preliminary study forms a part, is to formulate a diatom index for South African wetlands. The index is to be based on species commonly occurring in the examined wetlands, and will yield information on trophic status, levels of organic pollution, oxygen saturation, salinity and pH.

This index will then be used to assess the general quality or health of South African Wetlands. The present paper reviews methodology used in the study and preliminary results.

MATERIALS AND METHODS

Sample collection

All water quality and diatom samples were collected from a palustrine wetland in the farming district of Ventersdorp in the North-West province. Samples were collected throughout 2007 on a monthly basis from two different localities, the first being a Department of Water Affairs and Forestry (DWAF) site, access to which is granted at DWAF's discretion, thus there is very limited human activity around this site, however, cattle have free access to the site. The second locality (site 2-4), known as the Schoonspruit wetland, is on a private farm. Direct impacts on this area are also mostly limited to cattle grazing. Samples were collected from three spatially discrete sites within the wetland at this locality, the first of which is formed by a spring known as the "Schoonspruit eye". These two localities were chosen for a preliminary study because they were easily accessible and have limited anthropogenic influence.

Samples were collected from submerged substrata including rocks and from the submerged stems of living *Phragmites australis* (reeds) in a radius of 10 m2. Dead, floating reeds were avoided since their duration at the sampling site was not known. About five to ten rocks or reeds were collected randomly around the sampling area; these were then vigorously brushed with a toothbrush to remove diatom material. The material was then suspended in a small amount of water and placed in a sampling bottle and taken to the laboratory for processing and analysis. Physico-chemical analysis: Physical and chemical parameters, including pH, temperature, conductivity and dissolved oxygen were measured at each site using a hand held Cyberscan meter. Samples collected for additional chemical analysis were kept in a refrigerator until processed.

Additional chemical parameters were analysed with a Palintest photometer 8000, following the methods outlined in the photometer instruction manual. Variables measured included ammonia, chloride, phosphate, potassium, nitrite, nitrate, sulphate and total hardness.

Diatom Sample Preparation

The diatom samples were removed from the refrigerator, the supernatant was discarded and the remaining sample was thoroughly mixed. 2 ml of the sample was placed in a clean test tube, and an equal amount of potassium permanganate was added to the sample, the solution was then left overnight for the potassium permanganate to oxidise all the organic material present in the sample. After twenty-four hours, 2 ml of hydrochloric acid were then added to the test tubes.

The test tubes were then placed in a beaker with water and the beakers were placed on a hot plate to speed up the process of the digestion of potassium permanganate by the hydrochloric acid. The solutions were heated (~90°C) until they cleared to a straw yellow colour. To check for the presence

of any organic material or the presence of potassium permanganate, a few drops of hydrogen peroxide were added, with a vigorous reaction indicating the presence of either.

The samples were then left to cool to room temperature before they were centrifuged. After cooling the samples were transferred into centrifuge tubes and the solutions were centrifuged for ten minutes at a speed of 2500 rpm. After centrifuging for 10 minutes the supernatant was discarded and the pellets were rinsed with distilled water and the process of centrifuging and rinsing was repeated four times.

At the completion of the centrifugation process, ~2 ml of the sample material was placed in a clean test tube, in the same test tube with the sample solution, two drops of 10% ammonium chloride were added and a few drops of distilled water. The function of the ammonium chloride is to neutralize surface charges on the particles of dirt on the diatoms allowing them to spread evenly on the cover slip. The solution was placed on a cleaned cover slip and allowed to dry out overnight.

The dried cover slips were then placed on a hot plate (~350°C) to sublimate the ammonium chloride, thereafter they were checked under the microscope for the presence of diatoms. When present, the slides were then mounted using Pleurax (r.i. 1.73). If there were no diatoms observed, the process was repeated until at least twenty five diatoms could be counted per field of view under 400x magnification. To mount the slides, the cover slips were placed on a hot plate at a low heat, a drop of Pleurax was then placed on the cover slip and allowed to settle, a clean slide was then placed on top of the cover slip and carefully inverted, the Pleurax was heated and when cured, the slide was removed from the hot plate and cooled.

Counting and data processing

Diatom cells were counted under a Nikon 80i light microscope equipped with a 100x 1.4 N.A. objective lens and DIC optics according to a standard protocol (13). Diatoms were identified to species and form level (14). The count data was uploaded into OMNIDIA 4.2 and the Specific Pollution sensitivity Index score or SPI (15) was calculated. This index was deemed suitable for water quality indication as it has a very broad species base, including several thousand species, and has also been demonstrated to be able to successfully indicate water quality in South African rivers (16; 17; 18).

RESULTS

Table 1. Chemical environmental variables measured at the sampling sites (29/05/2007).

	Unit	Site 1 DWAF gauging weir	Site 2 Schoonspruit Eye	Site 3 Schoonspruit Eye	Site 4 Schoonspruit Eye
Ammonium (NH_4-N)	mg.l^{-1}	0.07	0.04	0.07	0.05
Chloride (Cl^{-})	mg.l^{-1}	4.00	2.50	2.10	3.80
Conductivity	µS/cm	455	648	590	518
Dissolved oxygen	%	84.50	55.30	38.70	113.70
pH	-	7.12	7.85	6.92	7.96
Phosphate (PO_4-P)	mg.l^{-1}	0.03	0.10	0.06	0.10
Potassium (K^{+})	mg.l^{-1}	9.50	3.60	5.00	9.60
Nitrate (NO_3^+-N)	mg.l^{-1}	0.04	0.15	0.05	0.12
Nitrite (NO_2^+-N)	mg.l^{-1}	0.01	0.01	0.00	0.00
Sulphate (SO_4^{2-})	mg.l^{-1}	4.00	3.00	3.00	2.00
Total Hardness ($CaCO_3$)	mg.l^{-1}	16.00	14.00	8.00	14.00

