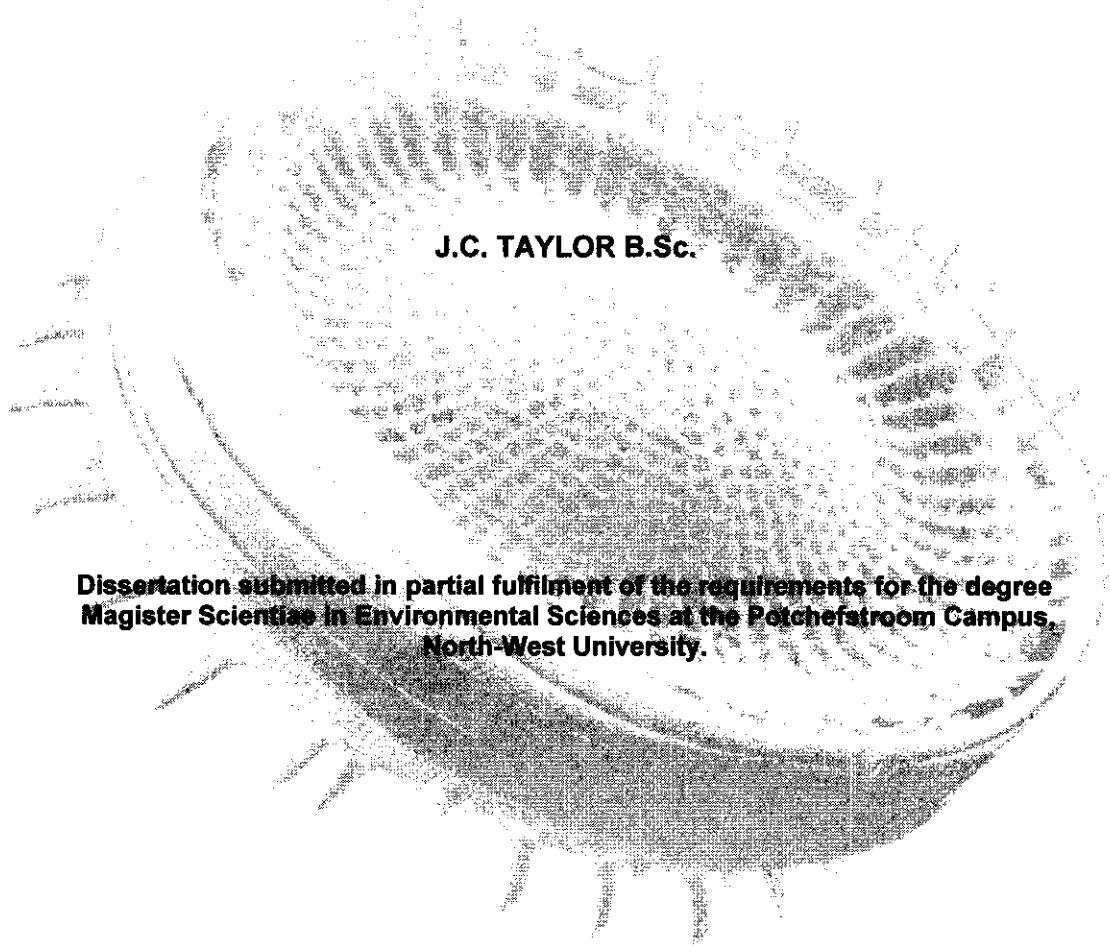
THE APPLICATION OF DIATOM-BASED POLLUTION INDICES IN THE VAAL CATCHMENT



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ABSTRACT

South Africa is a semi arid country and the provision of water clean water to a steadily growing population is currently one of the major challenges facing governmental organisations. Water resources in South Africa are subject to many forms of pollution, resulting in eutrophication and salinisation. Hence, there is a need to monitor chemical and organic pollution in South African rivers.

Chemical monitoring is expensive and not all the elements of water quality can be monitored and measured in a particular sample. The synergistic effects of water quality determinants cannot be demonstrated if only the chemical composition of a water resource is monitored. Biological monitoring can provide a rapid indication of water quality and at a lower cost than traditional monitoring. Organisms within a river are exposed to all water quality variables present in a system and can provide an integrated reflection of the health of their environment.

Diatoms are found in all aquatic ecosystems and have demonstrable responses to many of the elements of water quality that have been identified as causing aquatic pollution. These elements include total dissolved solids, pH and plant nutrients such as nitrates and phosphates. The relationship between the structure of a given diatom community and the water quality to which the community is exposed, has lead to the development of several indices of water quality. Diatom indices of aquatic pollution have been developed in France, Belgium, Germany, Britain and Japan. Existing diatom indices have been tested for use in Finland, Poland, Britain, the Himalayas and South America.

Several diatom indices were tested in this study for application in the Vaal and Wilge Rivers. The tested diatom indices correlated well with measured water quality variables such as pH and the chemical variables responsible for eutrophication and salinisation. The demonstrated correlations were comparable to those demonstrated by European authors. Several indices proved successful in indicating general water quality, namely the Biological Diatom Index (BDI), the Specific Pollution sensitivity Index (SPI) and the Generic Diatom Index (GDI). The Eutrophication and Pollution Index (EPI) successfully indicated levels of plant nutrients together with the ionic composition measured at various sites in the Vaal and Wilge Rivers.

It is recommended that these indices be further tested in different regions within South Africa.

KEY WORDS: BIO-INDICATOR, DIATOM, DIATOM INDICES, EUTROPHICATION, SALINISATION, WATER QUALITY

DIE TOEPASSING VAN DIATOOM-GEBASEERDE INDEKSE IN DIE VAAL-OPVANGGEBIED

OPSOMMING

Suid-Afrika is 'n semi-ariede land met 'n lae jaarlikse reënval en die voorsiening van skoon water aan 'n steeds-groeiede bevolking is tans een van die groot uitdagings waarvoor staatsinstansies te staan kom. Suid-Afrikaanse waterbronne is onderhewig aan besoedeling, wat tot eutrofikasie en versouting lei. Daar bestaan dus 'n behoefte om chemiese en organiese besoedeling in Suid-Afrika se riviere te moniteer.

Chemiese monitering is duur en alle faktore betrokke by die kwaliteit van water kan nie in een bepaalde watermonster gemeet word nie. Dit is nie moontlik om die sinergistiese uitwerking van waterkwaliteitdeterminante te bepaal as slegs die chemiese samestelling van water gemoniteer word nie. Deur biologiese monitering, daarenteen, kan 'n vinnige oorsig oor waterkwaliteit, teen 'n laer koste as tradisionele moniteringsprosesse, verkry word. Organismes in 'n rivier word aan al die waterkwaliteitsveranderlikes teenwoordig in die sisteem blootgestel en weerspieël dus die totale vitaliteit van die omgewing akkuraat.

Diatome kom in alle akwatiese ekosisteme voor en hulle reageer meetbaar ten opsigte van baie van die elemente wat verantwoordelik is vir waterbesoedeling. Hierdie elemente sluit die totale opgeloste soute, pH en plantvoedingstowwe, soos nitrate en fosfate, in. Die verwantskap tussen die samestelling van 'n gegewe diatoomgemeenskap en die waterkwaliteit waaraan die gemeenskap blootgestel is, het tot die ontwikkeling van verskeie waterkwaliteitsindekse, gebaseer op diatoomgemeenskappe, gelei. Diatoomindekse van waterbesoedeling is in Frankryk, België, Duitsland, Brittanje en Japan ontwikkel. Bestaande diatoomindekse se toepasbaarheid is in Finland, Pole, die Himalayas en Suid-Amerika getoets.

Verskeie diatoomindekse is gedurende hierdie studie vir aanwending in die Vaal- en Wilgeriviere getoets. Hierdie diatoomindekse het goed met die waterkwaliteitsveranderlikes soos pH en chemiese veranderlikes, verantwoordelik vir eutrofikasie en versouting, gekorreleer. Die korellasies was vergelykbaar met dié wat deur Europese outeurs verkry is. Verskeie indekse, naamlik die Biologiese diatoomindeks (BDI), die Spesifieke Besoedelingsensitiwiteitsindeks (SPI) en die Diatoomgenusindeks (GDI), het die algemene waterkwaliteit akkuraat weergegee. Die Eutrofikasie- en Besoedelingsindeks (EPI) het die konsentrasies van plantvoedingstowwe, asook die ioonkonsentrasie, gemeet by verskillende plekke in die Vaal- en Wilgeriviere, akkuraat weergegee.

Daar word aanbeveel dat hierdie indekse verder getoets word in ander streke in Suid-Afrika.

SLEUTELWOORDE: BIO-INDIKATOR, DIATOOM, DIATOME, DIATOOMINDEKSE, EUTROFIKASIE, VERSOUTING, WATERKWALITEIT

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"You are worthy, O Lord to receive glory and honour and power; for You created all things, and by Your will they exist and were created."

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ABBREVIATIONS

APDI - Artois-Picardie Diatom Index (Prygiel *et al.*, 1996)

BDI - Biological Diatom Index (Lenoir & Coste, 1996)

BOD_5 – Biological Oxygen Demand in five days

CEC - Commission of Economical Community Index (Descy & Coste, 1991)

DEAT – Department of Environmental Affairs and Tourism

DES - Descy's index (Descy, 1979)

DIN – Dissolved Inorganic Nitrogen

DIP – Dissolved Inorganic Phosphorus

DO – Dissolved Oxygen

DWAF – Department of Water Affairs and Forestry

EC – Electrical Conductivity

FHI – Fish Health Index

GDI - Generic Diatom Index (Coste & Ayphassorho, 1991)

LHWP – Lesotho Highlands Water Project

LM – Light microscopy

LMI - Leclercq & Maquet's Index (Leclercq & Maquet, 1987)

NBPAE – National Biomonitoring Programme for Aquatic Ecosystems

NIWR - National Institute for Water Research

OECD - Organisation for Economic Co-Operation And Development

SASS – South African Scoring System

SEM – Scanning Electron Microscopy

SHE - Schiefele and Schreiner's index (Schiefele & Schreiner, 1991)

SLA - Slàdeček's index (Slàdeček, 1986),

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

TDI - Trophic Diatom Index (Kelly & Whitton, 1995)

TDS – Total Dissolved Solids

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

WRC – Water Research Commission

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CHAPTER 1

Introduction

1.1 Water – A limiting resource in the development of South Africa

South Africa has long recognised that water is one of its prime limiting natural resources (DWAF, 1986; Huntley et al., *1987).*

Southern Africa is a subcontinent notorious for its unpredictable rainfall brought about by the extreme variability of climatic pattern and the generally high to very high evaporation rates across the region (Snaddon *et al.*, 2000). South Africa is a semi-arid country and receives only half the annual average rainfall of other countries in the region (500 mm yr^{-1}), and this is spread disproportionately across the country from east to west. Water availability now and in the future is heavily dependant on climate, water use and management and land-use practices (Walmsley *et al.*, 1999). South Africa's available freshwater resources are already almost fully utilised and under stress. At the projected population growth and economic development rates, it is unlikely that the projected demand on water resources in South Africa will be sustainable. Water will increasingly become the limiting resource in South Africa, and supply will become a major restriction to the future socio-economic development of the country, in terms of both the amount of water available and the quality of that which is available. At the present many water resources are polluted (changed from the natural condition) by industrial effluents, domestic and commercial sewage, as well as mine drainage, agricultural runoff and litter (Hohls *et al.*, 2002). These polluted water resources need to be constantly and effectively monitored by both the polluter (e.g. mining companies) and regulatory bodies such as the Department of Water Affairs and Forestry (DWAF) using cost effective accurate assessments based on both chemical and biological investigations of water quality.

The Vaal River catchment basin can be regarded as the most important water supply region in the country. The immense mineral wealth of the Vaal River supply area resulted in a concentration of economic activities in the area. Economic activities are dominated by the Gauteng Province, which contains the main metropolitan and mining complexes in South Africa. The world's richest gold and platinum mines are located on the West Rand (Gauteng), in the North West, Mpumalanga and Free State provinces. High-grade coal is utilised in, and exported, from the Vaal River catchment area earning valuable foreign exchange for South Africa along with gold and diamonds. An area of about 62 000 ha is also being irrigated directly from the river. The water required to generate the major part of South

Africa's electricity supply and to manufacture a large part of its synthetic fuel is also being supplied from the Vaal River system (DWAF, 1993).

The Vaal River rises on the western slopes of the Drakensberg escarpment in the vicinity of Lake Chrissie near Breyten and flows into the Orange River near Douglas after 1 200 km. The latter river flows into the Atlantic Ocean at Alexander Bay. The Vaal River has a large number of tributaries of which the main ones are the following: the Little Vaal, Klip (Upper Vaal), Watervals, Wilge, Suikerbosrand, Klip (Barrage catchment), Mooi, Renoster, Vals, Vet, Harts and Riet Rivers and Schoonspruit. Although the Vaal River has a catchment area of 196 290 km^2 and is one of the major water suppliers in the country, its annual runoff is only 8% (4300 m^3/a) of South Africa's total annual runoff (DWAF, 1993; DWAF, 2000)

In the following paragraphs brief mention will be made of the changes in water quality in the Vaal River, as a preamble to the central theme of this investigation, namely using diatom community associations as a biomonitoring tool.

The Vaal River is highly regulated by numerous dams and small weirs, which hold back water for irrigation; this regulation is so extensive that there is now only sporadic flow into the Orange River (Braune & Rogers, 1987).

Braune & Rogers (1987) divide the Vaal River catchment basin into five regions; the Vaal River upstream of Grootdraai Dam, the Vaal River between the Grootdraai and Vaal Dam, the Vaal River stretching from the Vaal Dam to the Vaal Barrage, the reach of the Vaal River between the Vaal Barrage and Bloemhof Dam and finally the stretch of the Vaal River from the Bloemhof Dam to the confluence of the Vaal with the Orange River at Douglas.

The Vaal River upstream of the Grootdraai Dam experiences a constant addition to the base flow through water transfer from the Usutu and Tugela basins.

The flow in the Vaal River, which stretches from the Grootdraai Dam to the Vaal Dam, is greatly reduced due to extensive abstraction from the Grootdraai Dam to supply water users in Mpumalanga; this use is so extensive that a period of zero flow was recorded in this stretch in November 2002 (authors observation). Land use in this region is mostly agricultural.

The Vaal River from the Vaal Dam to the Vaal Barrage, is highly influenced by man; the average return flow from the urban and industrial sectors exceeds the natural mean annual

runoff (Pitman, 1985). The Klip River tributary provides the major content to the Barrage catchment. It is highly mineralised, the principal ions being sulphate, chloride, sodium calcium and magnesium which originate from the highly urbanised industrialised and intensely mined areas of Southern Gauteng.

The Vaal River, extending from the Vaal Barrage to Bloemhof Dam, is highly regulated to meet demands for water supply. High sulphate loads with a corresponding contribution of total alkalinity dominate the Bloemhof Dam catchment. The high sulphate loads and higher alkalinity, are due to the contribution of the Vaal Barrage and to high inputs from the tributaries draining the Northern part of the catchment, which are heavily polluted by intensive mining and industrial activities. Mine service water can have a low pH, a high salt content as well as elevated levels of sulphate (coal mining). There is an upward trend in salinity in this reach. The by-products of fuel processing are discharged into the Vaal River below the Barrage. These effluents contain different pollutants including sodium, fluorides and a number of non-biodegradable organic compounds. The effluent from satellite industries may contain extremely high concentrations of phosphorus, nitrates and ammonia (see Chapter 3, section 3.3.3). Taste and odour problems in drinking water are regularly experienced by some municipalities in this region and are attributed to eutrophication.

The stretch of the Vaal from Bloemhof Dam the confluence of the Vaal and Orange Rivers, is highly regulated as a result of releases from the Bloemhof Dam. Downstream of this dam the upstream pollution effects are ameliorated to some extent by the inflow of water of low sulphate and high bicarbonate and chloride levels from the East. However, irrigation return flows contribute to high TDS waters rich in chlorides via the Harts River in the North. Thus at the lower end of the Vaal River the sulphate problem has been replaced to some extent by one of high chloride and alkalinity, due principally to irrigation return flow (Braune & Rogers, 1987). The map of water quality trends in the Vaal River based on diatom index scores to be found in Chapter 4 (section 4.7) illustrates the trend in water quality described above.

Flows in the Wilge River are not greatly changed as a result of abstractions; the only major changes being experienced during periods of release from the Sterkfontien Dam. After the confluence with the Liebenbergsvlei River turbidity becomes higher due to the lower salinity of the high mountain water. This low salinity of the mountain water enhances riverbank erosion. Land use in the catchment of this tributary is mainly agricultural implying diffuse loads of nutrients from fertilised land, although early season flushes may cause increased peaks of nutrients in return flows to the river.

In general, the best quality water of the Vaal River catchment is still found upstream of the Vaal Dam and quality deteriorates downstream. Long-term pollution threats to the Vaal River catchment are atmospheric pollution, diffuse agricultural sources and further industrial development. Long-term pollution impacts and deterioration in surface water quality need to be effectively monitored if these trends are to be noted and arrested. Biological monitoring techniques, such as those based on diatom community composition are ideal for monitoring both long and short-term pollution effects (Round, 1993).

The transport of water across catchments has been a component of river regulation in Southern Africa for at least three decades, and almost all of the inter-basin transfer schemes fall within the borders of South Africa. South Africa accounts for the bulk of the water consumption on the subcontinent, while only 10% of the water is located here (Snaddon *et al.*, 2000). As the water demands of all economic sectors in the supply area of the Vaal River increased, it had to be augmented by a number of schemes that transfer water from other catchments. The Vaal River supply area thus became part of a complex system consisting of various subsystems that are linked together and that are interdependent (Figure 1.1; DWAF, 2000).

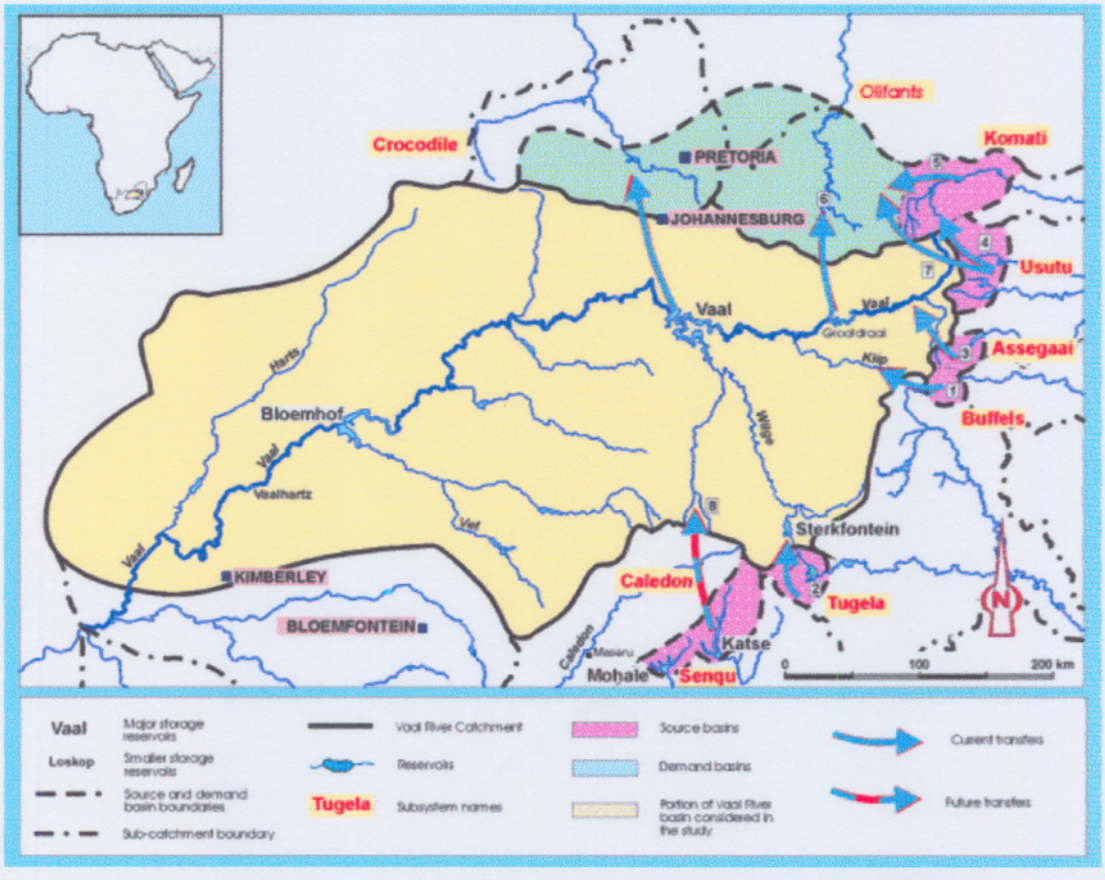

***Figure 1.1**: Map of the Vaal River Catchment basin showing current and projected inter-basin transfer schemes (DWAF, 2000).*

Water within the Vaal River is presently augmented by several river basins, including the Tugela, Orange, Usutu, Komati, Olifants and Buffels Rivers. The most recent inter-basin scheme to be implemented is the Lesotho Highlands Water Project (LHWP). This international inter-basin transfer scheme is designed to divert 2.2 x 10^9 m^3 yr^{-1} of water from the headwaters of the Orange River in Lesotho, into the Ash/Liebenbergsvlei tributary of the Vaal River in the Freestate province. This water is primarily used for industrial and domestic consumption in Gauteng and generates hydro-electric power for use in Lesotho. On completion of the final phase of the Lesotho Highlands Water Project more than 75% of the flow of the Vaal River will be imported from other catchments (Snaddon *et al.*, 2000).

No other single catchment in the country supports as much agricultural, mining, industrial and urban developments, all of which require water and have polluted return-flows to the river. This intensive utilisation of the Vaal River places great stress on the system, and has resulted in severe degradation of the river as a water resource. For effective management of the Vaal River, which has such a complex assortment of pollution sources, water quality monitoring programmes need to be far more intensive than currently undertaken by DWAF (13 points in almost 1 000 km of the Vaal River). For intensive monitoring to become practical, an accurate and relatively cheap method of monitoring needs to be implemented in South Africa. The implementation of such a system, using the micro-algae known as diatoms, has been the primary goal of this study. However, such a study cannot be undertaken without knowledge of the major problems and challenges facing the studied river system. Two of the greatest problems experienced in the river are eutrophication and salinisation (Walmsley, 2000).

Salinisation or mineralisation implies the addition of various dissolved mineral substances into a river system. Mineral substances arise both naturally from soil erosion and the resultant washout of mineral substances present in the soil, as well as the contribution from human settlements and activities. Land use activities including both domestic (leading to nutrient enrichment or eutrophication) and industrial (the contamination of surface waters by acid mine drainage). Mine drainage water may contain constituents such as sulphate arising from the accelerated oxidation of sulphur bearing minerals in exposed rock consequent to mining operations (Hohls *et al.*, 2002). Due to return-flow from the industrial heartland of Gauteng, the Vaal Barrage and Vaal River directly downstream of the Barrage especially experience high dissolved solid concentrations (DWAF, 1993). The addition of mineral substances to a freshwater resource is detrimental on several levels. Firstly, aquatic organisms are adapted to live at certain levels of mineral substance concentration - as mineral substance levels increase, communities of fish, insects, zooplankton and algae

change. These changes may be detrimental, such as the increasing occurrence of dinoflagellate algae in the Vaal River. Certain dinoflagellate species are bloom-forming and are responsible for red tide in the ocean (potentially toxic). Secondly, a high mineral substance concentration decreases turbidity (Grobler *et al.*, 1986). The turbid nature of the Vaal River decreases light penetration, with the consequence that less light is available for algal photosynthesis. Thus, although the middle and lower Vaal River has nutrient concentrations allowing for almost continuous blooms of different groups of algae, the effect of these high levels of nutrient are ameliorated by turbidity, as lower levels of light penetration limit algal growth. As light limitation ameliorates to some extent the negative effects of high nutrient concentrations in the middle and lower Vaal, an accurate assessment of the ecological effects of a certain concentration, or combination, of plant nutrients can only be made by examining the structure of the autotrophic communities within the river (see Kelly, 1998). Algae actively assimilate plant nutrients for growth and reproduction. Diatoms, which compose 40% of any given algal community (Round, *et al.*, 1990), provide a representative group of species indicative of the effects of a particular concentration of plant nutrients on riverine "health".

Eutrophication is a serious problem not only in the Vaal River, but also in many other South African inland waters. Eutrophication, a process whereby water bodies become progressively enriched with plant nutrients, especially nitrogen (N) and phosphorus (P), can occur naturally over geological time. However, the process of eutrophication may be accelerated by allochthonous anthropogenic impacts. The latter process is often referred to as cultural eutrophication. Eutrophication is most often found in highly populated and developed areas where sewage discharge and agricultural practices contribute to elevated loads of nutrients into receiving water systems. Phosphorus, and to a lesser degree nitrogen, have been identified as the major causes of eutrophication in fresh surface waters (Rast & Thornton, 1996). The Vaal River, often called "The hardest working river in Africa" (Vaal River Catchment Association, 1981), bears the full brunt of policy dictated effluent return in the form of increasing nutrient levels of a concentration leading to eutrophication (Pieterse, 1986a). Effluents from various point and non-point sources carry plant nutrients (mainly nitrates and phosphates), which stimulate the growth of both undesirable algae (bloom-forming cyanobacteria, diatoms etc.) as well as macrophytes such as the water hyacinth (*Eichornia crassipes*), which in turn detrimentally affect the water quality. Algal blooms cause problems such as unpleasant odours and tastes in the water, the blockage of sand filters when water is purified and may be potentially toxic. The water hyacinth degrades water quality because of the creation of anaerobic conditions (>20% O_2 saturation, author's observation) caused by thick floating mats of plants. Toxic water conditions may then result

because of the release of ammonia and hydrogen sulphide produced by the mineralisation of organic material by anaerobic bacteria (DWAF, 1993).

South Africa has some of the most highly enriched surface waters in the world (Toerien, 1974; Toerien *et al.*, 1975; NIWR, 1985). Until now the focus of governmental agencies has been monitoring techniques developed for the monitoring of organic pollution and associated aquatic health. Walmsley (2000) is of the opinion that it is only by the stringent management and monitoring of nitrate and phosphate levels within the aquatic environment that the eutrophication problem can be solved. He further goes on to state: "It is felt that immediate national research should be directed at quantitatively assessing the eutrophication problem in terms of its extent and trends; the source of nutrients and the levels entering aquatic systems....". Therefore, this study will attempt to apply an efficient and cost effective biological method (using diatom communities) to accurately assess the trophic status of inland rivers in South Africa using the Vaal and Wilge Rivers as a case in point.

Governmental policy has recently attempted to ameliorate the steady decline in water quality by the introduction of a new national water policy. The National Water Act 36 of 1998, repealed and replaced over one hundred previous acts dealing with water, so that we now have two consolidated Acts, namely the National Water Act and the Water Services Act 108 of 1997. The tenor of the democratic reform processes and the underlying cornerstone of the government's water law reform process is encapsulated in a preliminary section of the Act, which states that the National Government is the public trustee of the nation's water resources to "...ensure that water is protected, conserved, managed and controlled in a sustainable and equitable manner for the benefit of all persons in accordance with its constitutional mandate." Diatom-based methods for monitoring water quality can make a contribution to the management of aquatic resources by providing a preliminary indication of pollution types and concentrations without detailed and expensive chemical analysis. Thus the quality of a particular body of water can be classified and a decision taken as to whether there is a level of pollution which could be detrimental to the aquatic environment or eventually to water users.

Under the National Water Act certain activities, which pollute or degrade water resources, require a water use license from the Department of Water Affairs and Forestry (DWAF). It is stipulated in the Act that an applicant may be required to provide "...an assessment by a competent person of the likely effect of the proposed license on the resource quality...", which can be subject to independent review. A license is not issued in perpetuity, but rather for a fixed period, which may not exceed 40 years. Provision is made for the periodic review

of the license at intervals, which do not exceed five years. Water quality monitoring forms an essential part of the conditions of many such water licenses.

The assessment of the general quality of a water resource requires regular monitoring. The monitoring of South African waterways has traditionally been carried out by two means, firstly the chemical analysis of water quality and more recently by the use of various biomonitoring techniques such as the South African Scoring System (SASS) and the Fish Health Index (FHI). These techniques were introduced as part of routine monitoring programmes due to certain shortcomings in standard physical and chemical methods. These monitoring techniques form part of the National Biomonitoring Programme for Aquatic ecosystems (NBPAE) which was initiated by DWAF, the Water Research Commission (WRC) and the Department of Environmental Affairs and Tourism (DEAT) (Hohls, 1996). It has become important to use these various methods as alternatives to chemical analysis for a number of reasons. Chemicals and chemical compounds constantly fluctuate in the river system; they are broken down, and dissolved by environmental conditions such as light and heat energy, they are also constantly removed from the system via uptake by organisms and sedimentation. Chemical components in a river system may also be diluted by inflows of rainwater or augmented from runoff from point (mine, sewage, storm water drainage) and diffuse sources (agricultural run-off, ground water seepage from settlement ponds), or become concentrated during times of drought and low flow. These factors make it difficult, if not impossible, to provide anything other than a fragmented overview of the state of a river along its complete length using conventional chemical monitoring techniques. Rapid efficient and cost-effective techniques, such as diatom-based pollution indices, are therefore required for the routine monitoring of rivers.

Biological communities reflect overall ecological integrity by integrating various stressors over time and thus provide a broad measure of their synergistic impacts (Laas, 2002). Aquatic communities (e.g. fish, riparian vegetation, macro-invertebrates and algae) can integrate and reflect the effects of chemical and physical disturbances that occur in river ecosystems over extended periods of time. These communities can provide a holistic, and integrated measure of the integrity or health of the river as a whole (Chutter, 1998). Dixit *et al.* (1992) list the ideal characteristics of biological indicators: they should be simple, be able to quantify the rate of degradation (or recovery) in water quality, be applicable over large geographic regions; and furnish data on background or reference conditions.

Numerous methods have been developed for the bio-assessment of the integrity of aquatic systems. Some of these are based on some or other aspect of a single species, but most are based on the attributes of whole assemblages of organisms such as fish, algae or

invertebrates. Although some methods have been available for a number years, benthic algal community analyses, such as diatom-based indices, have not yet been included due to lack of expertise in the identification of these organisms, a lack of standard protocols for sampling and data generation and perceived difficulties in the general use of this group. This study attempts to dispel some of the misconceptions surrounding water quality monitoring using diatom pollution indices and to try to demonstrate the value of existing tools (both diatom indices and associated electronic databases) into the suite of biomonitoring tools presently in use in South Africa.

Diatoms occur in all types of aquatic ecosystems, also extending into damp sub-aerial habitats. A golden-brown mucilaginous film on the surface of a substrate indicates the presence of benthic diatoms. Planktonic diatoms occur free-living in the water column of rivers and dams. The diatoms (Bacillariophyceae) comprise a ubiquitous, highly successful and distinctive group of essentially unicellular algae, whose most obvious distinguishing characteristic is the possession of siliceous cell walls (frustules). The frustule is a type of cell wall unique to the diatoms. The frustule is composed chiefly of hydrated amorphous silica, but may contain other trace elements. The frustule comprises two almost equal halves. The outer or older of the two halves is the epivalve - this older valve gives rise, during asexual reproduction, to the inner or younger half, which is known as the hypovalve. Each valve is composed of two parts: the valve face and valve mantle which is connected at right angles to the valve face. Closely united to the valve mantle are the girdle bands or copulae (Lee, 1997). As autotrophs diatoms contribute significantly to the productivity of ecosystems, frequently forming the base of aquatic food chains (Cox, 1996).

The diatom flora of Southern Africa has received much attention in the past from a number of specialists. These investigations were initiated in the middle 19th century by workers such as Ehrenberg (e.g. Ehrenberg, 1845) and Cleve (e.g. Cleve, 1881). This work was continued into the 20th century by notable specialists, including Fritsch (e.g. Fritsch, 1918) and co-worker Rich (e.g. Rich, 1932). During the 1950's and 1960's the acclaimed diatom specialist, Dr. B.J. Cholnoky, produced over 40 papers dealing with many of the diatom species found in Southern Africa (e.g. Cholnoky, 1960). Giffen published much valuable work in the 1960's and 1970's dealing with marine and estuarine diatoms along with several accounts of freshwater species to be found in the Eastern Cape region (e.g. Giffen, 1966). The work of Schoeman and Archibald in the late 1970's and early 1980's has made an invaluable contribution to the knowledge of both the systematics and ecology of the diatoms. The most noted work of these two authors being 'The diatom Flora of Southern Africa', the first volume of which was published in 1976 (Schoeman & Archibald, 1976-1980). Further important contributions by these two authors include a detailed investigation of the Genus *Amphora* in

a series of papers entitled 'Observations on *Amphora* species (Bacillariophyceae) in the British Natural History Museum' (e.g. Schoeman & Archibald, 1986). More recently Pienaar (1988) made a valuable contribution to the understanding of the taxonomy and occurrence of the centric diatom species in the Vaal River.

The potential of diatoms as indicators of water quality was early realised in South Africa. Cholnoky (1968) describes the application of the Thomasson (1925) community analysis, which he adapted to determine water quality using benthic diatom community composition. Using Thomasson community analysis allows for comparisons to be made between sites in the same river, or it may be used to track changes at a single site. One aspect of water chemistry is chosen for study e.g. the amount of nitrogenous pollution. First the sum of all the species of the genus *Nitzschia* within a particular diatom community is calculated as an abundance value. The genus *Nitzschia* is known generally to be nitrogen heterotrophic, and therefore the relative abundance of this genus in a sample gives a reflection of the amount of nitrogenous pollution at the study site. Similarly, abundance values of the acidobiontic diatom *Eunotia* can be used to track a pH gradient in a river system. Cholnoky (1968) obtained good results using this index, but the user of the Thomasson analysis method needs to have an in depth knowledge of the autecology of individual diatom species to draw accurate environmental conclusions based on diatom community composition. Cholnoky's application of the Thomasson analysis method was a forerunner of modern autecological indices (such as those dealt with in this study), which have become more accurate due to the development of correspondence analysis, with the advantage of being able to assign exact tolerance limits for chemical variables to not only genera, but also species.

Archibald (1972) attempted to relate diversity in some diatom communities to water quality. The diversity index approach proved to be unsuccessful, as Archibald concluded that diversity of species within a particular diatom community provides an unreliable reflection of water quality. Although Archibald's attempt to use diatoms as bio-indicators failed, the diversity approach was a parallel development in water quality monitoring with European countries in using microalgae to monitor water quality.

Schoeman (1976) used diatom indicator groups in the assessment of water quality. Schoeman simplified the community analysis method of Cholnoky (discussed above) by dividing diatom associations into four groups, each with their own particular ecological requirements. Only the groups or associations were then reflected in the table of results, instead of the lengthy tables used by Cholnoky. Schoeman concluded that these diatom associations or groupings could be successfully employed to assess the quality of running

waters especially in regard to the trophic status. Round (1993) also came to the conclusion that Schoeman (1976) found a good fit between groups of diatoms and chemical levels in the Jukskei-Crocodile River system, and went on to comment that the species used were similar to those in Europe.

In 1979 Lange-Bertalot developed a monitoring system based on groups of diatoms with similar tolerances towards pollution. Lange-Bertalot's "saprobian" classification system proved after certain modifications to be highly successful. Schoeman (1979) tested Lange-Bertalot's (1979) method in the upper Hennops River and found the method successful, with a good correlation between the species composition of the diatom communities studied, and the water quality. Unfortunately, this parallel development with Europe in the study of the application of diatoms as bio-indicator organisms terminated in South Africa with Schoeman's (1979) work.

Diatoms, as indicators of water quality, were only again investigated in depth in South Africa by Bate *et al.* (2002). The investigation attempted to relate a descriptive index, based on a dataset for the environmental tolerances of diatom species found in the Netherlands, to water quality in South Africa. The environmental variables generated by the Van Dam *et al.* (1994) index include: pH, conductivity, oxygen requirements, trophic status, saprobian status and habitat requirements of a selected number of diatom species found in waters of the Netherlands (Van Dam *et al.*, 1994). Bate *et al.* (2002) came to the conclusion that benthic diatoms could be a useful addition to the NBPAE as the diatoms give a time-integrated indication of specific water quality components. However, Bate and co-workers went on to state that the particular data set tested in their study (that of Van Dam *et al.*, 1994), could not be transposed directly for use under South African conditions. For this reason the present study investigates the potential use of several other numerical, rather than descriptive, diatom indices developed in Europe, Great Britain and Japan in the Vaal River system as part of the first phase of a national investigation into the efficacy of these indices for use in bio-monitoring of South African rivers.

No single group of organisms is everywhere best suited for detecting the diversity of environmental perturbations associated with human activities (Kelly, 2002). If the maintenance of ecosystem integrity is the aim of environmental management of a river system, the need to monitor the status of different taxonomic groups is vital. Diatoms provide interpretable indications of specific changes in water quality, whereas invertebrate and fish assemblages may better reflect the impact of changes in the physical habitat in addition to certain chemical changes (McCormick & Cairns, 1994).

Round (1991) lists several reasons why animal (fish and aquatic macroinvertebrates) components of an ecosystem may not provide a satisfactory index system. Animals have complex reproductive cycles which are often linked to the seasons; animals are largely motile and this may cause difficulty during sampling; animals may have many different life stages and may undergo metamorphosis; animals have specific habitats and niches; they are actively grazed; and closely linked to flow conditions and thus will not usually be evenly distributed from headwaters to estuaries. In addition, watercourses which are too deep to wade across such as the Vaal and Wilge Rivers sampled in this study, may prove difficult if not impossible to evaluate using a macro-invertebrate index along the length of the river.

Diatoms have several advantages over the animal (fish and aquatic macroinvertebrates) component of streams and rivers. Diatoms are an abundant, diverse and important component of algal assemblages in freshwater bodies. Diatoms comprise a large portion of total algal biomass over a broad spectrum of trophic statuses (Kreis *et al.*, 1985). While diatoms collectively show a broad range of tolerance along a gradient of aquatic productivity, individual species have specific habitat and water chemistry requirements (Patrick & Reimer, 1966; Werner, 1977; Round *et al.,* 1990). In addition, diatom communities live in open waters of lakes (plankton), or primarily in association with plants (epiphyton), rocks (epilithon), sand (epipsammon) or mud (epipelon) in littoral, nearshore habitats.

As mentioned above, eutrophication of surface waters has a severe influence on general water quality in South Africa. Numerous problems are posed in the chemical monitoring of eutrophication. Criteria for assessing trophic status from total phosphate concentrations are based on annual average values (OECD, 1982) and in turn criteria for assessing trophic status from total nitrogen are based on averages for the summer months (DWAF, 1995). The ratio between these two elements needs to be determined before an accurate assessment of trophic status can be made.

In addition to eutrophication, DWAF intends to address the problems related to water quality in South Africa. It is the stated intention of DWAF to firstly, review the existing chemical monitoring network and to terminate sampling at unnecessary sites and then expand the network to cover more adequately sensitive problem areas with insufficient sampling sites. Secondly to implement a more extensive Eutrophication Monitoring Programme as part of the Trophic Status Programme. The Eutrophication Monitoring Programme will be extended throughout the country to encourage appropriate land use management practices and to prevent or minimise large loads of nutrients entering the aquatic environment (Hohls *et al.*,

2002). Schoeman (1976), when assessing the trophic status of rivers in the Jukskei-Crocodile system came to the conclusion that diatom associations may be successfully employed to assess the quality of running waters especially in regard to the trophic status, as diatom associations reflect the trophic level of the water over a period of time. Modern, accurate diatom indices could be used as part of the Eutrophication Monitoring Programme to provide an integrated indication of concentrations of plant nutrients within a water resource over time, something which can only otherwise be achieved by implementing long term and expensive chemical monitoring programmes.

It is generally accepted that invertebrate-based indices (such as SASS) do not provide a reliable indication of eutrophication. For this reason it is better to take direct measurements of the photosynthetic community (Kelly, 1998). Diatoms are the preferred organisms used in bio-monitoring of eutrophication as they are sensitive to change in nutrient concentrations (Pan *et al.,* 1996), supply rates and ratios (e.g., Si:P; Tilman, 1977; Tilman *et al.*, 1982). Because diatoms are primarily photo-autotrophic organisms, they are directly affected by changes in nutrient and light availability (Tilman *et al.*, 1982). Each taxon has a specific optimum and tolerance limit for nutrients, which can usually be quantified to a high degree of certainty (e.g. P: Hall & Smoll, 1992; Reavie *et al.,* 1995; Fritz *et al.*, 1993; Bennion, 1994, 1995; Bennion *et al.*, 1996; N: Christie & Smol 1993).

Diatom assemblages are typically species rich. This diversity of diatoms in different population densities, composition and overall abundance, contains considerable ecological information. Moreover, the large number of taxa provides redundancies of information and important internal checks in datasets, which increase confidence of environmental inferences (Dixit *et al.,* 1992).

In addition to the above factors the response of diatoms to perturbation and recovery is rapid (Zeeb *et al.*, 1994). Diatoms have one of the shortest generation times of all biological indicator groups (Rott, 1991). They reproduce and respond rapidly to environmental change and provide early warnings of both pollution increases and habitat restoration success. Rapid immigration rates and the lack of physical dispersal barriers ensure that there is little lag-time between perturbation and response (Vinebrooke, 1996).

Although diatom taxonomy is currently in a state of flux, this should pose no unsolvable problems for the application of diatom indices, as the taxonomy of diatoms is generally well documented (Krammer & Lange-Bertalot, 1986-91) and full lists of synonyms are available in the afore-mentioned identification volumes and works such as that of Kellogg & Kellogg

(2002) and in the electronic database OMNIDIA v.3 (Lecointe *et al.*, 1993). Diatom species identifications are largely based on frustule morphology (Ross & Mann, 1984). Even in studies in which DNA is isolated from diatom cells, it is always necessary to study the ultrastructure of any given assemblage to make sure that all the diatom cells belong to the same species (Sherbakova *et al.*, 2000). Because the frustule is composed of resident opaline silica, diatom valves are usually well preserved in most samples. Consequently, by taking sediment cores and analysing diatom assemblages, it is possible to infer past environmental conditions using paleolimnological techniques.

Round (1993) lists numerous reasons why diatoms are useful tools for bio-monitoring, amongst which the following bear special relevance to the South African situation: diatom-based methods are cost effective; data is comparable (national and international); techniques are rapid and accurate; identifications and counts can be done by non-specialists, if they are provided with illustrated guides. Diatom-based indices could be particularly valuable in assessing rivers because a one-time assay of species composition of diatom assemblages in the system could provide better characterisations of physical and chemical conditions than one time measurement of those conditions (Stevenson & Pan, 1999). In addition, by sampling stream biota a reflection of the biological integrity of the stream may be gained. The structure of the community may not directly reflect the measured concentrations of water quality variables. This may be due to a number of reasons: either the chemical constituent was not sampled for or, if sampled it was below the levels of detection in the particular laboratory performing the analysis, or, either synergistic or antagonistic reactions took place between several chemical constituents within the stream or river. For this reason, measuring the integrity of the biotic community sampled, rather than just the relationship between biota and chemical concentrations, provides an indication of general stream health, as stream biota are directly exposed to all the elements within the particular water body which they inhabit. The community structure of a selected group of organisms provides an integrated reflection of all the chemical variables that influence that particular group of biota.

Concerns have been raised as to the transfer and comparison of bio-monitoring data between the Northern and Southern Hemispheres. It is well known that some species have the same morphology, but questions still remain regarding the range of ecological tolerances of the various species. Concerns about ecological tolerances are valid when distance, climatic condition, and other environmental pressures are taken into account (Round, 1991). However, the present study will demonstrate the concept discussed by Kelly *et al.* (1998), namely that diatoms are "sub-cosmopolitan" meaning that they occur anywhere in the world

where a certain set of environmental conditions exist (Padisák, 1998). The sub-cosmopolitan concept suggests that geographical location is not the determining factor in the distribution of diatom species and the composition of communities, but it is rather the specific environmental variables at a site that determine this distribution. Hence the sub-cosmopolitan concept implies not only a cosmopolitan distribution, but it also that together with a cosmopolitan distribution diatom species would have similar tolerances, where ever they are encountered (Northern or Southern Hemisphere), to water quality variables. The application of the sub-cosmopolitan concept will be tested in this study in South Africa by comparing not only the species compositions of the diatom communities encountered in the Vaal and Wilge Rivers to those encountered in European habitats but also the environmental tolerances of the diatoms species to specific water quality variables will be compared between hemispheres by determining whether diatom index scores (based on the environmental tolerances of diatoms from the Northern Hemisphere) yield the same or similar results.

Criticism of diatom-based techniques has been expressed regarding the difficulty involved in accurate species identification necessary for the effective use of diatom indices. Descy & Coste (1991), however, are of the opinion that species identification problems can be solved by editing complex identification keys to allow for accurate identification of a limited number of taxa. The accuracy of the opinion that identification problems could be solved by the production of simplified identification keys was later demonstrated by the publication of just such a guide for the identification of common diatom taxa from French inland waters (Prygiel & Coste, 2000). This guide provides a means for identification of all of the diatom taxa used in the Biological Diatom Index (BDI) of Lenoir & Coste (1996) developed for use in national river quality monitoring networks in France. Kelly (2000) developed a similar guide for the identification of common benthic diatoms in Great Britain.

Taxonomic difficulties may also be avoided by using a simplified diatom index such as the Generic Diatom Index (GDI) of Coste & Ayphassorho (1991). GDI allows for the determination of water quality at a particular site, based on the identification of diatoms to the genus level. GDI index has been found comparable to indices such as the Specific Pollution sensitivity Index (SPI; CEMAGREF, 1982), which is based on a large number of taxa (Kelly *et al.*, 1995; Kwandrans *et al.*, 1998). The genus level approach has also proved to be successful in Taiwanese waters using a specific index based on only six genera and the ratio of occurrence between these six genera. Strong correlations were found between the Taiwanese generic index and other diatom-based indices of water quality (Wu & Kow, 2002).

Within the last decade diatom indices have gained considerable popularity throughout the world as a tool to provide an integrated reflection of water quality, which can form the basis of management decisions regarding rivers and streams. Work on the use of diatoms as bio-indicators has in fact proceeded to such an extent that in some cases diatom indices have replaced invertebrate indices as the biomonitoring method of choice (Prygiel & Coste, 1993b). After the perquisite testing of European diatom indices for application in South Africa, a portion of which work is completed in the present study, these diatom indices may provide a valuable addition to the suite of bio-monitoring tools currently in use in South Africa. Diatom-based indices could be particularly useful for monitoring habitats in which it is difficult or inappropriate to use other types of monitoring tools (e.g. macro invertebrate based indices such as SASS are inappropriate for use in canals and effluent streams).

The vast majority of the development and testing of diatom indices has been carried out in French drainage basins. The fact that these French diatom indices have been tested on the scale of territory as large and as typologically diversified as France enabled the more general application on the European continent (Prygiel & Coste, 1999). The design of software programmes such as OMNIDIA for the calculation of diatom indices has also facilitated the use of diatom based bio-monitoring methods (Lecointe *et al.*, 1993). A variety of diatom indices have been adopted and tested by many European countries including Finland (Eloranta & Andersson, 1998) and Poland (Kwandrans *et al.*, 1998). European and British diatom indices were derived, applied and tested in temperate regions, and there is little information regarding their application in the tropics and subtropics (Wu & Kow, 2002). Thus the need exists for the evaluation of these indices before they can be routinely applied in warmer climates. Recently Jüttner *et al.* (2003) found that the TDI index of Kelly & Whitton (1995), developed to demonstrate trophic levels in British inland waters, showed consistent responses in TDI scores between Europe and the Himalayas. This has many important implications for research into the use of diatom indices in Southern Africa. Bate *et al.* (in press) express the opinion that before an index system can be attempted, there is a need to gather information on South African dominant diatom species, their locations and the water quality at the sampling localities. Bate and co-workers go on to say that when data are available, the possibility might exist to re-analyse these data into an index system. However, if European indices such as the TDI can be used in their current state or slightly modified this will negate the need for highly detailed research into the ecological tolerances and distribution of diatom species encountered in South Africa, as the direct implication would be that diatoms are cosmopolitan (or "sub-cosmopolitan") in their distribution. If diatoms are truly "sub-cosmopolitan" in their distribution, data concerning ecological tolerances of these species already exist and are encapsulated in (European/British) diatom indices. The

present study will test whether European indices may be implemented in the Vaal River system with the same degree of success experienced by examining, for example Himalayan inland waters.

There is currently a strong international effort towards the testing and development of diatom indices specific to different countries. Countries, which are currently engaged in this work, are among others Taiwan (Wu, 1999), Malaysia (Maznah & Mansor, 2002), Argentina (Gómez, 1999) and Australia (John, 2000). Within Europe and Great Britain a concerted effort is being made to standardise the routine sampling and processing of diatoms for water quality assessments (Kelly *et al.*, 1998; Prygiel *et al.*, 2002).

The various diatom indices fall into different classes. A discussion of each class relevant to the present study and representative index(s) follows. The majority of the indices used are based on the weighted average equation of Zelinka & Marvan (1961) and have the basic form:

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

where $\mathbf{a_j}$ = abundance (proportion) of species **j** in sample, $\mathbf{v_j}$ = indicator value and $\mathbf{s_j}$ = pollution sensitivity of species **j**. The performance of the indices depends on the values given to the constants **s** and **v** for each taxon and the values of the index ranges from 1 to an upper limit equal to the highest value of **s**. Diatom indices differ in the number of species used (Table 1.1) and in the values of **s** and **v** which have been attributed after compiling the data from literature and from ordinations (Prygiel & Coste, 1993b).

Diatom indices, such as those used in the present study, function in the following manner: In a sample from a body of water with a particular level of a water quality determinant, diatom taxa with their optimum close to that level will be most abundant. Therefore an estimate of the level of that determinant in the sample can be made from the average of the optima of all the taxa in that sample, each weighted by its abundance. This means that a taxon that is found frequently in a sample has more influence on the result than one that is rare. A further refinement is the provision of an 'indicator value' which is included to give greater weight to those taxa which are good indicators of particular environmental conditions. In practice, use of diatom indices involves making a list of the taxa present in a sample, along with a measure of their abundance. The index is expressed as the mean of the optima of the taxa in the sample, weighted by the abundance of each taxon. The indicator value acts to further increase the influence of certain species (Kelly, 1998).

TABLE 1.1.

Number of taxa taken into account by seven diatom indices

Index*	SPI	GDI	DES	SLA	TDI	BDI	CEC
Number of taxa	2035	174	106	323	86	209	208

*SPI; Specific Pollution sensitivity Index, GDI; Generic Diatom Index, DES; Descy's index, SLA; Sládeček's index, TDI; Trophic Diatom, BDI; Biological Diatom Index, CEC; Council for European Communities index.

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

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

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

Leclerq & Maquet (1987) applied the method of Descy (1979) to the Belgian Ardennes watercourses (the Samson catchment area). The authors proposed new **s** and **v** values for 210, species following an exhaustive compilation of the autecological data in scientific literature. The index was updated (Leclercq, 1995), and now includes 403 species.

In 1991 Descy & Coste developed a diatom index for use in general water quality monitoring across Europe. The Commission for Economical Community index (or CEC) is calculated from a two-entry table, which contains 208 taxa. Horizontally, there are 8 groups of taxa ranked according to decreasing tolerance for pollution by biodegradable organic matter from left to right and vertically, there are 4 subgroups of the more stenoecous species representing the upstream-downstream succession along a theoretical running water ecosystem.

The Artoise-Picardie Diatom Index (APDI; Prygiel *et al.*, 1996) was the result of the need expressed by French water management specialists for a technique for wide application in monitoring networks. The APDI was designed to combine ease of use and reliability with standardised techniques. An attempt was made to reduce the number of units to be counted, the level of identification and a reduction in number of taxa to those of the most significance (i.e. those taxa with a high indicator value). The requirements for ease of use and reliability were met by combining the most recent version of the GDI index and the SPI index, yielding an index based on the identification of 45 genera and 91 species.

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

Dell'Uomo (1996) proposed an index known as the Eutrophication/Pollution Index (EPI). The EPI was designed on the basis of investigations concerning 8 measurement stations in the river Chienti, a watercourse in the Central Apennines, Italy. The EPI is a specific sensitivity index, which integrates the saprobic (pollution tolerance), the trophic (trophic levels) and halobic (specific salinity requirements) aspects attributed to 93 diatom species.

Slàdeček (1986) applied the method of Descy (1979) in the context of the saprobic system. Saprobity refers to the differing levels of tolerance or sensitivity towards organic pollution (domestic and industrial). The values within the formula of Zelinka & Marvan (1961) of **s** (pollution sensitivity) and **v** (indicator value) are attributed to 323 species according to their affinity for organic material expressed in the measurement of BOD_5 (Slàdeček, 1973, 1986).

Watanabe (1982) proposed a saprobic index known as the Diatom Community Index (or DCI) based on an altogether different formula to that of Zelinka and Marvan (1961). The DCI values are calculated using the sum of the relative frequency of pollution tolerant taxa added to the relative frequency of indifferent (ubiquitous) species. The DCI index underwent several refinements and resulted in the Diatom Assemblage Index of Organic Water Pollution or DAIpo (Watanabe, 1990), which takes into account 87 species (Prygiel *et al.*, 1999).

Schiefele and Kohmann in Hofmann (1996) proposed a Trophic Diatom Index (TDI) on the basis of a three year study of 31 sampling sites in 5 German federal states. Indicator values relating to dissolved inorganic phosphate (DIP), total phosphate (TP), nitrate and ammonia were calculated for 105 diatom species. The formula of the trophic diatom index conforms to the saprobic index of Zelinka & Marvan (1961), and is intended to be its trophic counterpart. As a measure of the indicator quality, species-specific tolerances are weighted (1 to 7) and included into the calculation. Analogous to the saprobic system, the TDI divides quality status into seven levels covering oligotrophic to hypereutrophic conditions. This TDI index is only calibrated for mesotrophic to hypereutrophic conditions (Prygiel *et al.*, 1999).