Table 2. Relative abundance of the dominant diatom species and index scores calculated for each site

	Domininat taxa >2%	% Abundance	SPI Score	% sp. Incl in SPI calculation
Site 1	Achnanthidium pyrenaicum (Hustedt) Kobayasi	60	18.3	82
	Cocconeis placentula var.euglypta (Ehr.) Grunow	11		
	Achnanthidium minutissimum (Kützing) Czarnecki	9		
	Encyonopsis minuta Krammer & Reichardt	6		
	Encyonopsis krammeri Reichardt	4		
	Epithemia adnata (Kützing) Brébisson	3		
	Cymbella kappii (Chonoky) Cholnoky	2		
Site 2	Achnanthidium pyrenaicum (Hustedt) Kobayasi	74	17.8	93
	Amphora pediculus (Kutzing) Grunow	9		
	Eolimna minima(Grunow) Lange-Bertalot	5		
	Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	4		
	Achnanthidium minutissimum (Kützing) Czarnecki	3		
Site 3	Achnanthidium pyrenaicum (Hustedt) Kobayasi	46	17.5	81
	Achnanthidium minutissimum (Kützing) Czarnecki	13		
	Denticula kuetzingii Grunow	13		
	Encyonopsis krammeri Reichardt	10		
	Navicula microcari Lange-Bertalot	2		
	Eolimna minima (Grunow) Lange-Bertalot	2		
Site 4	Achnanthidium pyrenaicum (Hustedt) Kobayasi	60	16.2	97
	Amphora pediculus (Kützing) Grunow	10		
	Eolimna minima (Grunow) Lange-Bertalot	6		
	Caloneis bacillum (Grunow) Cleve	4		
	Achnanthidium minutissimum (Kützing) Czarnecki	2		

Table 3. Interpretation of diatom index scores

Interpretation of index scores	
Index score	Class
>17	high quality
13 to 17	good quality
9 to 13	moderate quality
5 to 9	poor quality
<5	bad quality

DISCUSSION

The values outlined in Table 1 represent the chemical water quality properties of the wetlands at the time of sampling (29/05/2007). The dominant diatom species as well as diatom index scores are presented in Table 2. The interpretation of the index scores is according to Table 3.

The first and most important result is, simply, that diatoms were found in each of the samples collected, regardless of substratum. Availability of the indicator organism is the key factor in developing successful methods and indices for reflection biological integrity. As diatoms are not limited by the availability of different habitats, season and food availability and are found under all hydrological conditions, they show good potential for use as bioindicators of wetland biotic integrity (19).

All these four sites harboured similar diatom communities, with *Achnanthidium pyrenaicum* (Hustedt) Kobayasi and *Achnanthidium minutissimum* (Kützing) Czarnecki dominant (> 2%; Table 2), at all four sampling sites.

Other commonly occurring diatoms included; *Amphora pediculus* (Kützing) Grunow, *Encyonopsis minuta* Krammer & Reichardt, *Encyonopsis krammeri* Reichardt, *Epithemia adnata* (Kützing) Brébisson, *Eolimna minima* (Grunow) Lange-Bertalot, *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot, *Denticula kuetzingii* Grunow. Most of these diatoms were found at all the sites but were not necessarily dominant. All of these taxa are common to riverine environments and no specialist wetland taxa, if such taxa exist, were found.

Diatom index scores were calculated for the Specific Pollution sensitivity Index (SPI; (15). The scores, when interpreted with the aid of table 3, showed that the first three sites fell into the category "high quality", while site four was classified as "good quality". It should be noted that site 2 and 3 were at the bottom end of the range for classification as high quality. This result is in general borne out by the concentration of the chemical water quality variables (Table 1). Most chemical parameters were

low /acceptable (with the exception of phosphate) when compared to the DWAF's water quality guidelines (3).

The percentage of species included in the calculation is also indicated in Table 2. This is very important quality control measure; if only a few species were used for the calculation the result may be doubtful. Conductivity was moderately elevated at all of the sites (Table 1) and thus the dominance of species such as *Achnanthidium pyrenaicum*, *Denticula kuetzingii, Encyonopsis minuta, Epithemia adnata* etc. all of which are found in good quality waters with moderate to elevated electrolyte content (~500 µS/cm). *Planothidium frequentissimum* is also found in such waters; the abundance at which this species was found in the investigated sites gives an indication of slightly elevated levels of nutrients. These nutrients may result from intermittent influx of organic material from cattle or simply from decaying vegetation.

CONCLUSIONS

The use of diatoms as indicators of water quality in riverine ecosystems has been substantiated by a number of studies worldwide, these organisms have found to be good indicators of water quality, with specific reference to eutrophication (20). Several studies on wetlands have also shown that diatoms respond to ecosystem alterations and that diatom community composition shows strong correlations to changes in physical and chemical parameters (21).

Conclusions which may be drawn from the present study are that:
- Diatoms do occur in wetlands
- Samples may be easily collected from these environments and,
- Diatom community structure seems to be dictated by the prevailing physical and chemical water quality.
- The structure of these communities can then in turn be used to infer water quality.

As the project continues however, there are several questions which will need to be answered: Will diatom species have the same tolerances to environmental variables in wetlands as in rivers, and, to what extent will the diatoms accurately reflect water quality in wetlands after empirical testing?

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