A similar Trophic Diatom Index (TDI) was proposed by Kelly & Whitton (1995) which is based on investigations at 70 sites representing 14 hydrographical basins located in England and Scotland. The TDI index is not a general quality index, but should be considered an auxiliary tool for decision-making on phosphorus treatment in wastewater plants. The index should not be used on its own but should be complimented by the percentage of organic pollution-tolerant taxa. Easy identification and high indicator values were the criteria for the selection of 86 taxa. A sensitivity value between 1 and 5 was given to each taxon, depending on the concentration at which taxa were most abundant. The final value is comprised between 1 (very low nutrient concentrations) and 5 (very high nutrient concentrations). This technique is original in that, while working with species and genera in a way, which is analogous to APDI (Prygiel *et al.*, 1996), it also takes into account the cell size of the species. A number of changes have been implemented since the 1995 Kelly & Whitton paper, namely scale extension from 1-5 to 1-100, removal of predominantly planktonic taxa from the calculation of the index and slight changes to pollution sensitivity and indicator values for some taxa (Prygiel *et al.*, 1999).

All the diatom indices mentioned above underwent rigorous statistical testing to confirm significant relationships between the species included in calculation of the index value and the actual environmental conditions to which the diatom communities were exposed. Similarly, when diatom indices are applied outside of the region of origin, strict testing is required to ensure that diatom index scores give a realistic reflection of the specific type of environmental pollution being tested for. Several considerations need to be taken into account when testing diatom indices, amongst which both time and pollutant concentrations are important. Individual diatom cells are known to have a generation time below 30 hours in both field and laboratory conditions (Baars, 1983). The direct assumption would then be that individual cells will respond very rapidly to changes in environmental conditions which would benefit certain species with specific tolerances (either negatively or positively). Can this

assumption be considered true if transferred from the level of the individual cell to the community as a whole? A longer period of time is needed for a reaction to be noted on community level. The reaction of a single cell in the form of either increased or decreased reproduction is not measured by diatom indices, rather the structure of the community as a whole is examined and usually the dominant species (more than 5% of the community) determine the score produced by any specific index. Kelly *et al.* (1998), in a discussion of recommendations concerning diatom sampling from artificial substrates, postulated a minimum exposure time of four weeks prior for the artificial substrate to the first sampling (Cattaneo & Amireault, 1992; Hürlimann & Schanz, 1993). From exposure period recommended by Kelly and co-workers the conclusion may be drawn that, although the response of an individual cell to an environmental stressor may be very rapid, it takes a longer period of time for the overall community to develop changes in composition in such a way as to reflect changes in water quality accurately. The time period taken for diatom communities to respond to changes in water quality variables can be explained using growth kinetics. The period of time that is necessary for the diatom community to adapt to new environmental conditions is known as the lag-phase. Historical data (collected at least two weeks prior to sampling) is regarded as ideal by Bate *et al.* (2002) for the basis of comparing diatom community structure and water quality. Prygiel & Coste (1993b) found that the best correlations are noted with chemical analysis carried out just before sampling. Hence, for an accurate comparison of diatom indices and water quality, water quality data collected slightly previous or concurrent to the sampling needs to be examined. This is, however not always possible as financial constraints may, for example, dictate that a site be visited only once per sampling occasion and hence it would be necessary for analysis of water quality to be performed on a sample taken at the same time as the diatom sample. In the present study once-off chemical water quality data collected a few days prior or concurrently with diatom sampling will be used (as in Prygiel & Coste, 1993b), the average chemical data for a period of one month ending two weeks before sampling for diatom communities and the average chemical data for a period of one month ending six weeks before sampling for diatom communities will be used for comparative purposes.

A further difficulty encountered when relating diatom index scores to actual chemical conditions is encountered when considering the reaction of diatom communities to differing concentrations and peaks in water quality variables. Chutter (1998), in a discussion of the effects of water quality variables on macro-invertebrates, is of the opinion that extreme physical and chemical conditions govern the biology of the stream. In other words changes within the macro-invertebrate communities are driven by spikes or peaks in physicochemical constituents of the environment. In discussing the perceived problem encountered when

using canonical correspondence analysis on diatom community data, Bate *et al.*, (in press) pose the question as to whether diatoms in a system have reacted to a high value of mineral element(s) or "spikes" in the system. For this reason comparisons between the results of chemical analysis and the structure of diatom communities will be carried out on several differing data sets for the same periods of time, in addition to both average data and available data on extreme conditions or "spikes" will be used (see Chapter 4).

As is outlined in the previous paragraphs, the present study will adopt three different approaches when testing European diatom-based pollution indices for use in South Africa. The fist approach will be to compare index results based on diatom community structure to once-off chemical data for the rivers a few days prior to biological sampling (physical data will be taken concurrently with diatom sampling). The second approach will be to compare average chemical water quality data to diatom index scores over different periods of time and the third approach will be to test whether diatom communities (and hence diatom index scores) react to peaks or "spikes" in chemical water quality variables.

1.2 Aims and objectives of the study

From the above introductory paragraphs the study aims firstly to determine the extent of water pollution at the studied sites in the Vaal River catchment basin. The principal components of the physical and chemical quality attributes of the Vaal River encountered during the period of the study will be evaluated on the basis of their particular effect on aquatic resources and potential influence on the structure of diatom communities as reflected by diatom index scores.

Diatom community composition at each of the study sites will be analysed and absolute abundance data generated. From the abundance data the OMNIDIA database will be used to calculate diatom index scores. Using the index scores generated by OMNIDIA, rigorous statistical testing of European/British diatom indices will be performed. The indices will be tested by the comparison of diatom index scores, generated by community analysis, to the measured determinants of water quality at the study sites. The experimental rational of the present study is based on the testing of the relationship between diatom index scores and chemical and physical water quality variables over different periods of time and at different concentrations and will take place on several levels. Firstly diatom index scores will be compared physico-chemical data obtained a few days prior or concurrently with diatom sampling. The second will be an evaluation of index scores by comparison to chemical data obtained from the study sites a few weeks prior to the time of sampling, as well a second set

of variables acquired six weeks previously. Thirdly, an evaluation will be performed on the index scores using the maximum concentrations of chemical variables noted at the study site two weeks prior to the time of sampling. The results of the statistical testing will be used to firstly, determine whether diatom communities react to average physico-chemical conditions or to spikes in these components and secondly, to draw conclusions concerning the time period required by diatom communities to change in reaction to fluctuations in water quality.

From the results of comparisons between scores yielded by European/British diatom index and the water quality of the study region, an evaluation will be conducted to determine which, if any, of the investigated diatom indices are best suited for use as a bio-monitoring tool to evaluate water pollution in South Africa, not only in terms of accuracy but also taking into account the usability of the particular index. The ecological condition (general water quality as well as trophic status) of the Vaal River and Wilge River for the month of June 2002 will be determined based on diatom index scores and graphically illustrated using maps of the Vaal River catchment.

Diatom indices, which correlate well to the suite of chemical and physical components used in this investigation, may be in turn used to answer concerns voiced about the transfer of bio-monitoring data using diatoms between the Northern and Southern Hemispheres. Furthermore, this data will be used to demonstrate the sub-cosmopolitan concept of diatom species distribution.

CHAPTER 2

Materials and Methods

2.1 Sites and frequency of sampling

The sampling sites are situated in the Vaal River catchment basin (Fig. 2.1) and extend from upstream sites in Mpumalanga Province (Site V1) to the Northern-Cape Province (Site V13). A site in the Wilge River, which carries water from Lesotho (Katse Dam) into the Vaal Dam (Site W1), was also sampled.

All sampling was performed monthly for the period March 2002 to February 2003 at the sites briefly described below:

Site V1: Vaal River at Bloukop DWAF (Department of Water Affairs and Forestry) No. C1H007 co-ordinates: 26°50'16"S 29°40'23"E.

Site V2: Grootdraai Dam downstream of wall, DWAF No. C1HO19, co-ordinates: 26°55'00"S 29°15'29"E.

Site V3: Vaal River at Gladdedrift, DWAF No. C1H012, co-ordinates: 27°00'13"S 28°45'19"E.

Site V4: Vaal River downstream of Vaal Dam wall, DWAF No. C2H122, co-ordinates: 26°50'34"S 28°05'36"E.

Site V5: Vaal River at Goose Bay Estates, DWAF No. C2H140, co-ordinates: 26°40'45"S 27°35'37"E.

Site V6: Vaal River at Schoemansdrift, DWAF No. C2HO18, co-ordinates: 26°55'38"S 27°10'11"E.

Site V7: Vaal River, at Orkney, DWAF No. C2H007, co-ordinates: 27°00'22"S 26°40'20"E.

Site V8: Vaal River at Kliplaatdrift, DWAF No. C2H061, co-ordinates: 27°20'46"S 26°25'50"E.

Site V9: Vaal River, downstream of Bloemhof Dam at weir, DWAF No. C9H021, co-ordinates: 27°35'48"S 25°35'10"E.

Site V10: Vaal River, downstream of Vaalharts Dam at weir, DWAF No. C9H008, co-ordinates: 28°05'33"S 24°50'55"E.

Site V11: Vaal River at De Hoop, DWAF No. C9H009, co-ordinates: 28°30'22"S 24°35'13"E.

Site V12: Vaal River at Gamagara, DWAF No. C9H010, co-ordinates: 28°25'00"S 24°15'13"E.

Site V13: Vaal River, downstream of Schmitsdrift weir, DWAF No. C9H024, co-ordinates: 28°40'33"S 24°00'56"E.

Site W1: Wilge River at Frankfort, DWAF No. C8H001, co-ordinates: 27°13'16"S 28°25'45"E.

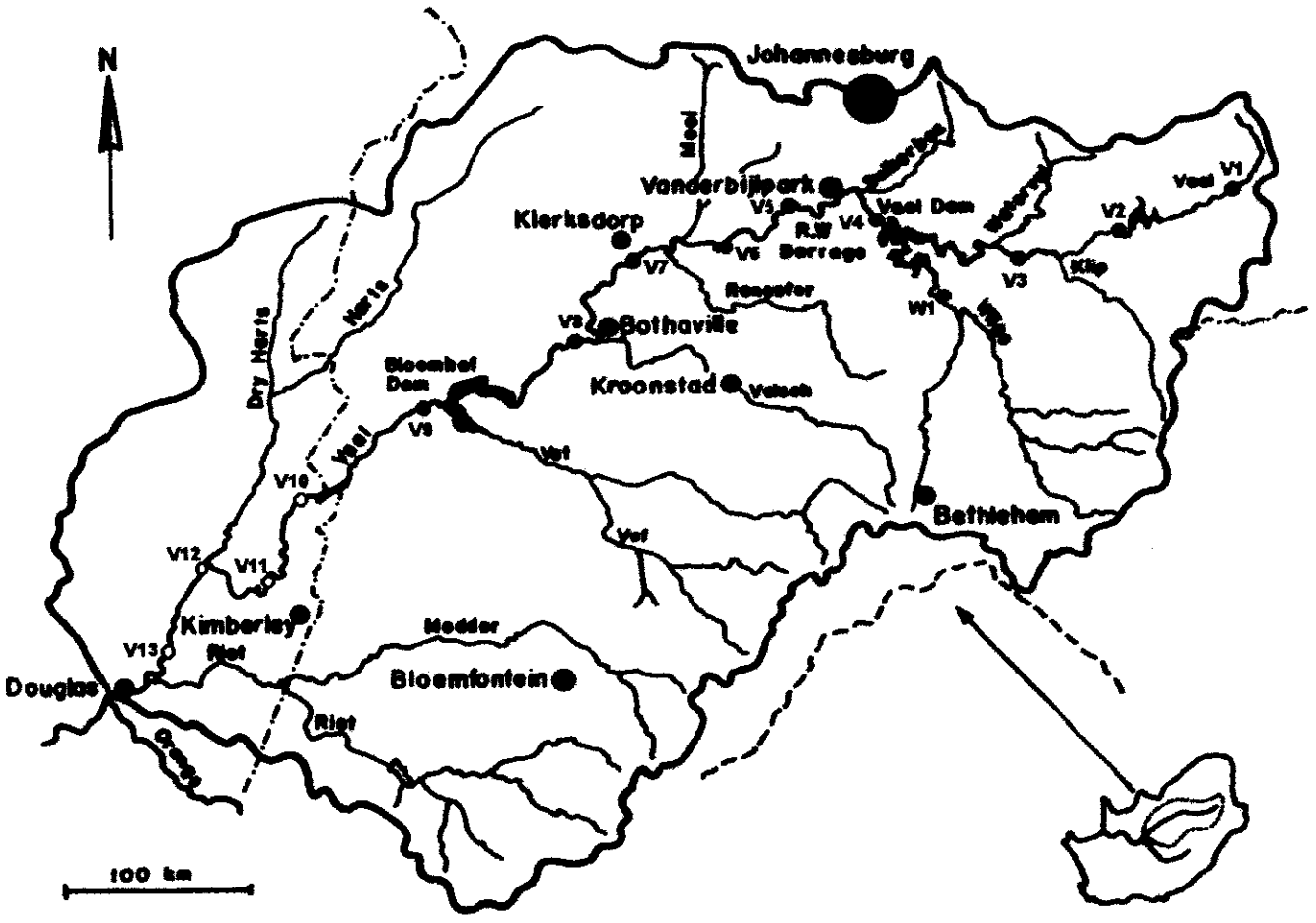

Figure 2.1: Map showing the location of the various sampling sites along the Vaal River. Sites marked in red represent those used to test various diatom indices; sites marked in yellow represent those used to classify the ecological condition of the river (adapted from Braune & Rogers, 1987).

The distances of the sampling sites (except the single site in the Wilge River - W1) from the source of the Vaal River and their respective elevations above sea level are given in Fig. 2.2.

2.2 Sampling techniques

2.2.1 Biological sampling

Site selection for stream biomonitoring should be in a riffle part of the stream where the water is flowing over stones (Round, 1993). Prygiel *et al.* (2002) state more specifically that the site should be selected in the flowing area of the river, possibly in the middle of the river. These recommendations have, however, been made for wadeable rivers.

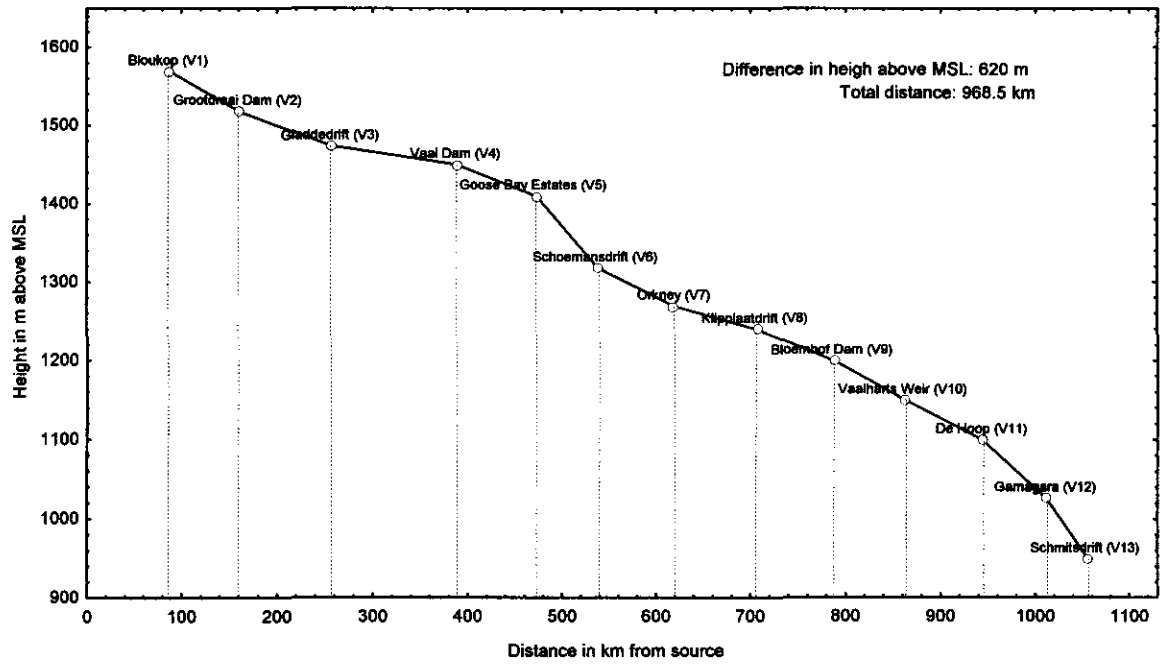

Figure 2.2: *Height above mean sea level (MSL) and distance from the source of the selected sampling sites within the Vaal River*

For the present study, site selection was dependant on the locality of existing DWAF sampling sites for the National Chemical Monitoring Programme. Added to this lack of flexibility of site selection, is the nature of the Vaal River. At most points (except the upper reaches) it is broad, slow flowing and by no means wadeable. Due to the nature of the Vaal River, the sampling procedure of Fore & Grafe (2002) was followed. Rocks were collected closer to the riverbank, in riffles with flowing water. The flowing water at the edge of the main stream (littoral zone) was assumed to be of both the same physical and chemical quality as the main steam. A further assumption that this littoral zone would have the same species composition, as the main stream, is strengthened by Round's (2001) statement that "...river diatoms can colonize massive rivers but also 'rivers' millimetres deep and centimetres wide..."

According to Round (1993), Kelly *et al.* (1998) and Prygiel *et al.* (2002) the epilithon (diatom communities growing on rocks) is the preferred substrate for monitoring diatoms in the riverine environment. All samples were removed from three or more boulders (>256mm) where possible. At some sites there were very few boulders and samples from these sites were very often collected from a single boulder (Kelly & Whitton, 1995). Boulders free from filamentous algae were chosen as filamentous algae support unique diatom communities. If samples are taken from a substrate, which includes filamentous green algae, then those diatoms that are epiphytes, growing on the surface of the filamentous green algae, are also

included. In this case the sample is no longer a true epilithic sample, but rather a mixture of the epilithon and epiphyton. Boulders covered with a layer of sediment were also avoided, as this phenomenon tends to influence the species composition of the samples by confusing epipelic and epilithic habitats (Kelly, 2003). In the absence of boulders, due to the nature of the river at the particular site, the sampling substrate used was dead wood (Kelly *et al.*, 1995). It is important to sample from similar substrates along a river as diatom communities vary according to substrate (Patrick, 1977). Samples were taken in such a way as to obtain the greatest possible degree of uniformity between sites.

Samples were taken at each site by scrubbing the substrate with a small brush and rinsing both the brush and the substrate with distilled water. The resulting diatom suspension was then poured into a 150ml plastic sample bottle. The use of distilled water was seen to be necessary as the usual practice of suspending the diatoms in river water was deemed impractical for the Vaal River due to its nature as a broad slow flowing river. The phytoplankton component of the Vaal River is composed of a large proportion of diatoms (Janse van Vuuren, 2001), which are undesirable in a sample comprising riverine benthos. Cell death due to sudden changes in the ionic composition between river and distilled water was not deemed to be a problem, as preparation for microscopy results in the destruction of the organic components of the cell. The diatom samples were transported to the laboratory in an icebox and stored in a cool dark place to prevent cell division until processing.

2.2.2 Sampling for physical and chemical variables

Simultaneously with biological sampling, pH (using a Wissenchaftlich-Technische Werkstäten WTW Model pH 330/SET-1digital pH meter) and turbidity (using a Hach Company Model 2100P portable turbidity meter) were measured. Oxygen concentration and water temperature were measured using a Yellow Springs YSI Model 54A oxygen/temperature meter.

The Department of Water Affairs and Forestry (DWAF) collected the water quality data used in this study of the relationship between diatom communities and the surrounding aquatic environment as part of their National Chemical Monitoring Programme. This programme has been in operation since the early 1970's and samples are regularly collected at approximately 1600 monitoring sites at a frequency that varies from weekly to monthly. The samples collected for this programme are analysed in the laboratories of the Institute for Water Quality Studies (IWQS), Pretoria, and the data is stored on DWAF's database and information management system, namely the Water Management System (WMS) from which the environmental data used in this study was drawn.

The water quality variables used by DWAF were deemed suitable for the present study as they were chosen to represent a number of specific problems that may be associated with specific land uses and economic activities. These water quality problems include salinisation (measured by EC), acidification by mines and atmospheric deposition (measured by pH) and potential toxicity, the impact of erosion (measured by turbidity), nutrient and other problems associated with sewage treatment works and excess fertiliser application in agriculture (measured by constituents such as NH_4 and dissolved inorganic nitrogen) (Hohls *et al.*, 2002).

Unfortunately although all of the 14 sites chosen for this study were sampled monthly for diatoms for a period of twelve months, environmental data made available for the 4 downstream sites in the Vaal (V10 – V13) is not of a standard which would lend itself to detailed analysis of relationships between diatom species composition and environmental variables. Data for several months or the whole year is missing for these sites (V13) and cannot be obtained from DWAF. Each sampling occasion at each of the 14 sampling sites was viewed as a separate entity or sample. The loss of the above mentioned samples reduced the total number of samples from 168 to 117 (3 extra samples were lost due to the denial of access to one site, V2, and flood conditions at the time of collection for a further 2 samples). Rather than discard the diatom samples in their entirety from the study, some of these samples will be used in the ecological classification of the Vaal River (Figure 2.1) following a detailed analysis of the relationship between diatom communities and environmental variables using the other 10 sites.

The ecological classification of the Vaal River is based on diatom index scores for the month of June 2002. Diatoms bloom in the Vaal River during the winter months (Janse van Vuuren, 2001) making high quality samples with a large number of species possible. Turbidity of the Vaal River is also lower in winter due to reduced flow of the river in winter (Janse van Vuuren, 2001) and thus there are reduced amounts of sediment within the diatom samples. As water levels are decreasing during this time rather than rising, constant exposure of the substrate within the water column is a given. During the winter months in the Vaal catchment little or no rainfall is experienced and thus the chemical constituents of the river system are not diluted on a regular basis. These conditions make winter the ideal time for the ecological assessment of a river system.

2.2.3 Preservation of diatom material

No formalin preservative was added to the fresh samples because Kolbe (1948) pointed out that very weak formalin solutions might damage the fine structure of diatoms. It is known that formalin breaks down into alcohol and formic acid. Riemann (1960) demonstrated that even in water with extremely low formalin concentration, silicic acid is released from diatom valves. Samples were either stored in a refrigerator or, if circumstances dictated a longer period of storage, the samples were fixed with ethanol to prevent possible cell division. To eliminate the risks of silica erosion in samples kept for archive purposes, the formalin preservative used for long term storage was buffered with acetic acid in a ratio of 1:1 (Pienaar, 1988).

2.3 Cleaning techniques

2.3.1 Decalcification of the diatom suspensions

Excepting material from calcium-poor water (e.g. dystrophic water of Northern Europe and high moors), it is almost always necessary to dissolve calcium traces in the sample with hydrochloric acid and then rinse the sample (Krammer & Lange-Bertalot, 2000). This is particularly important if further processing with sulphuric acid is needed, otherwise a calcium sulphate diatom precipitate will form. Decalcification of samples emanating from the Vaal River is particularly important due to the high silt load of the river.

The decalcification procedure is described below:

i) Mix the diatom suspension together with 5 ml concentrated (32%) Hydrochloric acid (HCl) in a beaker.

ii) Boil the resulting solution for 1to 3 hours.

iii) Finally the sample is rinsed by centrifugation until circumneutral.

2.3.2 Removing organic remains

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

It is important to provide a summary of techniques for diatom frustule preparation if biomonitoring using diatoms is to gain general acceptance. Not all laboratories have the

facilities to perform a particular frustule preparation procedure. Thus, a description of various techniques, demanding different levels of technical facilities, is provided.

Acid oxidation is the most commonly used method of preparing diatoms. It effectively removes all organic parts of a cell, including the diatotepum covering membrane, but has the disadvantage that the silica structures of the cell wall are more likely to be damaged. The acids dissolve one of the solid phases of the silicic acid out of the cell wall, so that under higher SEM magnifications the cell wall appears more or less jagged in structure. In LM studies such damage is of little significance (Krammer & Lange-Bertalot, 2000). The use of acids is dependant on the available technical facilities. In the absence of a fume cabinet, all methods employing boiling acids can only be carried out with the greatest caution. A series of techniques, including both acid and non-acid techniques, are described below. The most common method employed to prepare the diatom solutions for light microscopy in this study was that in which a sulphuric, nitric acid mixture is used to clean the frustules (see paragraph a below). When material was required for SEM techniques the use of acid oxidation was avoided, and the more gentle method using hydrogen peroxide was employed (Round *et al.*, 1990).

a) Hot HNO_3/H_2SO_4 method

i) Mix the diatom suspension carefully and take a sub-sample (ca. 5 to 10 ml) to a (50 ml) boiling flask. The size of the sample is dependant on the sample density, which can be judged by the visible concentration of suspended material.

ii) Add 5 ml of a strong acid mixture (HNO_3 + H_2SO_4, 2:1) and place the flasks on a hot plate. The boiling flasks should be covered with a watch glass to prevent contamination between flasks if boiling becomes too vigorous and splashing occurs. Boil the samples for 2-3 hours depending on the amount of organic matter in the sample.

iii) After oxidation of organic material with acid, 1 ml of hydrogen peroxide is added to check if the oxidation process is complete, in which case the hydrogen peroxide will not cause lasting foaming.

iv) When oxidation is complete, the samples are rinsed by centrifuging with distilled water at 2500 rpm for 10 min.

v) The supernatant is then decanted and the washing is repeated a further 3 times. Care should be taken not to lose any material.

vi) The cleaned diatom suspension is placed in small vials (with sample information) in alcohol or distilled water.

b) Hot HCl and $KMNO_3$ method

i) Homogenise sample, place 5 to 10 ml of thick suspension in a beaker.

ii) Add 10 ml saturated potassium permanganate ($KMNO_3$) solution, and leave to stand for 24 hours.

iii) Add 10 ml concentrated HCl, heat on a hot plate at 90°C for 1 to 3 hours until the solution becomes clear.

iv) Rinse the samples as in the previous method a (iv-vi).

Hydrogen peroxide is not as effective as acid methods, but is much gentler as it is not as caustic as an acid mixture. It is best used with samples that require little cleaning, and where corrosion should be limited, as in SEM studies (Krammer & Lange-Bertalot, 2000). Choice of techniques (either hot or cold) depends on the availability of extraction fans. If available the peroxide can be boiled and, if not, a cold method should be used.

c) Hot H_2O_2

i) Mix the diatom suspension, place 5 to 10 ml of suspension into a beaker.

ii) Add 20 ml H_2O_2 and heat on a hot plate at 90°C for 1 to 3 hours.

iii) Add a few drops of HCl and leave to cool.

iv) Rinse the samples as in method a (iv-vi).

d) Cold H_2O_2

i) As in method c described above, with the exception of a hotplate.

ii) Cover beaker with watch glass and leave for a minimum of four days.

iii) Rinse the samples as in method a (iv-vi).

2.4 Preparation of diatom slides

Most of the ultrastructural details of diatoms lie at the limit of resolution of light. In addition, all normal mounting media used in cytology have a refractive index similar to that of diatom valves, so that slides with diatoms mounted in these media are too low in contrast for satisfactory investigation. For this reason diatoms must be enclosed in a medium of higher refractive index than the diatom valves. Three types of mounting media are generally used: "Hyrax" r.i. (refractive index) 1.71 (Hanna, 1930); "Naphrax" r.i. 1.69 (Fleming, 1954) and "Pleurax", r.i. 1.73 (Hanna, 1949; refractive indices after Meller, 1985). To use these mounting media, a hot plate capable of melting them is required.

Permanent diatom slides were prepared as follows:

i) The cleaned diatom suspension is diluted until it appears only slightly cloudy to the naked eye.

ii) A single drop of this suspension is then placed with a pipette on a cover-slip, previously cleaned with ethanol.

iii) After the diatom suspension has spread on the cover slip it should not be touched until dry, as any vibration will cause clumping of the diatom valves.

iv) Diatom coated cover-slips are placed on a heated surface to drive off the excess moisture.

v) After cooling a small quantity of mounting media is placed onto the cover slip with a glass rod or pipette.

vi) A glass slide previously cleaned with alcohol is then lowered onto the cover-slip, inverted and then heated until it 'boils' and all the solvent has evaporated.

2.5 Preparation for SEM

The use of a Scanning Electron Microscope (SEM) is necessary if and when taxonomic difficulties arise in the identification of diatoms under the LM - this is often the case with the smaller centric diatom species. The SEM is used to investigate the finer details of a frustule, e.g. strutted processes, areolae and spines, structures of taxonomic value. Either fresh or cleaned material is filtered through a 1.5 µm Millipore® membrane. The membrane is rinsed with ethanol and allowed to dehydrate completely in a desiccator, sputter coated with gold-palladium and examined under high vacuum at 15 kV, with a spot size ranging from 2.3 to 3.5 depending on required magnification (Taylor, 2003).

2.6 Measuring valve characteristics

During ecological studies the dimensions of valve characteristics may have to be determined in order to correctly identify the diatoms being studied. Valve lengths are measured from apex to apex. Valve widths are usually maximal at the center (Fig. 4a), but in some cases the apices can be broader than the center (Fig. 4d), or the widest point is towards one pole rather than the other, particularly in the heteropolar diatoms (Fig. 4c). Depth of a cell is the distance between valve faces of a single cell (Fig 4b). The depth of the valve mantle (Fig. 4b) may also be important in some cases. Stria densities are measured as the number in 10 µm (Fig 4c, 4d), counted alongside the raphe slits in raphid pennates or halfway towards the apices in araphid diatoms. For centric diatoms the number of rows around the circumference is counted, divided by the circumference and multiplied by 10, to convert to numbers in 10 µm. (This method can also be used for other structures around the valve periphery). Hyaline areas (areas with no ornamentation) are also used as characteristics for identification.

Fibrulae and costa densities are determined in many specimens, while differences in fibrulae width or length, together with evenness of spacing is also useful for identification (Cox, 1996).

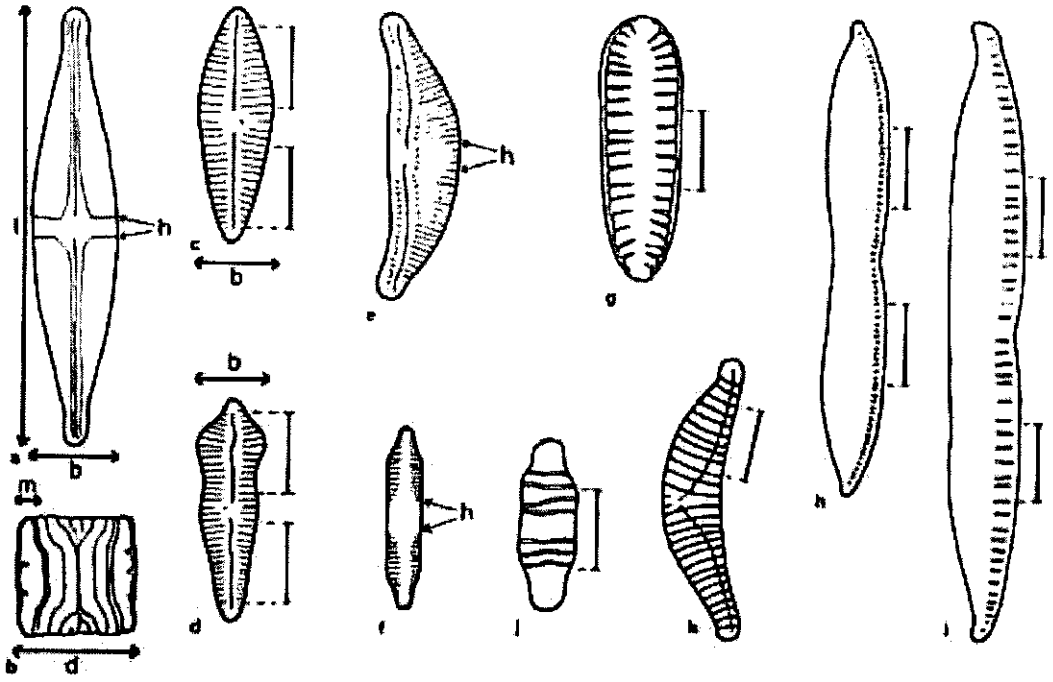

Figure 2.3: *Guide to measuring diatoms. Arrow bars show length (l), breadth (b), mantle (m) or cell depth (d); lines (10 µm) indicate position for measuring stria, costa and fibula densities; h indicates extent of hyaline areas.* ***a****, length and breadth of valve with naviculoid symmetry (transverse hyaline area extending from central areas as in* Stauroneis*);* ***b****, girdle view showing measurement of cell mantle depths;* ***c, d****, maximum breadth nearer one apex than the other (****c****) and very near the apex (****d****) (*Gomphonema *spp.);* ***e, f****, different positions of hyaline areas (****e****,* Amphora*;* ***f****,* Synedra*);* ***g-i****, measurement of fibula density in* Surirella *(****g****) and Nitzschia (****h, i****) sp.;* ***j, k****, measurement of costa density in* Diatoma *(****j****) and* Epithemia *sp.(****k****) (Cox, 1996)*

2.7 Identification, counting, data calculation and management

2.7.1 Identification

Round (1991) stated when discussing diatom identification for water quality monitoring: "The most valuable recent flora is that of Krammer and Lange-Bertalot (1986-91)". This flora was used for identification of all species and for confirmation of species identification by other authors. Other taxonomic guides consulted include Schoeman & Archibald (1976-80), Round *et al.*, (1990), Hartley (1996) and Prygiel & Coste (2000). For revised nomenclature the works of Lange-Bertalot (2001), Krammer (2002) and Kellogg & Kellogg (2002) were consulted.

Diatom taxonomy has recently and is currently undergoing many changes. This is mainly due to the splitting of large genera such as *Navcula* and *Nitzschia,* into which diatom species that exhibit a bilateral symmetry and diatoms with both a bilateral symmetry and a canal-raphe have been traditionally placed. There is now consensus amongst diatom taxonomists that the diatom genus *Navicula* is restricted to the group within the Naviculaceae known as the Lineolatae (linear shaped poroids – F.E. Round, pers. comm.*; Lange-Bertalot, 2001). This has lead to the creation of new genera by encapsulating species, which used to belong to the genus *Navicula.* Examples of these new genera are *Luticola* (Mann in Round *et al.,* 1990), *Fallacia* (Sickle & Mann in Round *et al.*, 1990) and *Microcostatus* (Johansen & Sray, 1998).

2.7.2 Counting

Counts of diatom valves on slides were made using a Zeiss microscope with phase contrast optics (1000x). A count of 300 to 500 valves per sample was used for data calculation, as recommended by Prygiel *et al.* (2002). The microscope field was used as the area to define the limits of the count. Adjacent fields were counted to obtain the total number of valves required per sample. Each valve is counted as 1 unit, the reason being that a single valve indicates the presence of a whole diatom frustule within the sample. When a whole frustule (2 united valves) is encountered it is counted as a single unit (1 whole diatom frustule). The reason for the adoption of this technique being that some diatom valves dissociate more easily than others, which could possibly skew count results. Broken valves are also included in the counts if more than 50% of the valve is unbroken and positive identification is possible. Valves in girdle view are also identified (when recognisable) and included in the counts. All slides used for counting as well a wet sample material is stored in the diatom collection of the Division Botany, School of Environmental Science and Development, Potchefstroom Campus, North-West University, and is available on request.

2.7.3 Calculation of diatom indices

The indices used here are known as Descy's index or DES (Descy 1979), the Generic Diatom Index or GDI (Coste & Ayphassorho, 1991), the Specific Pollution sensitivity Index or SPI (Coste in CEMAGREF, 1982), the Biological Diatom Index or BDI (Lenoir & Coste, 1996), the Artois-Picardie Diatom Index or APDI (Prygiel *et al.*, 1996), Sládeček's index or SLA (Sládeček, 1986), Leclercq & Maquet's Index or LMI (Leclercq & Maquet, 1987), the Commission of Economical Community Index or CEC (Descy & Coste, 1991) Schiefele and Schreiner's index or SHE (Schiefele & Schreiner, 1991), the Trophic Diatom Index or TDI

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(Kelly & Whitton, 1995), and the Watanabe index or WAT (Watanabe *et al.*, 1986; Watanabe, 1990). In all cases except in the CEC, SHE, TDI and WAT index, the diatom indices are calculated using the formula of Zelinka & Marvan (1961) (See Chapter 1). For all of the above indices, except TDI (maximum value of 100), the maximum value of 5 (converted to 20 by the software package OMNIDIA; Lecointe *et al.*, 1993) indicates clean water.

2.7.4 Data management and statistical analysis

Several chemical determinants were derived from the original data set. Dissolved inorganic nitrogen (DIN) was calculated from the summation of NH_4-N, NO_3-N plus NO_2-N. For the purpose of this study, the dissolved inorganic phosphorus (DIP) concentration was considered to be equivalent to the PO_4-P concentration.

pH is defined as the negative logarithm of the hydrogen ion concentration. In order to represent pH data each value should be converted to its hydrogen ion concentration (Rector, 1995). However, this method of averaging pH makes the most difference if pH is measured to the nearest whole number. If pH is measured to the nearest 0.001 unit, simply averaging the pH values will give almost the same result as averaging the hydrogen ion concentration. Mattson (1995) is of the opinion that converting pH to hydrogen ion concentration is not the best approach in every situation. Studies of acid precipitation and acid inputs to watersheds are concerned with the rate of loading with hydrogen ions. These studies assume that hydrogen ions behave conservatively, which is a reasonable assumption because most rainwater has little or no buffering capacity. However, studies of surface water quality usually have another purpose. Most such studies (including this study) are not concerned with determining mass balances or loading rates of hydrogen ions. Surface waters contain buffers, and thus hydrogen ions do not behave conservatively and loadings cannot be logically calculated. Instead, the purpose of surface water studies is usually to summarise the central tendency at a number of sites, to compare these sites and to provide descriptive statistics. In most cases, surface water acidity data are more nearly normal in their distribution when expressed as pH rather than hydrogen ion concentration (Mattson, 1995). For this reason pH data in this study was not converted to hydrogen ion concentration as the distribution of the data was normal and the data could be used in correlation analysis without $\log_{10}$ transformation.

For the representation of average chemical variables over a period of one year non-parametric statistical methods were used. All the environmental data collected and used in this study had a non-normal distribution, except pH which is a log value. To resolve a

potential misrepresentation of the data and skewing of the estimates central tendency, parametric representations of central tendency such as means and standard deviation were altogether avoided as advised by Mattson (1995). Instead non-parametric statistical methods (median, range and interquartiles) were used to represent the average annual data.

Prior to statistical analysis for correlation, the water quality data was analysed for normality (STATISTICA version 6). Where the data showed a skewed distribution, the data was $\log_{10}$ transformed (Bate *et al.*, 2002). Standard two-way correlation techniques carried out using STATISTICA version 6 were used to determine the relationship between environmental variables and index scores. The numerical values were considered significant at $p < 0.01$ or higher (Kwandrans *et al.*, 1998).

In the case of results recorded below the detection limit for a specific chemical determinant (e.g. NO_2-N and NH_4-N) a 50% value of the detection limit +1 unit (e.g. a detection limit of 0.05 divided by two, plus one unit becomes 0.026) was used as an actual value in the statistical analyses.

It was observed during this study that many of the environmental variables considered were highly inter-correlated, or in other words, the variables were multi-colinear. The problem of colinearity is not unusual with environmental data of this nature (Jongman *et al.*, 1987). To overcome the problem of colinearity, as well as to simplify the understanding of the data, Pearson correlation (also called linear or product-moment correlation; Pearson, 1896) was used on the environmental data to determine how closely different variables were related to one another. The most widely-used type of correlation coefficient is Pearson r. Special attention was paid to those elements of water quality e.g. electrical conductivity (EC) and total dissolves solids (TDS), which have known correlations with major ionic compounds (see Chapter 3, Table 3.1). Applying correlation techniques produces key variables, which describe most of the variation in the environmental data. A reduction in the dimensionality of the data was, therefore, achieved.

Acronyms or code names for the species used in the study follow those used in the computer programme OMNIDA (Lecointe *et al.*, 1993). These in general follow the simple rule that the first letter of the acronym finds its origin the generic epithet of a given taxa which is followed by three letters which are usually the first three letters of the specific epithet. Exceptions to this rule occur and for this reason a full list of the taxa encountered in this study as well as the acronyms used is given in Appendix 1. The programme OMNIDIA also gives a full list of codes/acronyms.

2.7.5 Computer programs

Data on diatom community counts and environmental variables were entered into spreadsheets using MICROSOFT EXCEL 2000 for WINDOWS 98. This program was used to calculate the monthly and annual averages for the environmental data and to $\log_{10}$ transform non-parametric data.

Diatom index values were calculated in the database program OMNIDIA (Lecointe *et al.*, 1993).

Correlation and regression analyses were carried out using the statistical analysis software package STATISTICA version 6. Box and whisker plots of environmental variables and diatom index scores were also drawn with the aid of this program.

All resultant graphs were imported into the word processor program MICROSOFT WORD 2000 for WINDOWS 98.

All programs used during this study are registered versions available for use in the Division Botany, School of Environmental Sciences and Development, Potchefstroom Campus, North-West University, Potchefstroom.

CHAPTER 3

RESULTS AND DISCUSSION - CHEMICAL AND PHYSICAL VARIABLES

3.1 Introduction

The primary aim of the present study is to determine which of the tested diatom indices have the closest relationship to measured water quality variables. All of the diatom indices used in this study were developed outside South Africa and there is no data available prior to the present study as to how numerical diatom indices will react to South African water quality conditions. The present study is the first in what is hoped will be the beginning of a succession of studies to test, modify and develop diatom indices as part of the suite of biomonitoring tools for use in South Africa. To achieve the aim of diatom studies becoming part of a national monitoring programme, it is necessary to dispel certain doubts that have been, and are being expressed as to the validity of the use of such a biomonitoring tool in South Africa (see Chapter 1). The concerns expressed all have to do with the transfer of data between the two hemispheres concerning ecological tolerance limits of diatom species. In order to lay these concerns to rest, diatom indices need to be tested against prevailing water quality variables in South Africa.

The Vaal River system is suitable for testing of the diatom indices in that the condition of the river is known and also historically well documented. Even without statistical analysis one can gain a fairly good idea of whether the diatom indices function or not. It is known that the quality of the Vaal River deteriorates from the Vaal Dam downstream to Bloemhof Dam, and that the best quality waters can be found in the Vaal Dam catchment (Braune & Rogers, 1987).

In Chapter 3 an overview will be given of all the physical and chemical water quality variables measured in this study, with the aim of providing the reader with some idea of the extent of the degradation in the different sections or stretches of the Vaal and Wilge Rivers. However, it is not intended in this Chapter to give a detailed description of the impact of the various measured water quality variables on the riverine ecosystem. Detailed discussions of water quality variables and the relationships between these variables may be found in Dallas & Day (1993).

The environmental conditions prevalent in the Vaal River have been studied since 1984 (Pieterse, 1986a). Information on the types of algae present, the seasonality of their occurrences and environmental factors controlling their growth and succession became

available through the studies of Pieterse (1986a, 1986b, 1987). Janse van Vuuren (1996, 2001) gives an in-depth discussion of the relationships between water quality variables and the structure of planktonic algae communities in the Vaal River.

Aspects of each of the water quality variables measured will be discussed in order to present an overall picture of the condition of the river at each of the sampling sites for the duration of the study. Special attention will be given to the water quality variables upon which the diatom indices are based. Special attention will also be given to those water quality variables that are the cause of specific problems such as eutrophication and salinisation.

3.2 Physical variables

3.2.1 Temperature

Temperature as a water quality variable is briefly discussed below as it does not strongly influence water quality in the Vaal River or the results generated by diatom index scores. Although diatom species composition may change seasonally, the taxa forming the association remain indicative of a particular environmental condition. The association would therefore reflect the overall ecological condition of the water body rather than temperature changes coupled with the seasons (Schoeman, 1979).

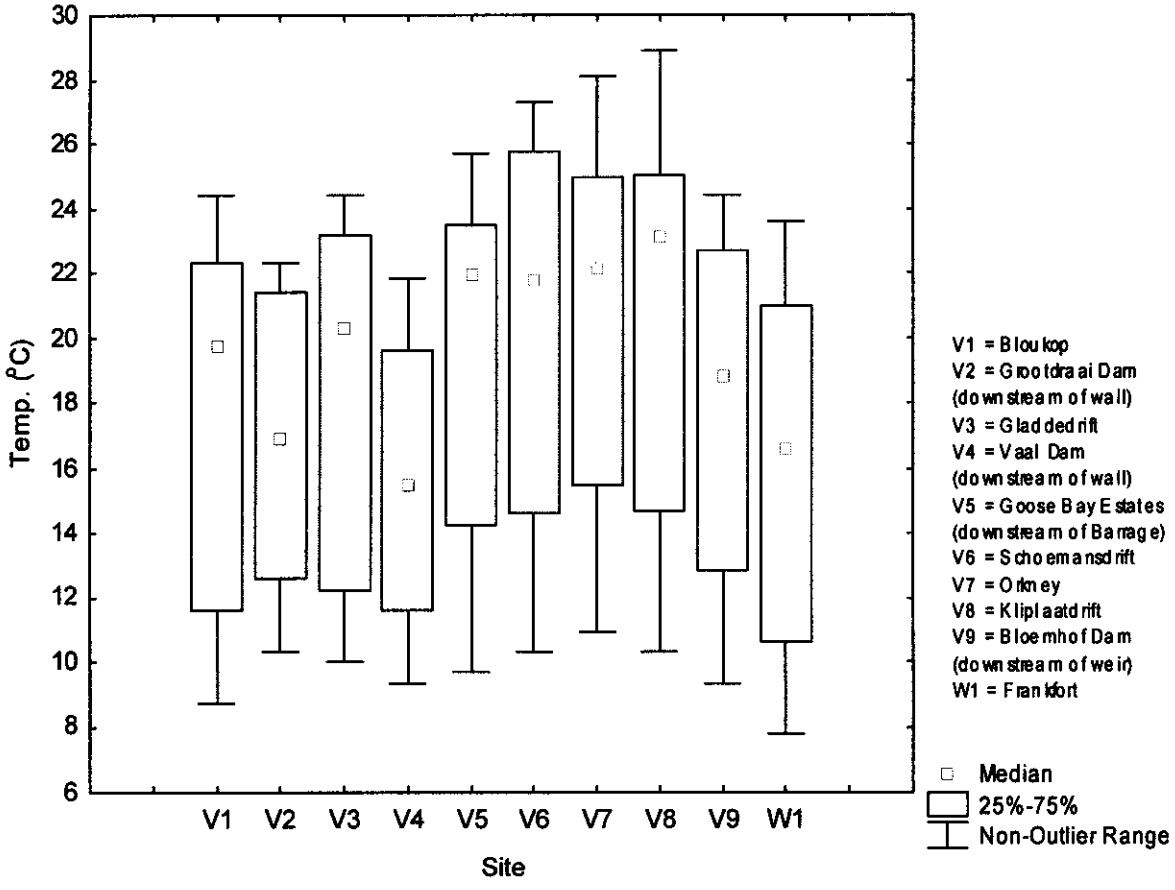

Figure 3.1: *Median water temperature values at selected sites in the Vaal and Wilge Rivers, for the period March 2002 to February 2003*

In general the temperature of a river system increases from the headwaters to the downstream zones. In addition, a temperature increase due to climatic changes can be expected in the Vaal River as it flows from east to west across South Africa. Additional temperature changes due to the influence of impoundments are discussed below.

The median values for water temperature measured in the Vaal River for the period March 2002 to February 2003 are presented in Fig. 3.1. When Fig. 3.1 is examined, a general temperature increase from the site nearest to the source of the Vaal River at Bloukop (V1) to site V8 at Kliplaatdrift before the Vaal River flows into the Bloemhof Dam can be observed. Median temperature decreases downstream of the Bloemhof weir.

Several noteworthy interruptions occur in the increasing temperature trend from sit V1 to site V8. These interruptions, in the form of lower median temperatures, can be related to the presence of bodies of impounded water. Site V2 is located immediately downstream of Grootdraai Dam, V4 immediately downstream of the Vaal Dam wall and site V9 immediately downstream of the Bloemhof Dam Weir. The coolest water is yielded by the Vaal Dam, which can be accounted for by the size and depth of the Dam in comparison the smaller shallower Grootdraai and Bloemhof Dams. The influence of impoundments, which have deep release (e.g. the Vaal Dam), is to decrease the temperature in summer and increase the temperature in the winter in rivers (Pitchford & Visser, 1975). The decrease in temperature also affects seasonality as experienced by the organisms resident within the river. Visible blooms of the chain-forming diatom *Diatoma vulgare* as well as blooms of *Cymbella* spp., that normally occur at the onset of winter, were displaced by several months at the site (V4) immediately downstream of the Vaal Dam. Hence the cooler water released from the Vaal Dam has a direct influence on diatom communities. This intraspecific selection for species with a particular temperature optimum within communities is one of the effects listed by Howells (1983) as caused by temperature change in riverine ecosystems.

Water flowing in the Wilge River (W1) is cooler as it has its origin in the Sterkfontein Dam. The Sterkfontein Dam is situated in the upper reaches of the Vaal Dam catchment on the Nuwejaarspruit, a few kilometres from the edge of the Drakensberg escarpment. The location of the Sterkfontein Dam, as well as the depth of the impoundment, dictates cooler water temperatures.

3.2.2 Turbidity

This section provides a short summary of the prevailing annual turbidity of the Vaal and Wilge Rivers. Special attention will be given to the positive effects of elevated levels of

turbidity in a nutrient rich riverine ecosystem. The immediate visual effect of a change in water turbidity is a change in the water clarity. Water turbidity in the Southern Hemisphere is generally considered to be equivalent to some measure of the concentration of inorganic suspensions (Dallas & Day, 1993). The natural seasonal variation in rivers often results in changes in turbidity (Harrison & Elsworth, 1958), the extent of which is governed by the basic hydrology (e.g. flow regime), rainfall and geomorphology (e.g. weathering, aspect of slopes) of the particular region. In South Africa most rivers become highly turbid and are laden with suspended solids during the rainy season (Chutter, 1969). Erosion of land surfaces in catchment areas by wind and rain is a continuous and historically natural process. However, land-use practices such as overgrazing and non-contour ploughing accelerate erosion and result in increased quantities of suspensoids in associated rivers (Dallas & Day, 1993).

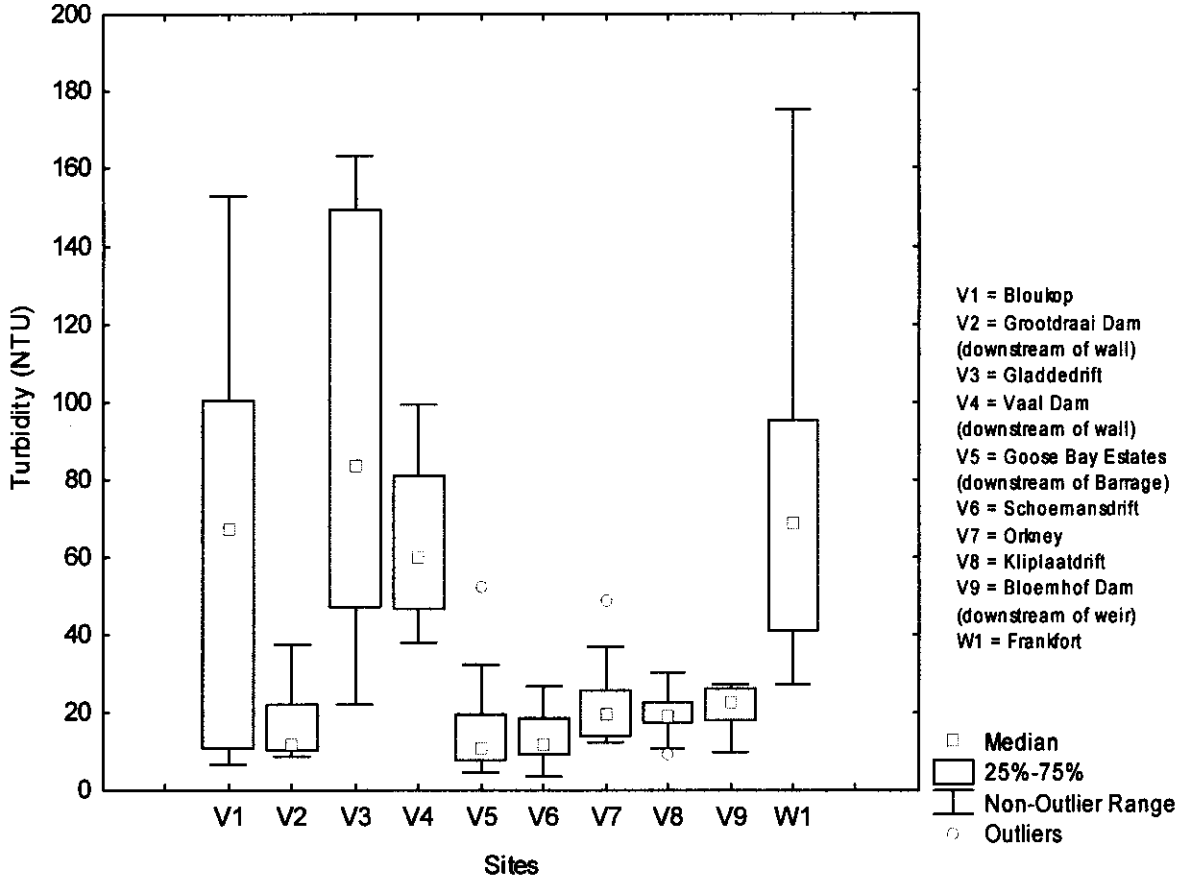

Figure 3.2: *Median turbidity values at selected sites in the Vaal and Wilge Rivers, for the period March 2002 to February 2003.*

The median values for turbidity measured in the Vaal River for the period March 2002 to February 2003 are presented in Fig. 3.2. Fig. 3.2 shows that four sites (V1-Vaal River at Bloukop, V3-Gladdedrift, V4-downstream of Vaal Dam Wall and W1-Wilge River at Frankfort) have particularly high median levels of turbidity.

Site V1 is located in the upper reaches of the Vaal where the river flows more rapidly. The turbidity is reduced due to the settling of suspensoids in the Grootdraai Dam (V2). High levels at site V3 may be due to intensive agriculture and irrigation in the region of this site. Water flowing from the Vaal Dam is turbid due to the fact that water is released from the lower part of the water column of the impoundment, and hence has an intrinsic sediment load; increased velocity caused by the release from the Vaal Dam increases turbidity directly downstream of the wall (site V4). Braune & Rogers (1987), discussing the possible impacts of the Lesotho Highlands Water Project on the Vaal Catchment, stated that the low salinity and turbidity of the water originating in the Lesotho Highlands would have a greater erosive power than Vaal catchment water, and could enhance riverbank erosion. The greater erosive power, together with an increased water volume and velocity from water transfer, are reflected in the high median turbidity value of water in the Wilge River at site W1(Fig. 3.2).

In Chapter 4 it will be shown that there is a positive correlation between turbidity and diatom index scores. Usually a negative correlation can be noted between water quality variables and diatom index scores (i.e. as index scores increase as concentrations or levels of water quality variables decrease). An attempt to explain why elevated levels of turbidity may have the effect of increasing water quality now follows.

In turbid waters light penetration is reduced (Kirk, 1985), leading to a decrease in photosynthetic rates and, in turn, reduced growth rates of submerged aquatic plants (Guenther & Bozelli, 2004). Turbidity levels as low as 5 NTU can decrease primary productivity by 3 to 13% (Ryan, 1991). Fig. 3.3 shows that the annual median turbidity values exceed 5 NTU. Although turbidity can have several deleterious effects (e.g. smothering and abrading of aquatic biota) if viewed from the perspective of decreasing light penetration, it may be advantageous for a highly polluted river system such as the Vaal. The decreased light levels penetrating the water, due to elevated levels of turbidity, to some extent ameliorates the effects of the almost constantly elevated levels of plant nutrients in the middle and lower Vaal River regions. Algae require light to photosynthesise as well as sufficient nutrient levels for growth and reproduction. Turbidity may also decrease the temperature of a river or stream as more heat is reflected and less absorbed by the water (Dallas & Day, 1993). A reduction in temperature may be limiting to cyanobacterial bloom-forming organisms such as *Oscillatoria* sp., which grows best at elevated water temperatures (Venter *et al.*, 2003). Janse van Vuuren (2001) demonstrated that increases in the turbidity of the Vaal River were often accompanied by decreases in algal unit (i.e. cells, colonies, etc.) and chlorophyll-*a* concentration. This decrease in the primary productivity of

the water was ascribed to limited light availability and adsorption of nutrients onto suspended material.

3.3 Chemical variables

3.3.1 Dissolved Oxygen (DO)

This section provides a short summary of the importance of dissolved oxygen for aquatic ecosystems as well as the concentration of DO measured during this study in the Vaal River. Oxygen concentration in the water is one of the most important abiotic factors relating to the survival of most aquatic organisms (Dallas & Day, 1993). Dissolved oxygen concentrations naturally fluctuate diurnally as a result of photosynthesis by plants (during the day) and respiration (day and night) of aquatic plants and animals (Lloyd & Swift, 1976). However, even though the concentration of dissolved oxygen is very important for the health of ecosystems, DO concentration was found to have no correlation with diatom index scores in this study (see Chapter 4). The median values for dissolved oxygen concentration measured in the Vaal River for the period March 2002 to February 2003 are presented in Fig. 3.3.

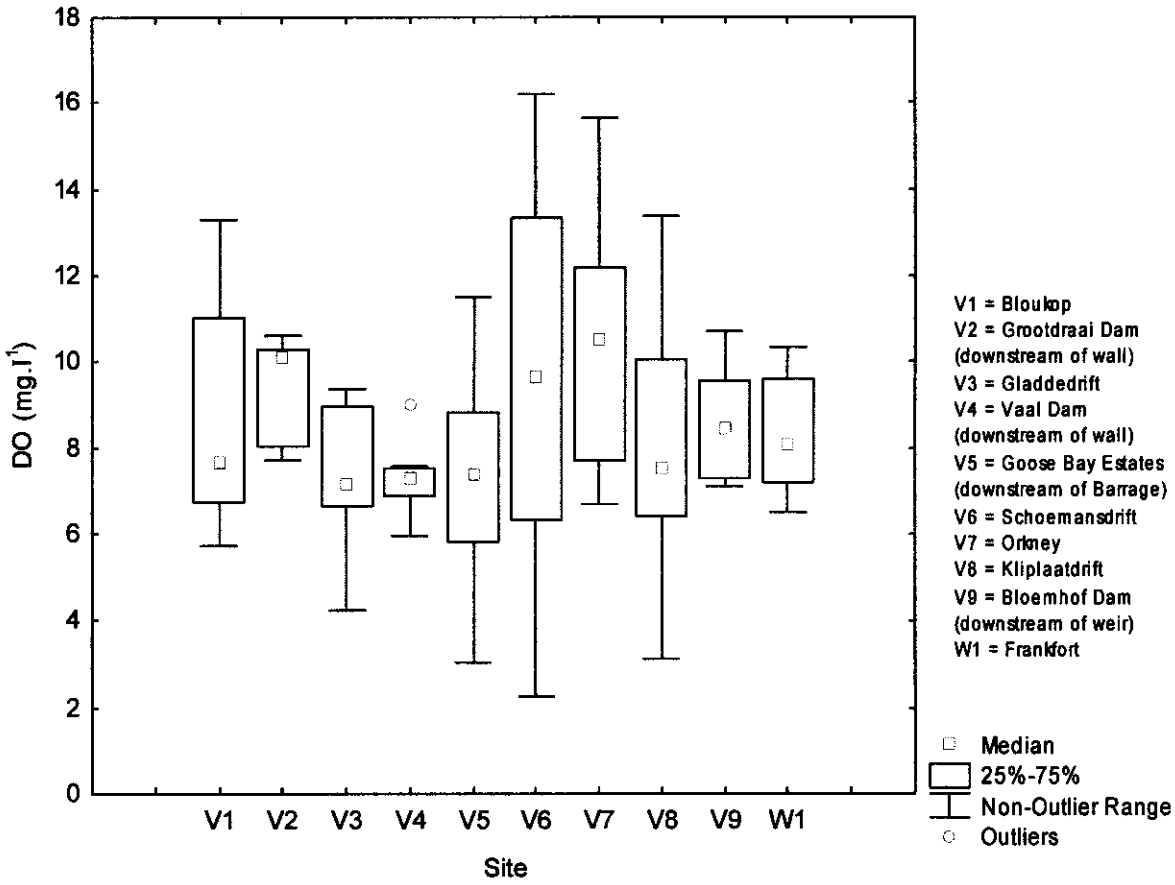

Figure 3.3: *Median DO concentrations at selected sites in the Vaal and Wilge Rivers, for the period March 2002 to February 2003.*

In general median dissolved oxygen concentrations do not fall below 7 $mg.l^{-1}$. The Vaal River has elevated levels of plant nutrients ensuring continuously high primary production with the

concomitant high levels of oxygen. Current standards in South Africa for the protection of aquatic life require that the concentration of dissolved oxygen be in excess of 4 $mg.l^{-1}$. In Australia (Hart *et al.*, 1992) guidelines state that dissolved oxygen should not decrease below 6 $mg.l^{-1}$. Fig. 3.3 shows that the annual median values for oxygen concentration in the Vaal River do not fall below the prescribed lower limit of 4 $mg.l^{-1}$ (DWAF, 1995). Some exceptions to the relatively high concentrations of DO measured at the study sites did, however, occur.

A low oxygen concentration (2.27 $mg.l^{-1}$) was observed at site V6 (Schoemansdrift) during November 2002. This low concentration may be ascribed to a large mat (several km's long) of water hyacinth (*Eichhornia crassipes*) floating on the Vaal River above the sampling point. Water hyacinths reduce the diffusion of oxygen at the surface and reduce surface turbulence. In addition, the thick mat of plants block light from entering the river. Without light, riverine algae and submerged macrophytes cannot photosynthesise and replenish oxygen by respiration.

3.3.2 pH and Alkalinity

The concentration of hydrogen (H^+), hydroxyl (OH^-), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions are some of the most important attributes determining the quality of water. The present study demonstrates that pH is one of the factors influencing diatom index scores and will be discussed in some detail below and again in Chapter 4.

pH is largely determined by the concentration of hydrogen ions (H^+) in the water and is defined as the negative $\log_{10}$ of the hydrogen ion activity. Because pH is a log scale, a change of one unit means a ten-fold change in H^+ concentration. If a given quantity of acid or alkali is added to pure water, the pH changes rapidly. The pH of a water sample determines the species in which numerous dissolved inorganic elements and molecules are found in that water. For instance, ammonium ions (NH_4^+) are not generally toxic, and indeed are the main form in which nitrogen is assimilated by most plants. At a pH above about 8, however, it is gradually converted to toxic un-ionised ammonia (NH_3^+; Gammeter & Frutiger, 1990).

The median values for pH measured in the Vaal River for the period March 2002 to February 2003 are presented in Fig. 3.4. The pH values recorded in the present study in the Vaal River ranged from 7.95 to 8.82, with a maximum value of 9.35 recorded at site V1 at Bloukop and a minimum value of 7.15 recorded at site V7 (Orkney). Median pH of the Vaal River increases downstream from site V1 (Bloukop, close to the source of the Vaal River) to site V8 (Kliplaatdrift) and decreases at V4 (downstream of the Vaal Dam) and V9 (downstream of

the Bloemhof Dam). pH in the Wilge River at site W1 ranged from 7.8 to 8.05 with a maximum of 8.3 and a minimum of 7.1 and the median pH in the Wilge River was lower than any of the studied sites in the Vaal River. The lower median pH at site W1 (Wilge River at Frankfort) could be due to the slightly acidic nature of water originating in the Lesotho Highlands (Braune & Rogers, 1987).

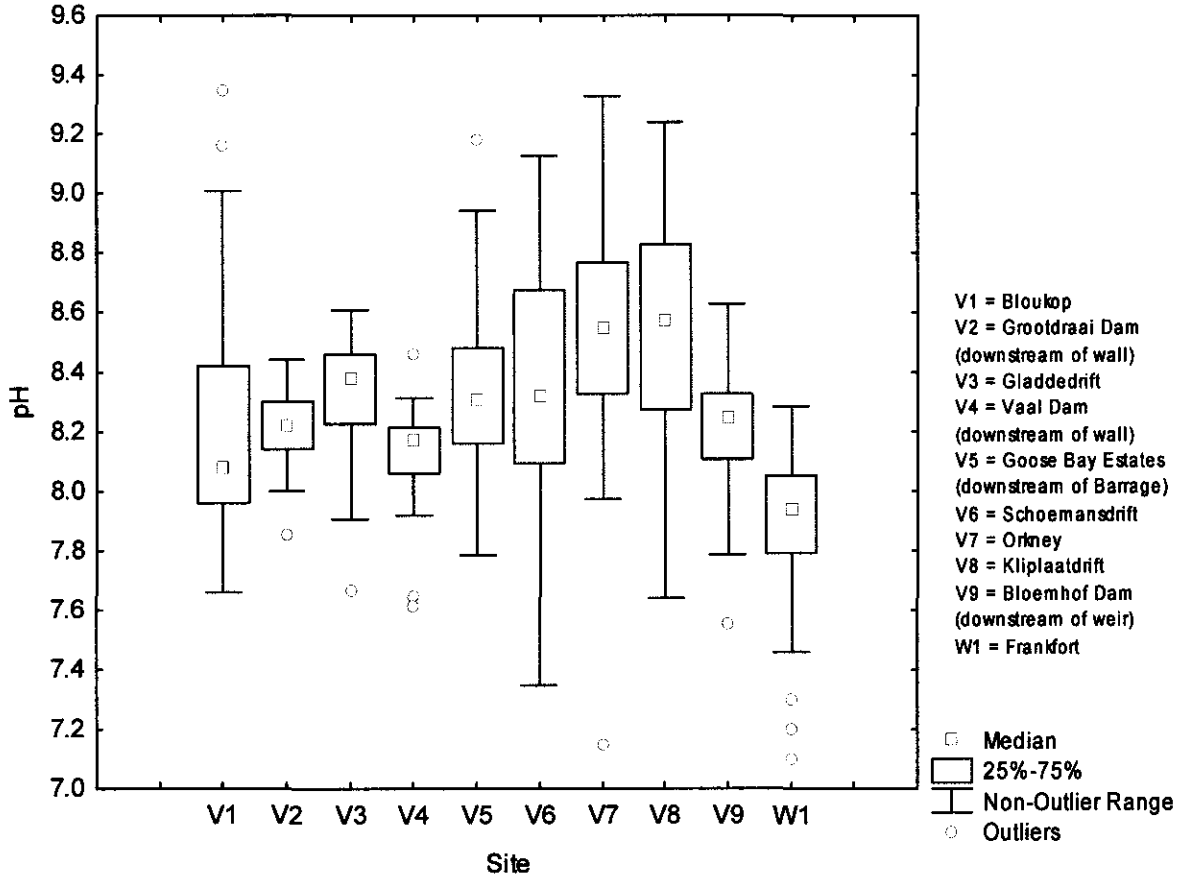

Figure 3.4: *Median pH values at selected sites in the Vaal and Wilge Rivers, for the period March 2002 to February 2003.*

The range of pH measured in the Vaal River during this study showed a similar distribution to that recorded by Janse van Vuuren (2001). Janse van Vuuren (2001) showed that pH at different localities in the middle Vaal River ranged between 7.0 and 10.0. A minimum pH of 6.8 was recorded and maximum of 10.2. The pH of natural waters is determined by geological and atmospheric influences. The pH of natural waters ranges between the extremes of < 2 to 12 (Wetzel, 1983). Most freshwaters, including most in South Africa, are relatively well-buffered and more or less neutral, with pH ranges of around 6.0 to 8.0 (Dallas & Day, 1993). Fig. 3.4 shows that at some sites (V6 and V8) in the Vaal River, the annual pH range is over one and a half pH units. Dallas & Day (1993) recommended that the pH of streams should not be permitted to change by more than one pH unit, as a change in pH normally encountered in unpolluted streams may have serious effects upon the biota.

Together with pH, total Alkalinity has been shown in the present study to influence diatom index scores significantly (see Chapter 4). Alkalinity refers to the sum of the anions (OH^-, CO_3^{2-}, HCO_3^-) of weak acids plus hydroxyl ions and bicarbonate, in a sample of water (Mackereth *et al.*, 1978). The milliequivalents of acid necessary to neutralise the hydroxyl, carbonate, and bicarbonate ions in a litre of water are known as total alkalinity (TAL; Wetzel, 1983). Alkalinity is thus usually an estimate largely of the concentration of bicarbonate and carbonate ions, although other ions contribute a small amount in some waters (Dallas & Day, 1993).

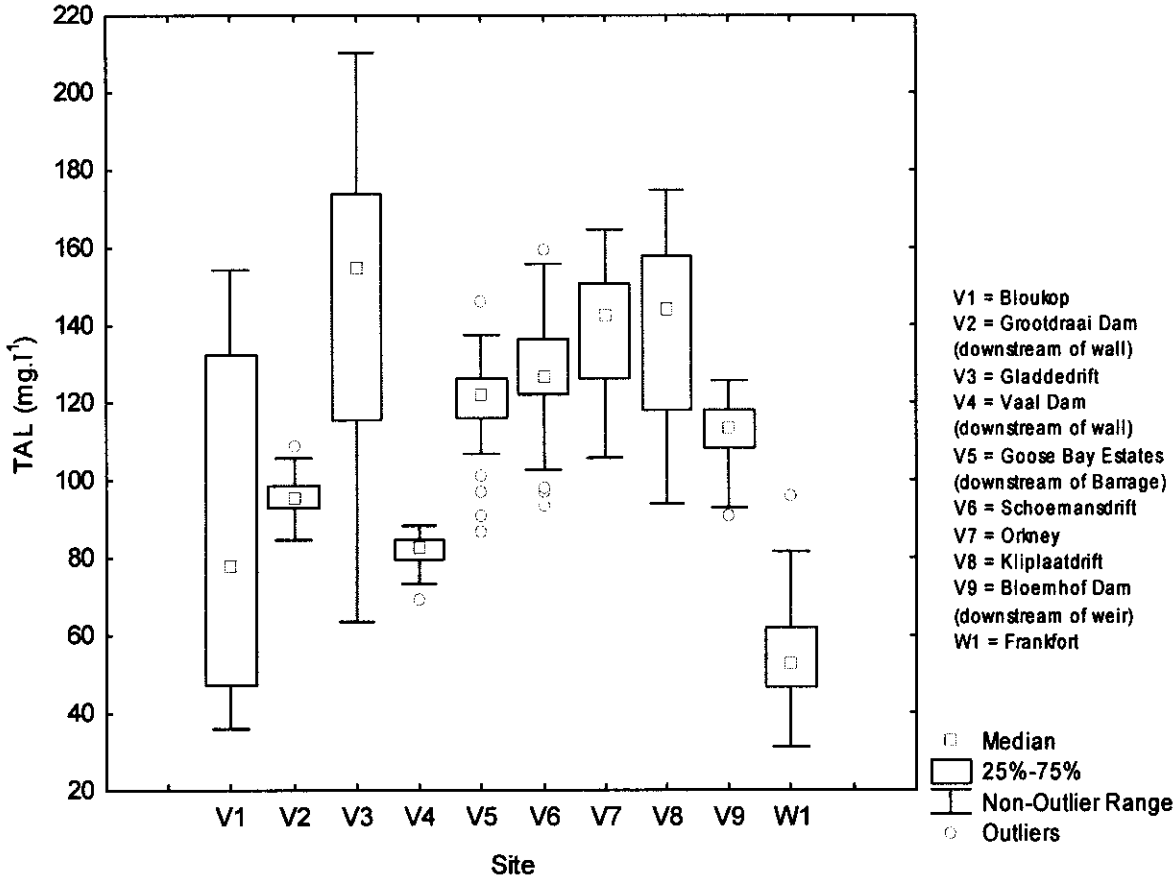

Figure 3.5: *Median total alkalinity (TAL) concentration at selected sites in the Vaal and Wilge Rivers, for the period March 2002 to February 2003.*

The median values for total alkalinity (TAL) measured in the Vaal River for the period March 2002 to February 2003 are presented in Fig. 3.5. Fig. 3.5 shows that total alkalinity (TAL) in general follows a similar trend to pH experienced at the sample sites. V3 at Gladdedrift however, displays the highest median value for TAL (155 $mg.l^{-1}$). In contrast to TAL, the highest median pH was experienced at site V8 at Klipplaatdrift. The lowest median TAL (52 $mg.l^{-1}$) was experienced at site W1.

Alkaline pollution of rivers is less common than acid pollution. Increases in pH can result from certain alkaline effluents from industries such as food canning and textile production, as

well as from anthropogenic eutrophication. Perhaps one of the above mentioned sources is responsible for the high TAL values measured at site V3 (Gladdedrift), although no information is available in the present study from which such deductions could be made.

3.3.3 Plant nutrients

The Vaal River is known to be eutrophic (i.e. elevated concentrations of plant nutrients, especially inorganic nitrogen and phosphorus, are present in the system) for most of its length. Plant nutrients include elements required for normal plant growth and reproduction. In this sense, plant nutrients include carbon, nitrogen, phosphorus, potassium, calcium, magnesium, sulphate and silica, as well as elements termed "micro-nutrients", required in much smaller quantities (e.g. Salisbury & Ross, 1992). Nutrient enrichment, termed eutrophication, can lead to an imbalance in biological communities, particularly to an increase in numbers of plant communities and associated water problems. The major nutrients that contribute to eutrophication are phosphorus as phosphate ions (PO_4^{3-}) and nitrogen as nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) ions (Davies & Day, 1986). PO_4^{3-} has been implicated more often than inorganic nitrogen as a limiting factor in freshwater systems (Hart *et al.*, 1992). As the listed nutrients contribute to eutrophication, the concentrations of these nutrients in aquatic ecosystems need to be constantly monitored. Diatoms community compositions may provide an accurate indication and prove useful for monitoring nutrient levels within aquatic ecosystems (see Discussion in Chapter 4). Most nutrients are not toxic (exceptions include nitrogen-derivatives ammonia and nitrite), even in high concentrations, but when present in aquatic systems in high concentrations, they may have a significant impact on the structure and functioning of biotic communities (Dallas & Day, 1993).

Organic discharges (also referred to as oxygen–demanding wastes), although not measured in this study, are important sources of plant nutrients. Organic discharges generally deplete or reduce the concentration of dissolved oxygen and increase the turbidity and concentration of suspended solids in the receiving water as a result of aerobic decomposition of the waste by micro-organisms. Compounds containing nitrogen and phosphorus are normally present in large amounts in organic discharges. The partial degradation of proteins and other nitrogenous material can lead to elevated ammonia, nitrite and nitrate concentrations in water (Hellawell, 1986). Organic discharges may result in the elimination of intolerant species, reduction in sensitive species and increased potential plant growth in otherwise nutrient-poor waters.

a. Dissolved inorganic phosphate (DIP)

The process of eutrophication has been primarily ascribed to elevated levels of dissolved inorganic phosphate (DIP) entering the aquatic ecosystem. Eutrophication affects the quality of water resources in several ways (see discussion in Chapter 1). In Chapter 4 a significant negative correlation has been demonstrated between diatom index scores and concentrations of DIP. A more lengthy discussion of DIP is, therefore, warranted and is given below.

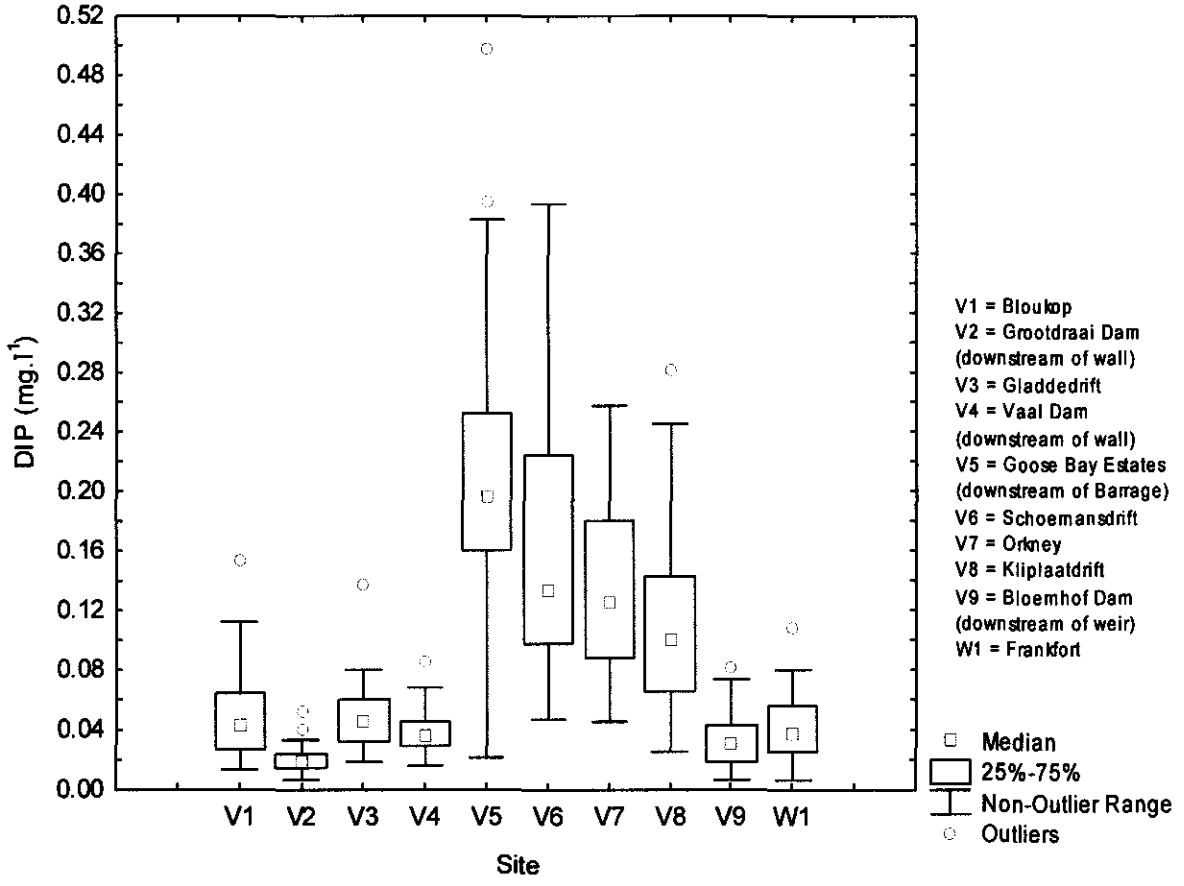

Figure 3.6: *Median DIP concentration at selected sites in the Vaal and Wilge Rivers, for the period March 2002 to February 2003.*

The median values for DIP measured in the Vaal River for the period March 2002 to February 2003 are presented in Fig. 3.6. Site V2 downstream of the Grootdraai Dam experienced the lowest median concentrations (0.02 $mg.l^{-1}$) of DIP during the study period. The highest median DIP concentration (0.195 $mg.l^{-1}$) was recorded for site V5 at Goose Bay Estates downstream of the Vaal Barrage, almost 10 fold the concentration experienced at site V2. Site V5 is located just below the Vaal Barrage. It will be seen in discussions in the following sections that DIP is not the only water quality variable that becomes drastically elevated after the Vaal Barrage. In general the decrease in water quality indicated by elevated concentrations of dissolved inorganic phosphate can be attributed to return flows from the industrial heartland of Gauteng (DWAF, 1993).

The high median value for DIP (0.035 $mg.l^{-1}$) experienced at site W1 in the Wilge River at Frankfort is worthy of note. It would not be expected that water, which has its origin in the Lesotho Highlands, would be eutrophic. However, although the water that flows into the Wilge River may not have originally been eutrophic, by the time it reaches site W1 it is enriched with enough phosphate to warrant the classification of eutrophic. The most probable source of the high levels of plant nutrients is agricultural runoff into the Wilge River as it flows through the Freestate Province. Most fertiliser applications are often in excess of actual requirement, and are an important source of plant nutrients (Walmsley, 2000).

Site V9, downstream of the Bloemhof Dam, is also worthy of note because median phosphate concentrations showed a recovering tendency so as to be lower than those experienced at site V4 below the Vaal Dam. The lower median value for DIP (0.03 $mg.l^{-1}$) at site V9 can be attributed to adsorption of DIP to sediments and phosphate removal by algae and macrophytes, such as water hyacinths, which form thick mats particularly in the downstream reaches of the river (see Janse van Vuuren, 2001), and on the Bloemhof Dam (author's observation). In addition, downstream of Bloemhof Dam the upstream pollution affects are ameliorated to some extent by inflow of water from the east (Braune & Rogers, 1987).

The trophic status of a system is classified according to either the annual average for total phosphorus (TP; OECD, 1982) or the summer concentration of phosphate (PO_4-P; DWAF, 1995). According to DWAF (1995) aquatic ecosystems with an average summer PO_4-P concentration ranging from 0.005 to 0.025 $mg.l^{-1}$ can be classified as mesotrophic. Eutrophic conditions are experienced at a PO_4-P concentration of 0.025 to 0.25 $mg.l^{-1}$. If the annual median values for PO_4-P concentration are examined (Fig. 3.6) it can be assumed that the classification of eutrophic can be applied to all of the sites studied (see also Janse van Vuuren, 2001) with the exception of site V2 downstream of the Grootdraai Dam, which can be classified as mesotrophic.

b. Dissolved inorganic nitrogen (DIN)

For the purposes of this study the sum of nitrate (NO_3-N), nitrite (NO_2-N) and ammonium (NH_4-N) ions will be referred to as dissolved inorganic nitrogen (DIN). Dissolved inorganic nitrogen is the second element deemed to be responsible for the elevated levels of aquatic plant reproduction and growth, commonly known as eutrophication. Because elevated levels of plant nutrients have an influence on diatom index scores, a short discussion of DIN will be given below.

The median values for DIN measured in the Vaal and Wilge Rivers for the period March 2002 to February 2003 are presented in Fig. 3.7. Fig. 3.7 shows that DIN undergoes a drastic increase between site V4 below the Vaal Dam and site V5 below the Vaal Barrage. This increase can be attributed to pollution associated with the intense economic activity, which takes place in Gauteng, as well as the very high population density in this region. It is known that several point source nutrient inputs enter the river, especially in the section between the Barrage and Parys (DWAF, 2000) i.e. close to the location of site V5. These nutrient inputs include effluents discharged from industries, mines and domestic waste water treatment plants.

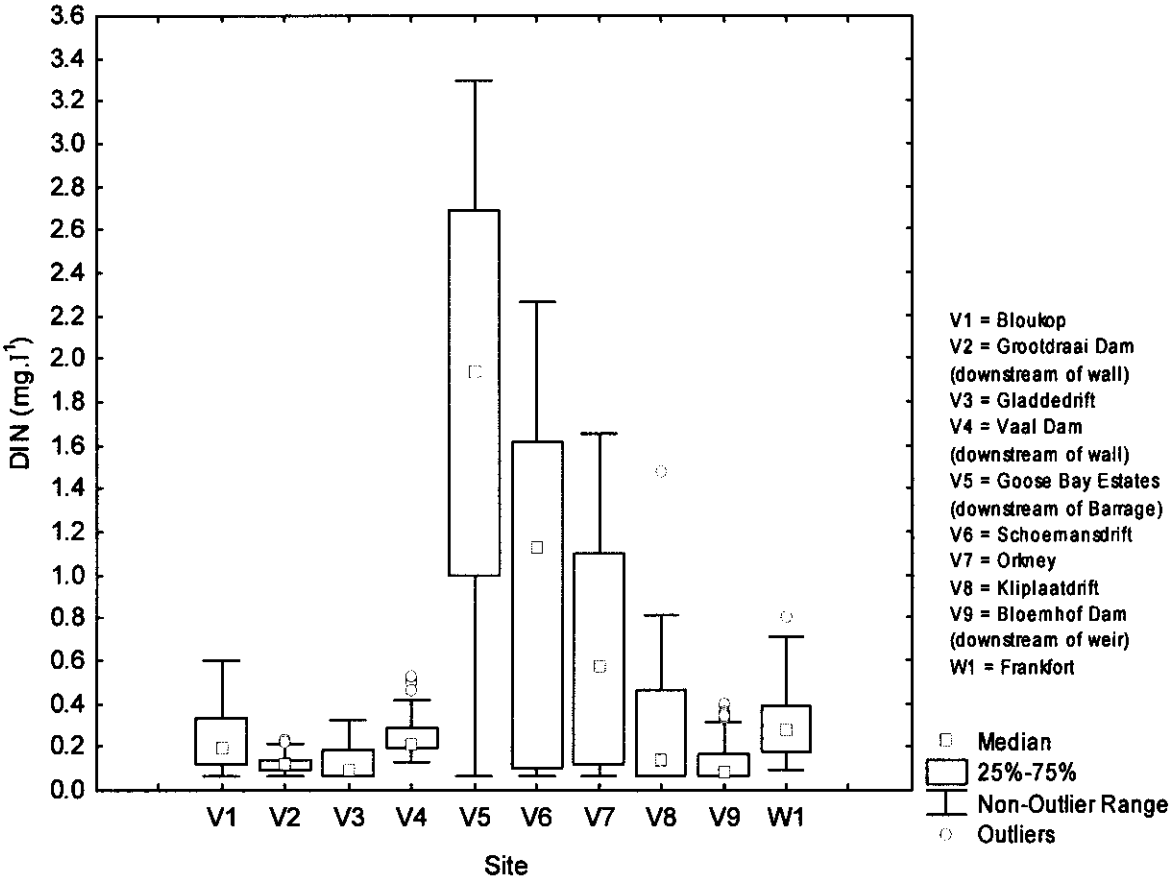

Figure 3.7: *Median DIN concentration at selected sites in the Vaal and Wilge Rivers, for the period March 2002 to February 2003.*

Surface runoff from the surrounding catchment area, the discharge of effluent streams containing human and animal excrement, agricultural fertilisers and organic industrial wastes are the major sources of inorganic nitrogen which enters aquatic systems (DWAF, 1995). Inorganic nitrogen is seldom present in high concentrations in unimpacted waters. This is because inorganic nitrogen is rapidly taken up by aquatic plants and converted into proteins and other organic forms of nitrogen in plant cells. In South Africa, inorganic nitrogen concentrations in unimpacted, aerobic surface waters are usually below 0.5 mg $N.l^{-1}$, but may increase to above 10 mg $N.l^{-1}$ in highly enriched waters (DWAF, 1995). Inorganic

nitrogen is primarily of concern due to its stimulatory effect on aquatic plants and algae growth and may indicate eutrophication (DWAF, 1995).

c. Silicate-silicon (SiO_2-Si)

Silicified structures occur in some aquatic organisms, but none approaches the importance of the frustule of the diatoms (Wetzel, 1983). All diatoms are enclosed in a silica frustule in which silicic acid has been dehydrated and polymerised to form silica particles (Lewin, 1962). Silicate-Silicon (SiO_2-Si) concentrations and their biogenic reduction from epilimnetic waters are major factors in diatom community regulation (Wetzel, 1983). It should be stressed however, that although SiO_2-Si may be limiting to diatom biomass accumulation, changes in SiO_2-Si concentration and its ratio to other elements such as nitrogen have little influence on the relative abundance of the most prevalent diatom species (Gilpin *et al.*, 2004). It follows then that SiO_2-Si limitation will not have any effect on diatom scores calculated using the relative abundance of diatom species. A positive relationship between diatom index scores and SiO_2-Si will be demonstrated in Chapter 4, making a discussion of SiO_2-Si concentrations and the origins of this element in the rivers studied necessary. The median values for SiO_2-Si measured in the Vaal River for the period March 2002 to February 2003 are presented in Fig. 3.8.

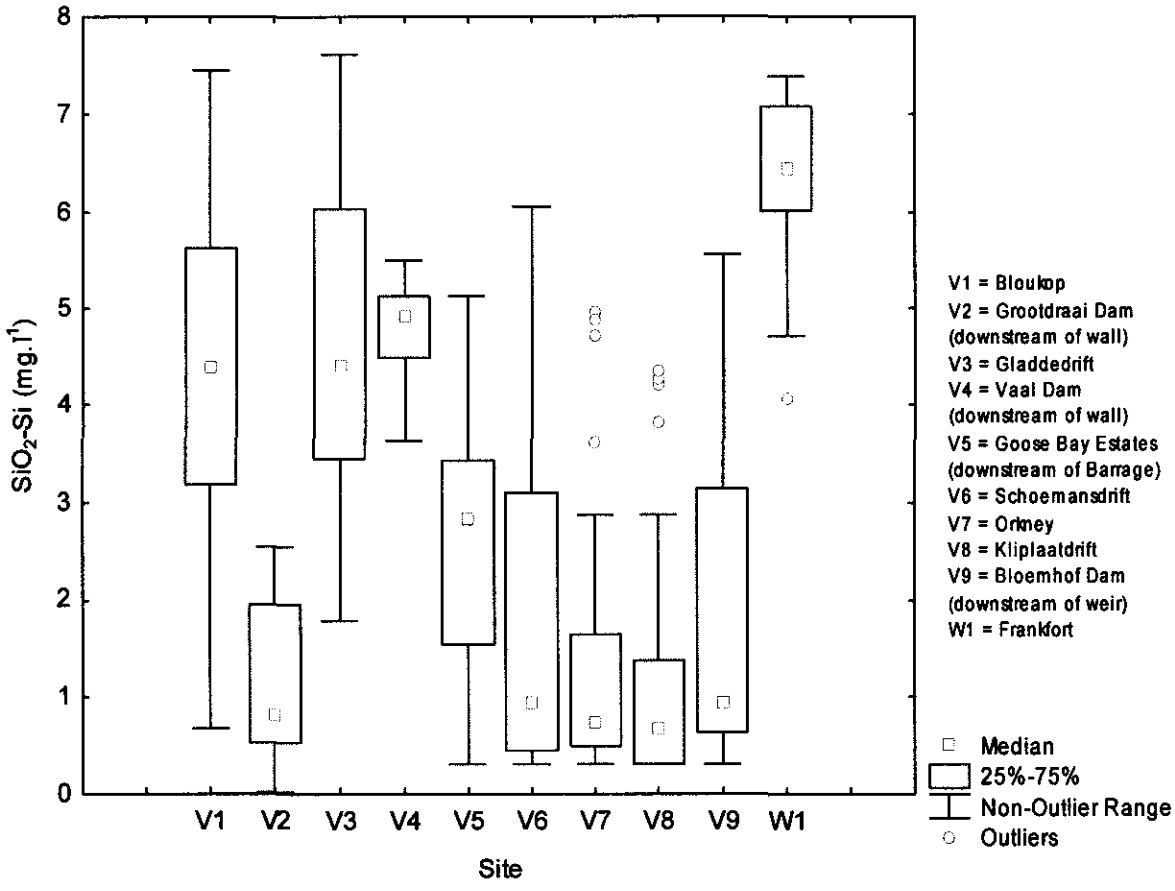

Figure 3.8: *Median SiO_2-Si concentration at selected sites in the Vaal and Wilge Rivers, for the period March 2002 to February 2003.*

Median SiO_2-Si concentrations (Fig. 3.8) in the Vaal River during the period of this study underwent large fluctuations. Silicate-silicon levels decreased to low levels (0.03 $mg.l^{-1}$) at site V2 downstream of the Grootdraai Dam during August 2002. This very low level of silica may have been due to a bloom of planktonic diatoms in the winter months in the Grootdraai Dam, which may have drastically reduced silica levels. In addition, conditions of low flow due to the season (winter; low rainfall and hence low runoff) could have influenced silica concentration.

Median silicate-silicon concentrations are also much reduced at sites V5 below the Vaal Barrage through to site V9 downstream of the Bloemhof Dam weir. The low median levels of silicate-silicon experienced at these sites can almost definitely be ascribed to consumption by phytoplanktonic diatom species throughout the year, but more especially in the winter months. For example, 75 % of the benthic diatom community at site V6 (Schoemansdrift) in the month of July 2002 was composed of planktonic diatoms. These diatoms included *Stephanodiscus agassizensis* (45%), *Stephanodiscus hantzschii* (14%), *Aulacoseira granulata* (10%) and *Cyclotella meneghiniana* (6%). Although these diatom species are planktonic, they completely dominated the benthic assemblage, possibly due to rapid rates of settling at the end of a diatom bloom. The site is also situated downstream of a small impoundment or weir, which could slow the flow of the river and favour the development of phytoplankton. The planktonic cells were, however, viable (contained chloroplasts) and were included in the diatom community counts. At site V7 at Orkney, V8 at Klipplaatdrift and V9 downstream of the Bloemhof Dam weir, winter low flow conditions, together with silica assimilation by planktonic diatom species, possibly became limiting to the benthic diatom community. No data is available from which calculations of cell density could be made. However, during the counting process it became necessary to use more optical fields to obtain the required number of diatom valves for index calculation, indicating a lower concentration of diatom cells in the sample. Instead of the usual 10 valves per field being visible, only one or two valves were visible in each field. It was not possible simply to increase the concentration of the material dried onto the cover slip, as the amount of detritus in the samples would then obscure the diatom valves. This low concentration of diatom valves within the sample might give some indication of the low concentration of diatom cells due to silicate limitation.

The supply of silicate-silicon is limited by the rate of rock and soil weathering, water flow, solubilisation of pre-existing diatom populations. SiO_2-Si is used up very rapidly during periods of rapid diatom growth, and therefore it can easily become limiting (Round, 1981). In eutrophic lakes, SiO_2-Si concentrations in the trophogenic zone (the superficial stratum of a

lake or dam in which photosynthetic production predominates) are commonly near analytical undetectability (Wetzel, 1983). In lakes in which silica concentrations are moderate to low (e.g. < 5mg.l^{-1}), progressive long-term enrichment with inorganic phosphorus and nitrogen can lead to rapid biogenic reduction in silica levels (Kilham, 1971). A strong interaction can exist between the population level of littoral diatoms and the development of planktonic diatoms. For example, in Lake Furesø (Sweden), the spring maximum of the planktonic diatoms, primarily *Stephanodiscus* sp., reduced the SiO_2-Si concentrations to < 0.04 mg.l^{-1}, levels experimentally demonstrated to inhibit the growth of the diatoms (Jørgensen, 1957). SiO_2-Si consumption by planktonic diatoms may provide an explanation for the low SiO_2-Si concentrations noted by the author in the Vaal River during the winter months. In turn, low levels of SiO_2-Si due to utilisation by planktonic diatoms in the Vaal River, may have limited the growth of benthic diatoms in the river at certain sites.

Janse van Vuuren (2001) found several interesting correlations when studying annual and seasonal concentration of silica in the Vaal River. Positive, significant correlations were found between silica and temperature. This correlation was ascribed to the influence of temperature on the solubility of silica that increases directly with temperature. The increase of silica during the summer months was also ascribed to its release from the sediment under warm water temperatures and from seepage water. Besides temperature, Janse van Vuuren (2001) also demonstrated a statistically significant correlation between discharge and SiO_2-Si concentration at three localities in the Vaal River, which would lead to the conclusion that the supply rate of SiO_2-Si is higher in the summer in rivers in a summer rainfall region such as the Vaal and Wilge Rivers.

3.3.4 Electrical conductivity (EC)

Along with pH values and the concentrations of nutrients within a water body, one of the major descriptors of the "quality" of a water sample is the total amount of dissolved material. Because of the importance of electrical conductivity (EC) in determining levels of pollution, and because the diatom indices tested in this study demonstrate a significant negative correlation to EC, a detailed discussion of this water quality variable is given below. The amount of dissolved material in a water sample is commonly measured in one of three ways: as total dissolved solids (TDS), as conductivity, or as salinity, all of which correlate closely in most waters (Dallas & Day, 1993). TDS is a measure of the total concentration of soluble material in a sample of water. The greatest mass of this material in natural waters comprises inorganic ions. The most common of these ions are usually the cations Na^+, K^+, Ca^{2+} and Mg^+ and the anions HCO_3^-, CO_3^{2-}, Cl^- and SO_4^{2-}. Together these are often referred to as the "major ions" (Dallas & Day, 1993).

Electrical conductivity (EC) is another measure of dissolved material and is often used as a surrogate for TDS, for the following reason. Since electrical conductivity of water is a function of the number of charged particles (ions) in solution, it is also a measure of the total quantity of salts, and therefore dissolved solids, in a sample of water. EC, in water quality terminology, is therefore a measure of the ability of a sample of water to conduct an electrical current; the higher the conductivity, the greater the number of ions in solution. EC is quoted in $mS.m^{-1}$, where S is a "Siemen", which is the reciprocal of an ohm (the unit of electrical resistance). Because the majority of material dissolved in most water is ionic, TDS and EC usually correlate closely (Dallas & Day, 1993):

$$TDS\ (mg.l^{-1}) = EC\ (mS.m^{-1}) \times 6.6$$

The median values for electrical conductivity (EC) measured in the Vaal River for the period March 2002 to February 2003 are presented in Fig. 3.9. The lowest median value for the Vaal River was recorded at site V4 just below the Vaal Dam wall. The highest median EC for the Vaal River was at site V7 at Orkney. The EC for site V5 just below the Vaal Barrage to site V8 at Klipplaatdrift remained continually elevated with a median value of about 80 $mS.m^{-1}$. Median EC decreased to about 46.5 $mS.m^{-1}$ at site V9 just below the Bloemhof weir. Median EC in the Wilge River at site W1 (Frankfort) was lower than any of the median values recorded for the Vaal River (15 $mS.m^{-1}$).

The range of EC at site V2 below the Grootdraai Dam, V4 below the Vaal Dam and to a lesser extent V9 below the Bloemhof Dam, is considerably lower than at the other sites in the Vaal River possibly due to a stabilising effect of these dams on water quality. The water quality deteriorates as the concentration of dissolved solids increases in a downstream direction by the input of mining, industrial and treated sewage effluents. The mineralising effect of the Klip River (Herold *et al.*, 1980) may also play an important role. The effect of the polluted water flowing into the Vaal River from various sources, including the Klip River, can clearly be seen at site V5 below the Vaal Barrage where the EC drastically increases and does not decrease until sedimentation of the major ions takes place downstream in the Bloemhof Dam.

In the Vaal River system industrial, mining and domestic wastes are of particular importance influencing EC in the river (Van Vliet & Nell, 1986). Several point sources enter the Vaal River between Orkney (V7) and Kliplaatdrift (V8). According to Braune & Rogers (1987), irrigation return-flow also makes a major contribution to increased TDS in the Vaal River,

particularly in the sections of the river below the Vaal Barrage. When the average conductivity of typical unpolluted rivers (35 $mS.m^{-1}$; Koning & Roos, 1999) is compared with conductivity ranges in the Vaal River, it is clear that the Vaal River can be regarded as polluted in terms of dissolved mineral substances concentration below the Barrage. Van Vliet & Nell (1986) state that most of the rivers in the upper Vaal catchment are generally of good mineral quality and rivers in upper catchments are characterised by a relatively low EC. Levels of EC are acceptable up to site V4 (Fig. 3.9). Below the Grootdraai and Vaal Dams EC is comparatively low as settling of major ions takes place within the dams.

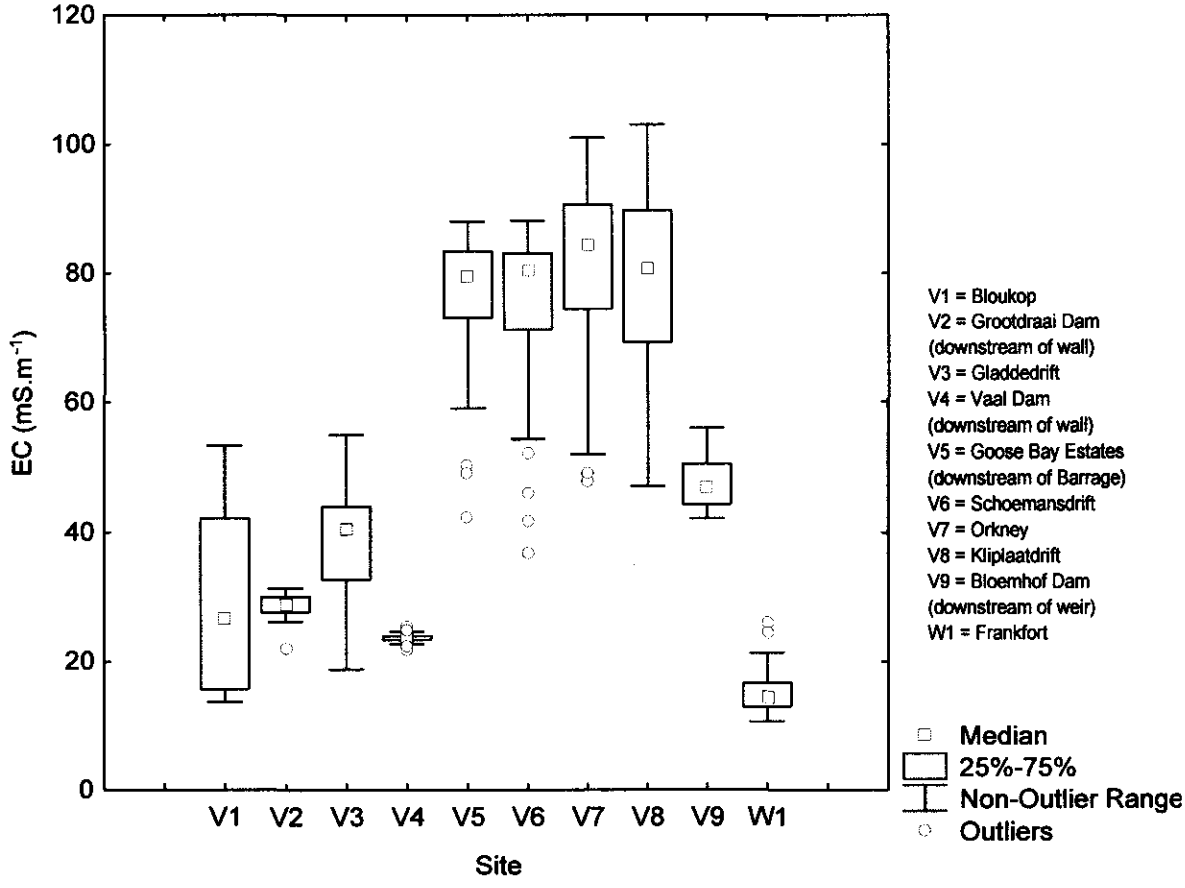

Figure 3.9: *Median EC values at selected sites in the Vaal and Wilge Rivers, for the period March 2002 to February 2003.*

The majority of ions in most natural waters derive from weathering of the rocks over which they flow or from which they drain. EC varies widely within South Africa. The lowest recorded values for South Africa are about 1.8 to 3.1 mS. m^{-1} (Britton, 1991) and the highest recorded value 9790 $mS.m^{-1}$ (Silberbauer & King, 1991). Human activities have severely increased the TDS concentrations of inland water worldwide, particularly in arid regions, including South Africa (Du Plessis & Van Veelen, 1991). Apart from the obvious effects of discharging saline industrial effluents into rivers, increasing EC (a process known as salinisation) may be

caused by irrigation, clear-felling and return of large quantities of sewage effluent to inland waters (Dallas & Day, 1993).

Irrigation causes salinisation in two ways. On the one hand, although irrigation water may have a low EC, the water itself is taken up by crops and evaporates, leaving behind its solutes. Ion exchange processes in the soil, particularly in base-poor soils, result in the accumulation of NaCl, which is washed out of the soil and into rivers during rain. Secondly, irrigation may result in a rise in the water-table and subsequent evaporation from the surface of the, now wet, soil (Williams, 1987; Hart *et al.*, 1990; 1991).

Although both the concentrations of TDS and the major ions were measured in this study, it was decided to use EC as the measure of ionic concentration at the sampling sites. After analysis using Pearson Correlation on $\log_{10}$ transformed data (to obtain a normal distribution – see Chapter 2), TDS and EC showed a correlation of 100 % at the sites sampled for this study (Table 3.1).

Table 3.1 presents as an illustration of the very close correlation between EC and the major ions and to justify the choice of EC to represent the ionic concentration at the study sites. For this reason only EC will be discussed further, and only EC and not TDS or any of the major ions (i.e. Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-}, K^{+} and SO_4^{2-}) will be used in correlation with the diatom indices. However, the major ions will be included in the regression analysis to determine which of the major ions, if any, influence diatom index scores (see Chapter 4, section 4.4).

TABLE 3.1

Pearson correlation coefficients between the average concentrations of some major ions, TDS and EC at sites in the Vaal and Wilge Rivers
Numerical values indicate significant correlations at $p < 0.01$ or higher
n=117 (Casewise deletion of missing data)

	EC	TDS	Na^{+}	K^{+}	Ca^{2+}	Mg^{2+}	Cl^{-}	SO_4^{-}
EC	1.00	1.00	0.99	0.95	0.98	0.96	0.98	0.96
TDS		1.00	0.99	0.94	0.99	0.97	0.97	0.95
Na^{+}			1.00	0.97	0.98	0.94	0.97	0.96
K^{+}				1.00	0.93	0.88	0.94	0.96
Ca^{2+}					1.00	0.93	0.97	0.96
Mg^{2+}						1.00	0.92	0.90
Cl^{-}							1.00	0.98
SO_4^{2-}								1.00

3.4 Summary

Before moving to the next chapter, in which the influence of the water quality variables discussed above on diatom indices will be evaluated, it is necessary to draw some conclusions about the general quality of Vaal River water.

This study finds itself in agreement with that of Braune & Rogers (1987) in that the best quality waters are found in the catchment of the Vaal Dam. Water quality decreases the further the river flows from the source. In general water quality decreases downstream of the Vaal Dam until the Vaal River flows into the Bloemhof Dam. Water flowing from the Bloemhof Dam is of a considerably higher quality that that found at sites immediately above the Dam.

Water quality variables considered to have a major impact on the Vaal River are pH, the plant nutrients dissolved inorganic phosphate and dissolved inorganic nitrate as eutrophication parameters and electrical conductivity. Increased levels of turbidity are considered to have a positive effect on water quality in the Vaal River. The trends in pH, EC and nutrient concentrations in particular are important to note for the interpretation of diatom index scores.

pH shows an increasing downstream trend until the Vaal River flows into the Bloemhof Dam where levels of pH decrease. Electrical conductivity increases after the Vaal Barrage and remains fairly constant until the Vaal River reaches Bloemhof Dam where levels of EC decrease. The plant nutrients dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) increase after the Vaal Barrage and then steadily decrease until the river reaches the Bloemhof Dam.

The Wilge River has low median values for pH and electrical conductivity, and in this case the degradation of the river is attributed to elevated levels of plant nutrients from diffuse agricultural sources.

The Vaal Barrage receives effluent via the Klip River from the industrial heartland of Gauteng. Water released from the Barrage would seem to be the main cause of the degradation of the Vaal River. Water from the Vaal Barrage has elevated levels of dissolved salts and plant nutrients. Increases in water quality, after the Vaal River flows through large dams, are attributed to sedimentation of ionic compounds and the utilisation of plant nutrients by algae in the dams and aquatic macrophytes in, and floating on the dams.

CHAPTER 4

RESULTS AND DISCUSSION - APPLICATION AND TESTING OF DIATOM INDICES

4.1 Introduction

Within the last decade, as shown in Chapter 1, diatom-based indices have gained considerable popularity throughout the world as a tool to provide an integrated reflection of water quality, which can form the basis of management decisions regarding rivers and streams. Work on the use of diatoms as bio-indicators has in fact proceeded to such an extent that in some cases diatom indices have replaced invertebrate indices as the biomonitoring method of choice in certain situations, e.g. in canalised waterways (Prygiel & Coste, 1993b).

A variety of diatom indices have been adopted and tested by many European countries including France (Lenoir & Coste, 1996), Finland (Eloranta, 1994, 1999; Eloranta & Kwandrans, 1996; Eloranta & Andersson, 1998), Poland (Kwandrans *et al.*, 1998) and England (Kelly *et al.*, 1995). The present study aims to test and apply different European, British and Japanese diatom indices in South Africa, using a similar rationale to that employed in the testing of diatom indices in Europe.

It was concluded in Chapter 3 that the water quality of the Vaal River decreases downstream from the source waters. This conclusion is based on the median data for a year (March 2002 to February 2003) at 10 sample sites in the Vaal catchment. In most cases the median chemical data for the year was based on weekly measurements of chemical water quality variables at each of the sites. Only regular measurements of chemical variables can provide an integrated overview of general water quality. Chemical analysis is costly, time consuming and, as discussed in Chapter 1, may not always give an accurate reflection of general water quality. Only a few sites within a river system can be monitored intensively at a rate of one sample per week (e.g. in the Vaal River DWAF only monitors 13 points in a distance of almost 1 000 km).

The present study highlights the need for a reliable indicator (an indicator with a demonstrable relationship to physico-chemical variables) of general water quality, which can be used with greater intervals of time between samples but still provide a similar, integrated reflection of general water quality to that gained from regular chemical monitoring. Because aquatic organisms can be considered to "monitor" their environment continually and

integrate the effects of water quality variables, biological samples can be taken less frequently than chemical samples to assess changes in ecological conditions. In addition, biota respond to the interaction and cumulative effects of a variety of environmental characteristics, not all of which could be monitored or interpreted individually by chemical analysis (Dixit *et al.*, 1992). A general water quality indicator should be simple, able to quantify the rate of degradation or recovery in a system and should be applicable over large geographic regions. Because diatoms satisfy these criteria more fully than any other group of aquatic organisms, diatoms are emerging as the preferred indicator in a variety of monitoring programmes and studies (Dixit *et al.*, 1992). Biological indices will never completely replace chemical water quality monitoring, but biological indices provide a valuable addition to a water quality monitoring programme. Existing chemical and biological monitoring programmes can benefit from the addition of a diatom-based index to the suite of monitoring tools already available and in use. Indices such as SASS, which is based on macroinvertebrates, demonstrate ecosystem health, while diatom indices provide a stronger reflection of water quality. Water quality variables can explain up to 70% of the variation in diatom index scores - the 30% of unexplained variability may be due to environmental factors such as light, current speed, mineralisation and the nature of the substratum (Lenoir & Coste, 1996). The diatom indices discussed below will provide a valuable addition to any private or governmental monitoring programme in which general water quality or trophic status needs to be determined.

Eutrophication is a specific water quality problem, the extent and trend of which needs to be monitored. Diatoms as autotrophic organisms directly use the plant nutrients (i.e. inorganic nitrogen and phosphorus) responsible for eutrophication. Diatom taxa have specific tolerance limits for these nutrients. The tolerance limits of diatoms to different nutrient concentrations have been collated to form specific indices for monitoring eutrophication (see Chapter 1 for examples).

In order to calculate a diatom index score needed to classify the water quality and trophic status of the rivers in question, the composition of the diatom community in each sample needs to be determined and the component taxa enumerated. Before the index results are discussed, various figures and tables will be presented which demonstrate the relationship between established diatom indices of water quality and diatom communities encountered in the Vaal and Wilge Rivers. Matrices representing the correlation between the diatom indices and certain water quality variables will be presented first. Secondly, the results presented in the correlation matrices will be compared to similar correlation matrices published by several international authors. The differences between the indices will be discussed, i.e. the way in

which each different index reflects specific aspects of water quality. Finally, the index showing the best correlation to general water quality will be used to classify the Vaal and Wilge Rivers in the month of June in 2002 and the index which shows the best correlation to dissolved inorganic phosphate (DIP) will be used to classify the trophic staus of the Vaal and Wilge Rivers in the month of June 2002.

Diatoms bloom in the Vaal River during the winter months (Janse van Vuuren, 2001), making high quality samples with a relatively large number of species possible. Turbidity of the Vaal River is also lower in winter due to reduced flow of the river in winter (Janse van Vuuren, 2001) and thus there are reduced amounts of sediment within the diatom samples. As water levels are decreasing during this time rather than rising, constant exposure of the substrate within the water column is a given. During the winter months in the Vaal catchment little or no rainfall is experienced and thus the chemical constituents of the river system are not diluted on a regular basis. These conditions make winter the ideal time for the ecological assessment of a river system employing diatom indices.

4.2 Species Composition

The score generated by a diatom index is expressed as the mean of the water quality optima (i.e the tolerance limits of diatoms to water quality variables) of the diatom taxa in the sample, weighted by the abundance of each taxon. In order to calculate the score with a particular index, the diatom taxa in each of the samples have, therefore to be identified using both light microscopy (LM) and scanning electron microscopy (SEM) if identifications made under LM are subject to doubt (Taylor, 2003). During the course of the study 245 diatom taxa were encountered, comprising 54 genera. A full species list of the taxa encountered is given in Table 4.1. Sixty-nine of the 245 taxa encountered were recorded as dominant (i.e. occurring as > 5 % of any given community). The 69 dominant taxa are illustrated with LM photographs in Appendix 2. The relative abundance of each of the diatom species in a particular sample may be found set out in Appendix 3.

Concern has been expressed as to the feasibility of transferring data concerning the ecological tolerance limits of diatoms between the Northern and Southern Hemispheres (Round, 1991; Kelly *et al.*, 1998; Bate *et al.*, in press); these concerns will be further addressed later in this chapter and in Chapter 5. However, if the species composition of the samples is examined, it is found that of the 69 dominant taxa (see Table 4.1), only two are possibly endemic to Southern Africa (*Navicula adamantiformis*; Schoeman & Archibald 1976-81 and *Eolimna* sp. nov.). The remaining 67 dominant taxa are without doubt

cosmopolitan species well-documented in international literature (Krammer & Lange-Bertalot, 1986-1991; see Table 4.1). Of the 176 non-dominant taxa encountered, a further four taxa are possibly endemic to Southern Africa namely *Amphora subacutiscula* (Archibald, 1983), *Navicula microrhombus* (Schoeman & Archibald, 1976-1980), *Thalassiosira duostra* (Pienaar & Pieterse, 1990) and a species as yet not identified* leaving a total of 240 out of 245 (98%) cosmopolitan taxa (i.e. taxa encountered both in Europe and South Africa).

TABLE 4.1

List of diatom species encountered in the Vaal and Wilge Rivers for the period March 2002 to February 2003 (* indicates dominant species)

**Achnanthidium exiguum* (Grunow) Czarnnecki	*Navicula perminuta* Grunow *in* Van Heurck
**Achnanthidium minutissimum* (Kützing) Czarnecki	*Navicula phyllepta* Kützing
**Achnanthidium saprophila* (Kobayasi & Mayama) Round & Bukhtiyarova	*Navicula pseudolanceolata* Lange-Bertalot
Amphora castellata Giffen	*Navicula radiosafallax* Lange-Bertalot
Amphora libyca Ehrenberg	**Navicula recens* (Lange-Bertalot) Lange-Bertalot
**Amphora montana* Krasske	*Navicula reichardtiana* Lange-Bertalot
Amphora ovalis (Kützing) Kützing	*Navicula rhynchocephala* Kützing
**Amphora pediculus* (Kützing) Grunow	*Navicula rostellata* Kützing
Amphora subacutiuscula Schoeman	**Navicula schroeteri* Meister
Amphora veneta Kützing	*Navicula scoliopleura* Schmidt
Asterionella formosa Hassall	**Navicula symmetrica* (Patrick)
Aulacoseira distans (Ehrenberg) Simonsen	*Navicula tenelloides* Hustedt
**Aulacoseira granulata* (Ehrenberg) Ralfs	**Navicula tripunctata* (O.Müller) Bory
**Aulacoseira granulata* var. *angustissima* (O.Müller) Simonsen	*Navicula trivialis* Lange-Bertalot
Aulacoseira muzzanensis (Meister) Krammer	*Navicula vandamii* Schoeman & Archibald
Caloneis bacillum (Grunow) Cleve	*Navicula veneta* Kützing
Caloneis branderi (Hustedt) Krammer	*Navicula viridula* (Kützing) Ehrenberg
Caloneis molaris (Grunow) Krammer	*Neidium globiceps* (Cleve-Euler) Cleve
Caloneis schumanniana (Grunow) Cleve	*Nitzschia acicularioides* Hustedt
Caloneis silicula (Ehrenberg) Cleve	**Nitzschia acicularis* (Kützing) W.M.Smith
**Cocconeis pediculus* Ehrenberg	*Nitzschia acula* Hantzsch
**Cocconeis placentula* Ehrenberg	*Nitzschia amphibia* Grunow
Cocconeis placentula var *euglypta* (Ehrenberg) Grunow	*Nitzschia amphibia* fo. *rostrata* Hustedt
Craticula accomoda (Hustedt) D.G. Mann in Round, Crawford & Mann	**Nitzschia archibaldii* Lange-Bertalot
Craticula halophila (Gunow *ex* Van Heurk) D.G. Mann in Round, Crawford & Mann	*Nitzschia aurariae* Cholnoky
**Craticula molestiformis* (Hustedt) Lange-Bertalot	*Nitzschia bergii* Cleve-Euler
Craticula submolesta (Hustedt) Lange-Bertalot	**Nitzschia capitellata* Hustedt *in* A.Schmidt
Cyclostephanos damasi (Hustedt) Stoermer & Håkansson	*Nitzschia communis* Rabenhorst
**Cyclostephanos dubius* (Fricke) Round	*Nitzschia commutata* Grunow *in* Cleve & Grunow
**Cyclostephanos invisitatus* (M.H.Hohn & Hellerman) Theriot, Stoermer & Håkansson	*Nitzschia compressa var. vexans* (Grunow) Lange-Bertalot
Cyclotella atomus Hustedt	*Nitzschia desertorum* Hustedt
Cyclotella atomus var. *gracilis* Genkal & Kiss	**Nitzschia dissipata* (Kützing) Grunow
**Cyclotella meduanae* Germain	*Nitzschia dissipata* var. *media* (Hantszch) Grunow
**Cyclotella meneghiniana* Kützing	*Nitzschia eglei* Lange-Bertalot

* The unidentified species is thought to belong to a new monospecific genus (F.E. Round pers. comm.), which is currently being described.

Cyclotella pseudostelligera Hustedt

Cymatopleura solea (Brébisson) W.Smith

**Cymbella affinis* Kützing *sensu* Krammer & Lange-Bertalot (1986)

Cymbella kolbei Hustedt

**Cymbella tumida* (Brébisson) Van Heurck

**Cymbella turgidula* Grunow

Diadesmis contenta (Grunow *ex* Van Heurck) D.G. Mann *in* Round, Crawford & Mann

Diadesmis gallica var. *perpusilla* (Grunow) Lange-Bertalot

**Diatoma vulgare* Bory

Diploneis elliptica (Kützing) Cleve

Diploneis oblongella (Naegeli) Cleve-Euler

Encyonema caespitosum Kützing

Encyonema gracile Rabenhorst

**Encyonema minutum* (Hilse in Rabenhorst) D.G.Mann *in* Round, Crawford & Mann

Encyonema perpusillium (Cleve-Euler) D.G.Mann *in* Round, Crawford & Mann

Encyonema silesiacum (Bleisch *in* Rabenhorst) D.G. Mann *in* Round, Crawford & Mann

**Encyonopsis microcephala* (Grunow) Krammer

**Eolimna minima* (Grunow) Lange-Bertalot

**Eolimna subminuscula* (Manguin) Lange-Bertalot & Metzeltin

**Epithemia adnata* (Kützing) Brébisson

**Epithemia sorex* Kützing

Fallacia indifferens (Hustedt) D.G.Mann *in* Round, Crawford & Mann

Fallacia insociabilis (Krasske) D.G. Mann *in* Round, Crawford & Mann

Fallacia monoculata (Hustedt) D.G.Mann *in* Round, Crawford & Mann

Fallacia tenera (Hustedt) D.G.Mann *in* Round, Crawford & Mann

Fallacia tenera (Hustedt) D.G.Mann *in* Round, Crawford & Mann

Fragilaria capucina Desmazières

Fragilaria capucina var.*gracilis* (Østrup) Hustedt

**Fragilaria capucina* var.*vaucheriae* (Kützing) Lange-Bertalot

Fragilaria crotonensis Kitton

Fragilaria miniscula (Grunow in Van Heurk) Williams & Round

Fragilaria nanana Lange-Bertalot

**Fragilaria spec.* entspr. *Synedra acus* var. *angustissima* order var. *radians* sensu auct. Nonnull

Fragilaria tenera (W.Smith) Lange-Bertalot

Frustulia vulgaris (Thwaites) De Toni

Frustulifera pelliculosa (Brébisson) Lange-Bertalot

**Frustulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot

Geissleria acceptata (Hustedt) Lange-Bertalot & Metzeltin

*Geissleria decussis (*Østrup) Lange-Bertalot & Metzeltin

Gomphonema affine Kützing

Gomphonema angustum Agardh

Gomphonema exilissimum (Grunow) Lange-Bertalot & Reichart

Gomphonema gracile Ehrenberg

**Gomphonema minutum* (Agardh) Agardh

**Gomphonema parvulum* Kützing

**Nitzschia filiformis* (W.M.Smith) Van Heurk

Nitzschia filiformis var. *conferta* (Richter) Lange-Bertalot

Nitzschia flexoides Geitler

**Nitzschia fonticola* Grunow *in* Cleve & Möller

Nitzschia fonticola var. *pelagica* Hustedt

**Nitzschia frustulum* (Kützing) Grunow

Nitzschia frustulum var. *bulnheimiana* (Rabenhorst) Grunow

Nitzschia ganderheimiensisk Kützing

Nitzschia graciliformis Lange-Bertalot

Nitzschia gracilis Hantzsch

Nitzschia inconspicua Grunow

Nitzschia intermedia Hantzsch *ex* Cleve &Grunow

Nitzschia intermedia var. *distans* Cleve-Euler

Nitzschia latens Hustedt

**Nitzschia liebetruthii* Rabenhorst

**Nitzschia linearis* (Agardh) W.M.Smith

Nitzschia linearis var. *subtilis* (Grunow) Hustedt

Nitzschia linearis var. *tenius* (W. Smith) Grunow

Nitzschia microcephala Grunow *in* Cleve & Möller

**Nitzschia palea* (Kützing) W.Smith

**Nitzschia paleacea* (Grunow) Grunow *in* Van Heurk

Nitzschia paleaeformis Hustedt

Nitzschia parvula W.M.Smith

Nitzschia perspicua Cholnoky

Nitzschia pumila Hustedt

Nitzschia pura Hustedt

**Nitzschia pusilla* (Kützing) Grunow

Nitzschia recta Hantzsch *ex* Rabenhorst

Nitzschia reversa W.Smith

Nitzschia rostellata Hustedt

Nitzschia sigma (Kützing) W.M.Smith

Nitzschia sigmoidea (Nitzsch) W.M.Smith

Nitzschia sinuata (Thwaites) Grunow

Nitzschia solgensis Cleve-Euler

Nitzschia subacicularis Hustedt *in* A.Schmidt

Nitzschia subtilis Grunow *in* Cleve Grunow

**Nitzschia supralitorea* Lange-Bertalot

Nitzschia tropica Hustedt

Nitzschia umbonata (Ehrenberg) Lange-Bertalot

Pinnularia borealis Ehrenberg

Placoneis clementis (Grunow) Cox

Placoneis dicephala (W.Smith) Mereschkowsky

Placoneis elgenensis (Gregory) Cox

Planothidium daui (Foged) Lange-Bertalot

Planothidium delicatulum (Kützing) Round & Bukhtiyarova

**Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot

Gomphonema truncatum Ehrenberg

Gyrosigma acuminatum (Kützing) Rabenhorst

Gyrosigma attenuatum (Kützing) Cleve

Gyrosigma scalproides (Rabenhorst) Cleve

Gyrosigma spencerii (Quekett) Griffith

Hantzschia amphioxys (Ehrenberg) Grunow *in* Cleve & Grunow

Hantzschia amphilepta (Grunow) Lange-Bertalot

Hippodonta capitata (Ehrenberg) Lange-Bertalot & Metzeltin *in* Witkowski

Hippodonta hungarica (Grunow) Lange-Bertalot & Metzeltin *in* Witkowski

Lemnicola hungarica (Grunow) Round & Basson

Luticola goeppertiana (Bleisch *in* Rabenhorst) D.G. Mann *in* Round, Crawford & Mann

Luticola mutica (Kützing) D.G. Mann *in* Round, Crawford & Mann

Luticola nivalis (Ehrenberg) D.G. Mann *in* Round, Crawford & Mann

Luticola obligata (Hustedt) D.G.Mann *in* Round, Crawford & Mann

Luticola ventricosa (Kützing) D.G.Mann *in* Round, Crawford & Mann

Mayamaea agrestis (Hustedt) Lange-Bertalot

Mayamaea atomus (Kützing) Lange-Bertalot

**Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot

Mayamaea lacunolaciniata (Lange-Bertalot & Bonik) Lange-Bertalot

**Melosira varians* Agardh

Microcostatus kuelbsii (Lange-Bertalot) Lange-Bertalot

**Navicula adamantiformis* Archibald

Navicula angusta Grunow

**Navicula antonii* Lange-Bertalot

Navicula arvensis Hustedt

**Navicula capitatoradiata* Germain

Navicula cari var. *linearis* (Østrup) Cleve-Euler

Navicula concentrica Carter & Bailey-Watts

Navicula constans Hustedt

Navicula cryptocephala Kützing

**Navicula cryptotenella* Lange-Bertalot

Navicula difficillima Hustedt

**Navicula erifuga* Lange-Bertalot

Navicula festiva Krasske

**Navicula germainii* Wallace

**Navicula gregaria* Donkin

Navicula integra (W.Smith) Ralfs

Navicula kotschyi Grunow

Navicula leptostriata Jørgensen

Navicula libonensis Schoeman

Navicula microcari Lange-Bertalot

Navicula microrhombus (Cholnoky) Schoeman & Archibald

Planothidium ellipticum (Cleve) Round & Bukhitiyarova

**Planothidium frequentissimum* (Lange-Bertalot) Round & Bukhtiyarova

Planothidium haukianum (Grunow) Round & Bukhitiyarova

**Planothidium lanceolatum* (Brébisson) Round & Bukhitiyarova

Planothidium rostratum (Østrup) Lange-Bertalot

Psammodictyon constricta (Gregory) D.G.Mann *in* Round, Crawford & Mann

Pseudostaurosira brevistriata (Grunow *in* Van Heurk) Williams & Round

**Reimeria sinuata* (Gregory) Kociolek & Stoermer

**Rhoicosphenia curvata* (Kützing) Grunow

Rhopalodia brebissonii Krammer

Rhopalodia gibba (Ehrenberg) O.Müller

Rhopalodia gibba var. *minuta* Krammer

Rhopalodia gibberula (Ehrenberg) O.Müller

Sellaphora nyassensis (O.Müller) D.G.Mann *in* Round, Crawford & Mann

Sellaphora pupula (Kützing) Mereschowsky

Sellaphora pupula fo. *rostrata* (Hustedt) Bukhtiyarova

Sellaphora seminulum (Grunow) D.G.Mann

Simonsenia delognei Lange-Bertalot

Stauroneis brasiliensis (Zimmerman) Compere

Stauroneis obtusa Lagerstedt

Staurosira construens Ehrenberg

Staurosira construens var. *venter* (Ehrenberg) Hamilton

Staurosirella pinnata (Ehrenberg) Williams & Round

**Stephanodiscus agassizensis* Hakansson & Kling

**Stephanodiscus hantzschii* Grunow *in* Cleve & Grunow

Stephanodiscus medius Håkansson

Stephanodiscus minutulus (Kützing) Cleve & Möller

**Stephanodiscus parvus* Stoermer & Håkansson

Surirella angusta Kützing

Surirella brebissonii Krammer & Lange-Bertalot

Surirella brightwellii W.Smith

Surirella splendida (Ehrenberg) Kützing

Synedra acus Kützing

Synedra ulna (Nitzsch) Ehrenberg

**Tabularia fasiculata* (C.Agardh) D.M. Williams & Round

Thalassiosira duostra Pienaar

**Thalassiosira pseudonana* Hasle & Heimdal

Thalassiosira weissflogii (Grunow) Fryxell & Hasle

Tryblionella angustata W.Smith

Tryblionella calida (Grunow) D.G.Mann *in* Round Crawford & Mann

Tryblionella coarctata (Grunow) D.G. Mann *in* Round, Crawford & Mann

Tryblionella hungarica (Grunow) D.G.Mann

Tryblionella levidensis W.M.Smith

The question is now posed, why is there so much uncertainty amongst aquatic ecologists (i.e. Bate *et al.*, in press) concerning the cosmopolitan nature of diatom flora of South Africa? When all relevant aspects are considered, it can be suggested that the answer lies not in confusion concerning the identification of diatoms, but rather in reference works used to identify the diatom species and the subsequent nomenclature imposed on those species. Much of the South African literature concerning diatoms is directed at identifying new and rare species in a particular sample. Common species thus receive only a mention in the text and illustrations are limited to novel species (e.g. Archibald, 1966), which is understandable when the amount of effort necessary to produce detailed line drawings is taken into account. Works of this nature may create the false impression that the majority of diatom species encountered in South African waters are in fact endemic to South Africa.

Schoeman (1982) gives a summary of diatom taxa occurring in the Jukskei-Crocodile River system with the following stated intention that "... others working on this river system may have some benefit by having some reference to the extent of the diatom flora." If Schoeman's check-list is examined, it is found that the majority of the taxa are encountered both in Europe and South Africa, and further that the diatom taxa listed bear remarkable similarity to the list of taxa encountered in the present study (see Table 4.1). The most apparent reason for this would be the similarity between the extent of the anthropogenic impact (such as high levels of pollution) on both the Vaal and Jukskei-Crocodile River systems.

If Schoeman's (1982) list is examined in the light of modern literature, a second problem, namely nomenclature, may be identified, which could be causing confusion about the cosmopolitan nature of diatom species encountered in South Africa. Diatom taxonomy is currently in a state of flux especially at the genus level. Many large and segregate genera have been, and are being, split into smaller groups. These changes are extensive, but usually the specific epithet is maintained or only slightly changed and the synonymy is well defined (Round *et al.*, 1990; Stoermer *et al.*, 1999; Kellogg & Kellogg, 2002). Due to the dramatic increase in the amount and availability of international diatom literature in recent years, many synonyms have been established especially between species described in South Africa by authors such as Cholnoky in the early 1950's, and species described from Europe and America. Much of this synonymy is documented by Krammer & Lange-Bertalot (1986-91). Synonyms are also included along with references in the OMNIDIA database from which it is possible to extract these synonyms with great ease. The creators of the OMNIDIA database (Lecointe *et al.*, 1993) extensively reviewed international diatom literature, including much of the literature emanating from South Africa. Many modern works

such as that of Stoermer *et al.* (1999) provide extensive species lists that are fully cross-referenced and provide a useful resource for determining the synonymy of species. Relevant synonyms for the diatom taxa encountered in this study are included in Appendix 1.

Following from the discussion of methodology in Chapter 2, the use of correct methods for both collection and identification are imperative to the success of an ecological study involving diatoms. Although in general, biomonitoring tools are perceived as a rapid and simple method for biological assessments, this is not always the case with studies using diatoms. In studies such as the present one, which attempt to test and apply diatom indices, utmost attention should be paid to making accurate identification, to the taxonomic level of species, form and variation, of all taxa encountered. Even when diatom inference models were in their infancy, Cholnoky (1968) expressed the view that correct identification of diatom taxa is vital to the success of diatom ecological studies.

4.3 Correlation analysis

4.3.1 Correlation between selected diatom indices and water quality variables

As stated in Chapter 2, Pearson correlation was the method used to determine the relationship between calculated diatom scores (see Appendix 4) and the environmental variables measured in this study. The water quality determinants used for correlation analysis with the diatom indices are pH, total alkalinity (TAL), dissolved inorganic phosphate (DIP), dissolved inorganic nitrogen (DIN), electrical conductivity (EC; chosen to represent the major ions as explained in Chapter 3) and silicate-silicon (SiO_2-Si). Dissolved oxygen (DO), temperature and turbidity were only used in the first correlation because these variables were from single measurements taken concurrently with biological sampling.

It will be noted that in the present chapter parametric statistics are used for correlation and regression analyses. In the previous chapter (Chapter 3) non-parametric statistical methods were used to describe trends in the environmental data. In the present chapter, both the environmental and index data was $\log_{10}$ transformed before performing statistical analysis in an attempt to normalise the distribution of the data (see Bate *et al.*, 2002). Non-parametric statistical methods will be used in the present chapter to describe the central tendency (i.e. the value that is "typical" or "representative" of all sample observations) of the scores generated by the different diatom indices.

4.3.1.1 Correlation between diatom indices and once-off physical and chemical water quality data

The results for a correlation analysis performed between several water quality variables and selected diatom indices are presented in Table 4.2. The chemical data were collected concurrently or a few days previously to diatom sampling. The data is from once-off samples and is not averaged. Dissolved oxygen concentration, temperature and turbidity were measured concurrently with biological sampling.

TABLE 4.2

Pearson correlation coefficients between water quality data a few days previously or concurrently to diatom sampling, and diatom indices at sites in the Vaal and Wilge Rivers (*index acronyms explained below)
Numerical values indicate significant correlations at $p < 0.01$ or higher
n=112 (Casewise deletion of missing data)

	SPI	SLA	DES	LMI	SHE	WAT	GDI	CEC	BDI	APDI	EPI
pH	..	..	..	..	..	..	..	-0.32	-0.35	-0.25	..
TAL	-0.31	-0.24	..	-0.28	..	-0.29	-0.28	-0.33	-0.44	-0.28	-0.19
DIP	-0.42	-0.48	-0.25	-0.45	-0.26	-0.37	..	-0.48	-0.36	-0.33	-0.52
DIN	..	-0.28	..	..	..	..	..	..	..	..	..
EC	-0.50	-0.48	..	-0.48	..	-0.46	-0.37	-0.52	-0.63	-0.42	-0.44
DO	..	..	..	..	..	..	..	..	..	..	..
Temp.	-0.35	..	-0.27	..	-0.29	-0.51	-0.29	-0.27	..	..	-0.36
Turb.	..	0.31	..	0.25	..	..	..	..	0.32	..	..

*SPI; Specific Pollution sensitivity Index, SLA; Sládeček's index, DES; Descy's index, LMI; Leclercq & Maquet's Index, SHE; Schiefele and Schreiner's index, WAT; Watanabe's index, GDI; Generic Diatom Index, CEC; Council for European Communities index, BDI; Biological Diatom Index, APDI; Artois-Picardie Diatom Index, EPI; Eutrophication/Pollution Index.

It can be clearly observed in Table 4.2 that significant correlations exist, to varying degrees, between most of the diatom indices tested in this study and water quality variables measured concurrently or a few days previous to diatom sampling. The elements of water quality that each index was designed to reflect, are boxed together in Table 4.2. The positive relationship between diatom index scores and the physical water quality parameter turbidity will be discussed in the following paragraph.

Positive significant correlations can be observed between several indices (Sládeček's index, Leclercq & Maquet's Index and the Biological Diatom Index) and turbidity (Table 4.2). It is important to explain the positive correlation between index scores and turbidity, as turbidity is a visible water quality variable that can be perceived as indicating pollution. Elevated levels of turbidity make river water "dirty" or "muddy". The positive correlation between turbidity and index scores may be due to the fact that turbidity in a river system like the Vaal can, in fact, ameliorates the effects of high levels of DIP. Because turbidity to some extent decreases the negative effects of DIP on aquatic ecosystems, a positive correlation between

the diatom index scores and turbidity can be observed. As turbidity increases, light penetration decreases so that low light conditions limit algal growth and reproduction rather than low nutrient concentrations (Guenther & Bozelli, 2004). In essence an increase in turbidity decreases the negative impacts of high levels of DIP on aquatic ecosystems in the form of high rates of aquatic plant (algae and macrophytes) growth and reproduction. High rates of growth and reproduction, and in turn the dieing out of algal cells, cause an increase in the concentration of dead particulate organic matter in a system. Increases in organic matter lead to a decrease in dissolved oxygen concentrations and a decrease in overall water quality because of the bacterial degradation of detritus. High turbidity levels prevent the uptake of DIP by aquatic plants and algae during growth and reproduction, as low light limits growth. Thus if aquatic plants are growing at a reduced tempo, because of light limitation by elevated levels of turbidity, DIP will in turn pass through the river system unutilised. Thus turbidity may be said to increase general water quality in a eutrophic aquatic ecosystem in spite of the detrimental aesthetic effects of elevated turbidity.

Although significant correlations may be found between most of the indices and water quality variables, diatom communities are considered to provide an integrated reflection of water quality over time (Schoeman, 1976), and thus average chemical data for the period of one month ending two weeks before biological sampling will be compared to diatom index scores in the next section.

4.3.1.2 Correlation between diatom indices and average water quality data

The results for a correlation analysis performed between several elements of water quality and selected diatom indices are presented in Table 4.3. The chemical data are the average for a period of one month ending two weeks before biological sampling was carried out.

It can be clearly observed in Table 4.3 that significant correlations exist, to varying degrees, between most of the diatom indices tested in this study and average water quality variables. The elements of water quality that each index was designed to reflect, are boxed together in Table 4.3. In all cases the correlations are stronger than those demonstrated in Table 4.2. and hence Table 4.3 will be used to discuss the reaction of diatom communities (as reflected by diatom index scores) to water quality variables rather than Table 4.2. The relationship between diatom index scores and certain individual water quality variables will be discussed below in section 4.3.2.

TABLE 4.3

Pearson correlation coefficients between average water quality variables and diatom indices at sites in the Vaal and Wilge Rivers (*index acronyms explained below)
Numerical values indicate significant correlations at $p < 0.01$ or higher
n=112 (Casewise deletion of missing data)

	SPI	SLA	DES	LMI	SHE	WAT	GDI	CEC	BDI	APDI	EPI
pH	-0.29	-0.37	..	-0.34	..	..	..	-0.34	-0.47	-0.43	..
TAL	-0.36	-0.27	..	-0.34	..	-0.32	-0.34	-0.40	-0.49	-0.34	..
DIP	-0.53	-0.51	-0.36	-0.49	-0.37	-0.50	-0.32	-0.61	-0.43	-0.33	-0.60
DIN	-0.26	-0.42	..	-0.31	-0.26	..	..	-0.27	..	..	-0.33
EC	-0.52	-0.50	-0.22	-0.50	-0.29	-0.48	-0.39	-0.59	-0.64	-0.47	-0.47
SiO_2-Si	..	0.33	..	0.30	..	0.28	0.15	0.29	0.48	0.35	..

*SPI; Specific Pollution sensitivity Index, SLA; Sládeček's index, DES; Descy's index, LMI; Leclercq & Maquet's Index, SHE; Schiefele and Schreiner's index, WAT; Watanabe's index, GDI; Generic Diatom Index, CEC; Council for European Communities index, BDI; Biological Diatom Index, APDI; Artois-Picardie Diatom Index, EPI; Eutrophication/Pollution Index.

Descy's index (DES) shows the weakest correlation to general water quality (as reflected by the variables pH, total alkalinity (TAL), dissolved inorganic phosphate (DIP), dissolved inorganic nitrogen (DIN), electrical conductivity (EC), and silicate silicon). Descy's index (DES) was developed in 1979 and was one of the first true autecological diatom indices of general water quality.

Since the creation of Descy's index (DES), many other diatom indices have been developed with the aim of making diatom autecological indices more generally applicable across France and Europe (Prygiel & Coste, 1993b). Most of the new indices are in essence modifications of the DES index. The success of these modifications can be seen in the Specific Pollution sensitivity Index (SPI; broad species-base) and the Biological Diatom Index (BDI; designed for general water quality monitoring across all geographical regions of France). The present study shows that both of these indices correlate significantly with water quality conditions in a different hemisphere to that in which they were developed.

Sládeček's index (SLA) correlates significantly with all measured variables of water quality (see Table 4.3). The SLA index, based on BOD_5 measurements, was developed to provide a reflection of the organic pollution present within a certain aquatic ecosystem. As mentioned previously (Chapter 3, section 3.3.3), compounds of nitrogen and phosphorus are normally present in high concentrations in organic discharges. The partial degradation of proteins and other nitrogenous material can lead to elevated ammonia, nitrite and nitrate concentrations and may account for the significant correlations between SLA and dissolved inorganic phosphate (DIP) and dissolved inorganic nitrogen (DIN) (Table 4.3).

Silicate-Silicon (SiO_2-Si) is positively correlated to a number of the indices (see Table 4.3). SiO_2-Si is an essential nutrient for the growth and reproduction of diatoms. Low concentrations of SiO_2-Si are known to limit diatom biomass accumulation (Round, 1981). However, it is important to note that, although the biomass of diatoms is limited by low SiO_2-Si concentrations, limitation on growth has little influence on the relative abundance of prevalent diatom species in a sample (Gilpin *et al.*, 2004). If low SiO_2-Si concentrations did in fact affect the relative abundance of diatoms, then diatom index scores would be skewed as a result of SiO_2-Si limitation in aquatic ecosystems.

In addition, SiO_2-Si concentrations are correlated with discharge (Janse van Vuuren, 2001). Discharge, due to rainstorms, usually occurs in the Vaal River system during the summer months, with a concomitant increase in water quality as river water is diluted. Therefore, scores yielded by the different diatom indices increase, and the increase in diatom scores is in turn reflected by a positive correlation with SiO_2-Si.

4.3.1.3 Correlation between diatom indices and maximum concentration of recent water quality variables

One of the aims of the present study was to determine whether diatoms reflect the influence of average concentrations of a particular water quality variable (i.e. their response to pollution is integrative), or whether the diatoms react to the maximum concentrations of water quality variables passing through the system. The results of a correlation between several diatom indices and the maximum concentration of chemical elements of water quality are presented in Table 4.4. The chemical data used represent the maximum values encountered during the period of one month ending two weeks before biological sampling. Data on variables such as dissolved oxygen, temperature and turbidity were not included in the correlation matrix because maximum values for these variables were not measured.

A difference test was used to evaluate the significance of differences between the correlation coefficients found in Table 4.3 and 4.4. The outcome of the difference test depends not only on the size of the raw difference between the two coefficients, but also on the size of the samples and on the size of the coefficients themselves (StatSoft Inc., 2003).

If Table 4.4 is compared to Table 4.3, it can be seen that for all chemical variables, with the exception of pH and total alkalinity (TAL), correlation coefficients obtained in both tables are similar i.e. there is no statistically significant differences between the correlation coefficients ($p < 0.01$). Correlation in Table 4.4 is between the diatom indices and maximum water quality variables encountered during the period of one month, while the correlation in Table

4.3 is between the diatom indices and average water quality variables during the same month.

TABLE 4.4

Pearson correlation coefficients between the maximum values for water chemistry variables and diatom indices at sites in the Vaal and Wilge Rivers (*index acronyms explained below)
Numerical values indicate significant correlations at $p < 0.01$ or higher
n = 113(Casewise deletion of missing data)

Green = better correlation than Table 4.2, yellow = similar correlation and red = a weaker correlation

	SPI	SLA	DES	LMI	SHE	WAT	GDI	CEC	BDI	APDI	EPI
pH	-0.29	-0.41	..	-0.40	..	-0.24	..	-0.36	-0.47	-0.47	..
TAL	-0.35	-0.26	..	-0.32	..	-0.33	-0.32	-0.40	-0.45	-0.36	-0.25
DIP	-0.46	-0.46	-0.28	-0.42	-0.30	-0.45	-0.25	-0.53	-0.37	-0.30	-0.56
DIN	..	-0.42	..	-0.26	..	..	..	..	..	..	-0.33
EC	-0.53	-0.52	..	-0.51	-0.29	-0.50	-0.39	-0.61	-0.62	-0.48	-0.50
SiO_2-S	..	..	..	..	..	..	..	..	0.32	..	..

*SPI; Specific Pollution sensitivity Index, SLA; Sládeček's index, DES; Descy's index, LMI; Leclercq & Maquet's Index, SHE; Schiefele and Schreiner's index, WAT; Watanabe's index, GDI; Generic Diatom Index, CEC; Council for European Communities index, BDI; Biological Diatom Index, APDI; Artois-Picardie Diatom Index, EPI; Eutrophication/Pollution Index.

It can be observed from Table 4.4 that peaks or spikes in pH values may alter the diatom community, as reflected in the diatom index scores, rather than the average values. The Watanabe index (WAT) shows a significant correlation to the maximum values for pH, but no significant correlation to average pH values. Sládeček's index (SLA) and Leclercq & Maquet's Index also both demonstrate better correlation to maximum values for pH rather than average values for pH. It should however be noted that the correlation coefficients for the SLA and LMI and pH in Table 4.3 and 4.4 are not significantly different ($p < 0.01$).

A possible region why diatom communities react to "peaks" or "spikes" in pH may be that diatoms are very sensitive to pH levels and have well-defined, and in some cases narrow, tolerance limits to this water quality variable (Cholnoky, 1968). pH is a log value so a change of one unit means a tenfold change in hydrogen ion concentration, spikes of pH can have a deleterious effect on riverine biota including diatoms especially if the resident diatom species have narrow pH tolerance limits.

Although correlation coefficients for the diatom indices and dissolved inorganic phosphate (DIP) are not significantly different ($p < 0.01$) in Table 4.3 and 4.4, the correlation coefficients for DIP and the diatom indices are all higher in Table 4.3. Algae growing in water that contains ample concentrations of inorganic phosphate take up 'excess' quantities and store it for use in periods of lower external phosphate concentration (Round, 1981). It would follow then that the diatoms would reflect the average levels of dissolved inorganic phosphate

(DIP) encountered in a stream or river rather than spikes, as the diatoms integrate and store excess DIP (luxury consumption; Round, 1981). DIP, even at high concentrations, is not toxic although high concentrations of DIP affect the integrity of the river system as a whole rather than only the riverine biota.

Electrical conductivity (EC) at the study sites changed gradually over time due to natural seasonal fluctuations, thus the similarity between correlation results found in the two tables (Table 4.3 & 4.4).

4.3.1.4 Correlation between diatom indices and average water quality data six weeks prior to biological sampling

The results of a correlation between diatom indices and chemical elements of water quality are presented in Table 4.5. The chemical data used in the correlation are the average for a period of one month ending six weeks before biological sampling was carried out. The correlation presented in Table 4.5 was carried out with the aim of determining the period of time it takes a specific diatom community to change in reaction to differing concentrations of water quality elements.

TABLE 4.5

Pearson correlation coefficients between the average values for water chemistry variables, collected 6 weeks prior to biological sampling, and diatom indices at sites in the Vaal and Wilge Rivers (*index acronyms explained below)
Numerical values indicate significant correlations at $p < 0.01$ or higher
n =113 (Casewise deletion of missing data)

Green = better correlation than Table 4.2, orange = slightly better correlation and yellow = similar correlation

	SPI	SLA	DES	LMI	SHE	WAT	GDI	CEC	BDI	APDI	EPI
pH	..	-0.42	..	-0.35	..	-0.29	..	-0.30	-0.47	-0.49	..
TAL	-0.38	-0.35	..	-0.38	-0.25	-0.39	-0.34	-0.41	-0.49	-0.38	-0.31
DIP	-0.52	-0.55	-0.32	-0.47	-0.36	-0.57	-0.30	-0.62	-0.49	-0.36	-0.64
DIN	-0.20	-0.46	..	-0.28	..	..	..	-0.28	-0.27	..	-0.37
SiO_2-Si	..	0.27	..	0.28	..	0.29	..	..	0.41	0.30	..
EC	-0.55	-0.55	..	-0.55	-0.34	-0.53	-0.42	-0.61	-0.64	-0.49	-0.51

*SPI; Specific Pollution sensitivity Index, SLA; Sládeček's index, DES; Descy's index, LMI; Leclercq & Maquet's Index, SHE; Schiefele and Schreiner's index, WAT; Watanabe's index, GDI; Generic Diatom Index, CEC; Council for European Communities index, BDI; Biological Diatom Index, APDI; Artois-Picardie Diatom Index, EPI; Eutrophication/Pollution Index.

If Table 4.5 is compared to Table 4.3, it will be seen that for most chemical variables, correlations between the average water quality variables for one month, six weeks prior to sampling, and between average water quality variables, two weeks prior to sampling, and the selected diatom indices are similar, i.e. there is no significant difference between correlation coefficients ($p < 0.01$).

The similarity between the correlation results, presented in Tables 4.3 and 4.5 is probably due to the nature of the Vaal River. It is mostly broad and slow flowing and hence relatively stable. Therefore, the diatom indices reflect an integration of the water quality variables to which they have been exposed over a period of six to eight weeks in this particular river system. It should be stressed, however, that this may not always be the case in shallower, faster flowing streams subject to point source impacts. Although a stable system like the Vaal River may be ideal for testing these indices, because stable concentrations of chemical variables can be more easily related to diatom index scores, these indices will also need to be tested in a similar way in smaller streams. It should be noted that the Biological Diatom Index (BDI) (boxed) has the most stable correlation over time, i.e. the correlation results from Table 4.3 and 4.5 are almost identical, with the exception of the correlation co-efficient for dissolved inorganic nitrogen (DIN).

Interestingly, correlations between total alkalinity (TAL) and the two diatom indices for organic pollution (Sládeček's index and Watanabe's index) correlate better or slightly better with the average quality data collected six weeks previous to sampling (Table 4.5). Large amounts of PO_4 are contained in river sediments. PO_4 is usually prevented from migrating from the sediments by an oxidised microzone. Oxygen levels tend to decrease with an increase in organic pollution. As the oxygen content of the water near the surface sediment interface declines due to microbial degeneration of particulate organic matter, the oxidised microzone barrier weakens and ferrous iron and phosphate are released into the water (Wetzel, 1983). Preceding the release of ferrous iron and phosphate, nitrate reduction occurs along with the slow release of bases (alkalinity), CO_2 and ammonia (Wetzel, 1983). This process of PO_4 release from sediments may provide some explanation as to why diatom indices of organic pollution demonstrate the best correlation to pH and alkalinity six weeks prior to biological sampling. Changes in the pH and alkalinity levels may precede an increase in the available amounts of dissolved inorganic phosphate (DIP). Similarly, the more significant correlation between the Biological Diatom Index (BDI) and dissolved inorganic nitrogen (DIN), illustrated in Table 4.4, may also be explained by the release of ammonia and nitrates from the sediment preceding the release of phosphate.

4.3.2 Correlation between different diatom indices and individual elements of water quality

This section will highlight differences between diatom indices and discuss how these different indices reflect differing degrees and forms of aquatic pollution. The different water quality variables discussed below have been shown to have an influence on the quality of surface waters, and to be important water quality determinants in the Vaal River system (see

Chapter 3). For this reason these water quality variables (including dissolved inorganic phosphate, pH and electrical conductivity) have been identified for a further discussion of their influence on diatom communities, as reflected by diatom index scores.

4.3.2.1 Correlation of some diatom indices with dissolved inorganic phosphate

The Trophic Diatom Index (TDI) of Kellly & Whitton (1995) displayed consistent responses in index scores between Europe and the Himalayas (Jüttner *et al.*, 2003). The Trophic Diatom Index (TDI), as the name implies, was developed to monitor trophic status or eutrophication. The TDI has a scale of 0-100, with higher values indicating progressively higher levels of nutrients (Kelly & Whitton, 1995). Interpretation of this index also requires calculation of a second value, Percentage Pollution Tolerant Valves (%PTV), which is the percentage of the total count that is composed of taxa described as "pollution tolerant". %PTV acts as a measure of the reliability of the TDI as a measure of eutrophication, with levels < 20% indicating that nutrients are probably the major factor influencing the composition of the flora (Kelly, 1998). Thus for the correlation calculation between TDI and DIP only those sites with a %PTV value of < 20% were taken into account (n = 73).

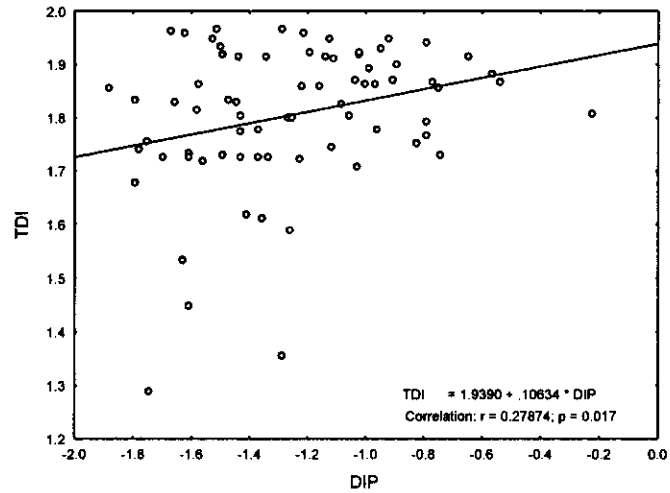

Figure 4.1: *Correlations between values for logDIP and the logTDI in the Vaal and Wilge Rivers (n = 113).*

Figure 4.1 shows the correlation between the Trophic Diatom Index and dissolved inorganic phosphate (DIP). Although the correlation can be considered significant at $p < 0.05$, it is not very strong ($r = 0.28$) when compared to the correlation between DIP and the TDI of $r = 0.79$ ($p < 0.001$) demonstrated by Kelly (1997). The relatively poor correlation between DIP and the Trophic Diatom Index in the Vaal and Wilge Rivers leads to the conclusion that this index is not useful for monitoring eutrophication in these rivers.

Fig 4.2 illustrates bi-variate correlation between DIP and four other diatom indices. The different diatom indices (see Chapter 1) were developed to either indicate general water quality (SPI, BDI etc.), organic pollution as reflected by BOD_5 (SLA) and trophic pollution (EPI, TDI). All of the indices illustrated in Fig. 4.2 demonstrate varying degrees of negative correlation to dissolved inorganic phosphate (DIP). SPI, BDI and GDI, as stated, are indices designed to provide an indication of general water quality, DIP is only one of the elements to influence index score. Other elements influencing these indices will be illustrated in Figures 4.3, and 4.4. EPI, however, was designed to indicate the presence of plant nutrients in a riverine eco-system and has the best correlation with DIP (60%) of the four indices illustrated in Fig. 4.2.

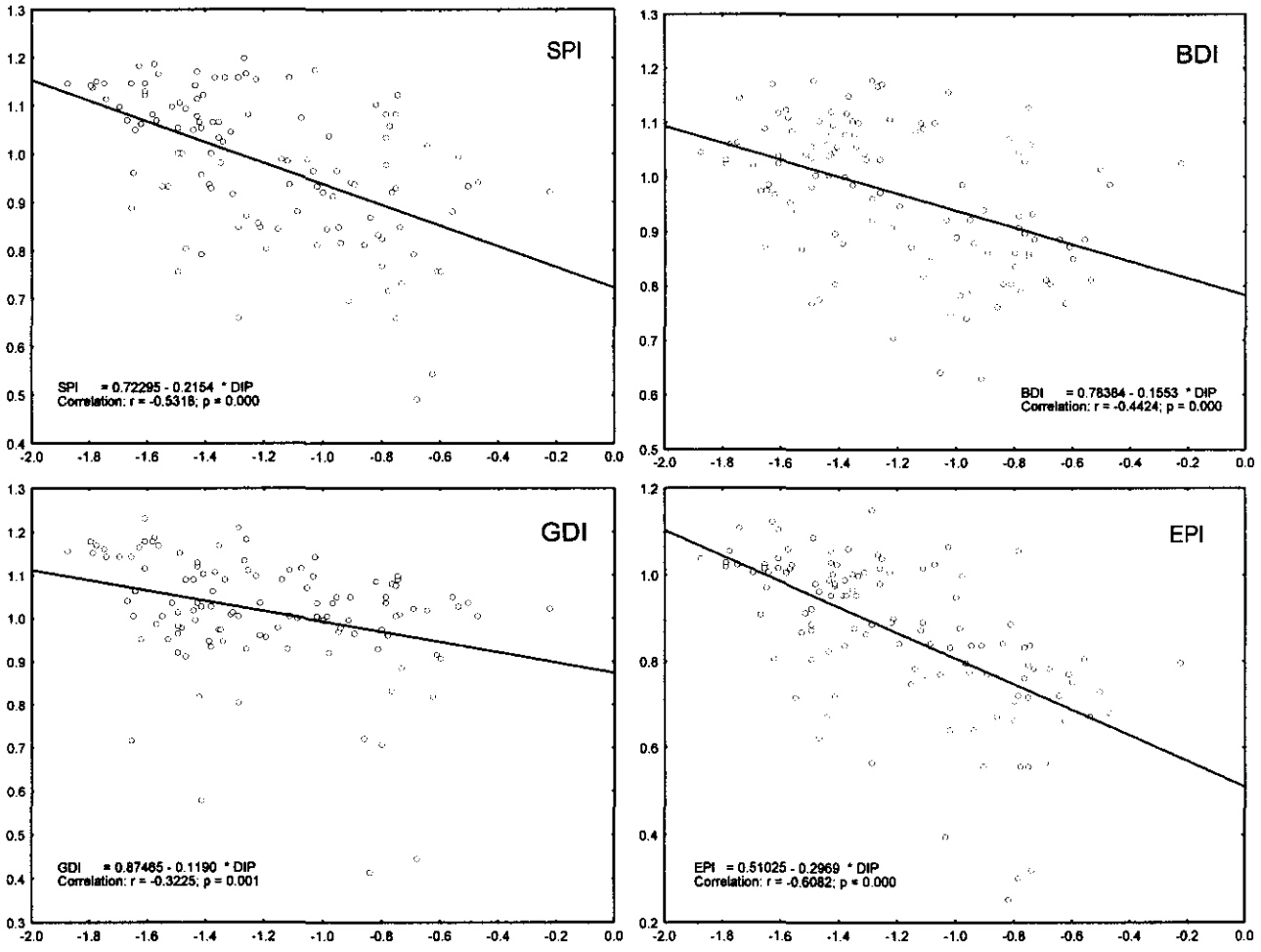

Figure 4.2: *Correlations between values for logDIP and four diatom indices (SPI; Specific Pollution sensitivity Index, BDI; Biological diatom index, GDI; Generic diatom Index, EPI; Eutrophication/Pollution Index) in the Vaal and Wilge Rivers (n = 113).*
X axis = logDIP, Y axis = log diatom index score
*(*This method will be used to indicate the axes in Figs 4.3 to 4.6)*

4.3.2.2 Correlation of some diatom indices with pH

Another of the major components of water quality, between which a significant correlation with several diatom indices can be demonstrated, is pH. Fig. 4.3 illustrates the correlation between pH and four diatom indices. As can be expected, the EPI showed no significant correlation with pH, as this index was developed specifically to reflect nutrient loading and the salinity of a specific watercourse. The two indices of general water quality (SPI, BDI) demonstrate a significant correlation with pH, but BDI demonstrates the strongest, most significant correlation. The SLA index, designed to reflect levels of organic pollution (see Chapter 1), also demonstrates a significant correlation to pH (see Fig. 4.3).

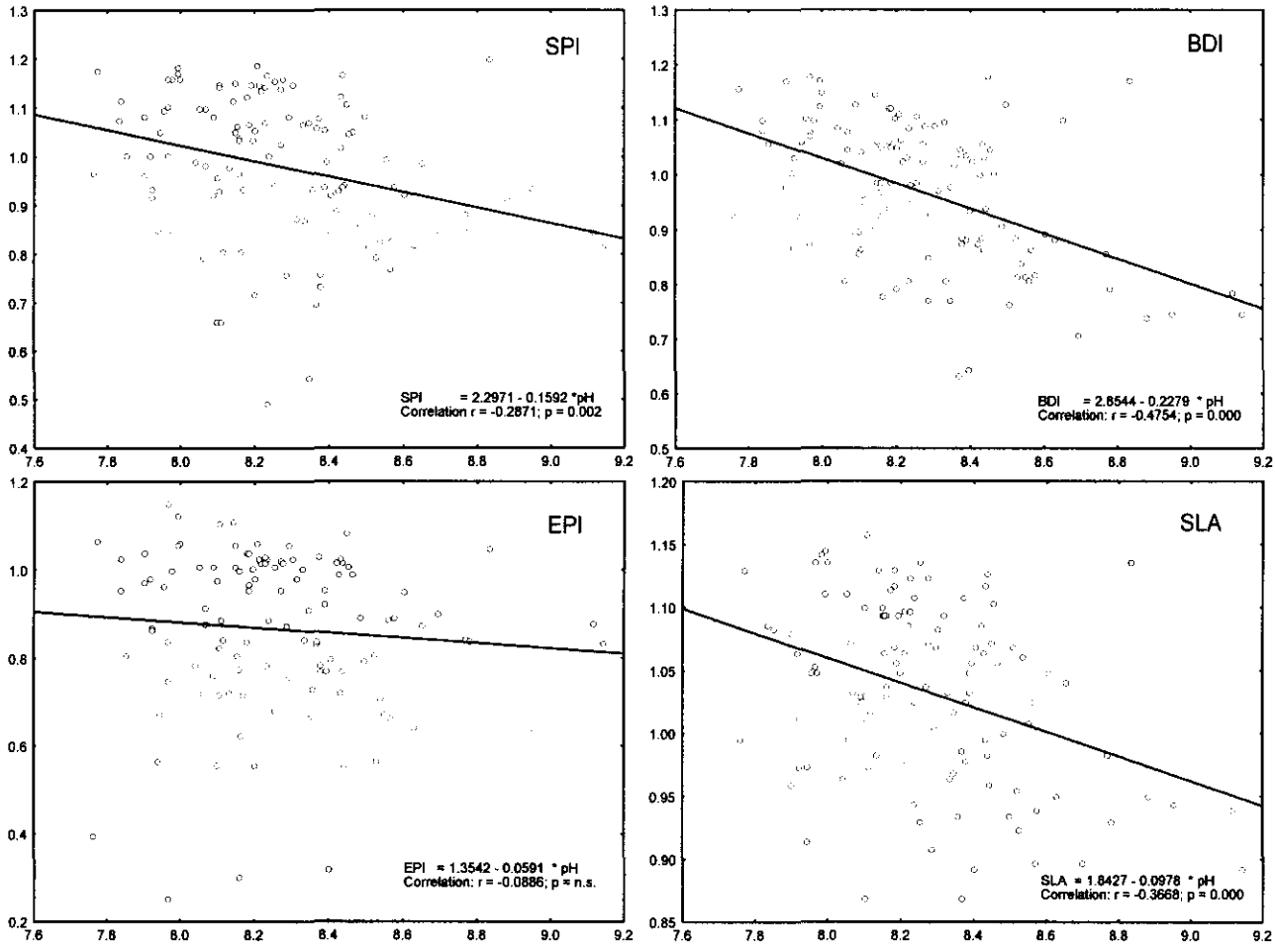

Figure 4.3: *Correlations between values for pH and four diatom indices (SPI; Specific Pollution sensitivity Index, BDI; Biological diatom index, EPI; Eutrophication/Pollution Index, SLA; Sládeček's index of organic pollution) in the Vaal and Wilge Rivers (n = 113).*

X axis = pH, Y axis = log diatom index score

4.3.2.3 Correlation of some diatom indices with electrical conductivity

A third major component of water quality that changed considerably with an increasing distance from the source of the Vaal River, is electrical conductivity (EC). EC, as a measure of the major ionic components of a river, provides a good indication of increasing organic

and industrial effluent inflow along the length of the Vaal River. Fig. 4.4 illustrates the correlation between EC and four diatom indices.

All four of the diatom indices show a significant negative correlation with EC. The Generic Diatom Index (GDI) showed the weakest correlation to EC and the Biological Diatom Index (BDI) showed the strongest correlation to EC making the BDI useful for the evaluation of levels of EC.

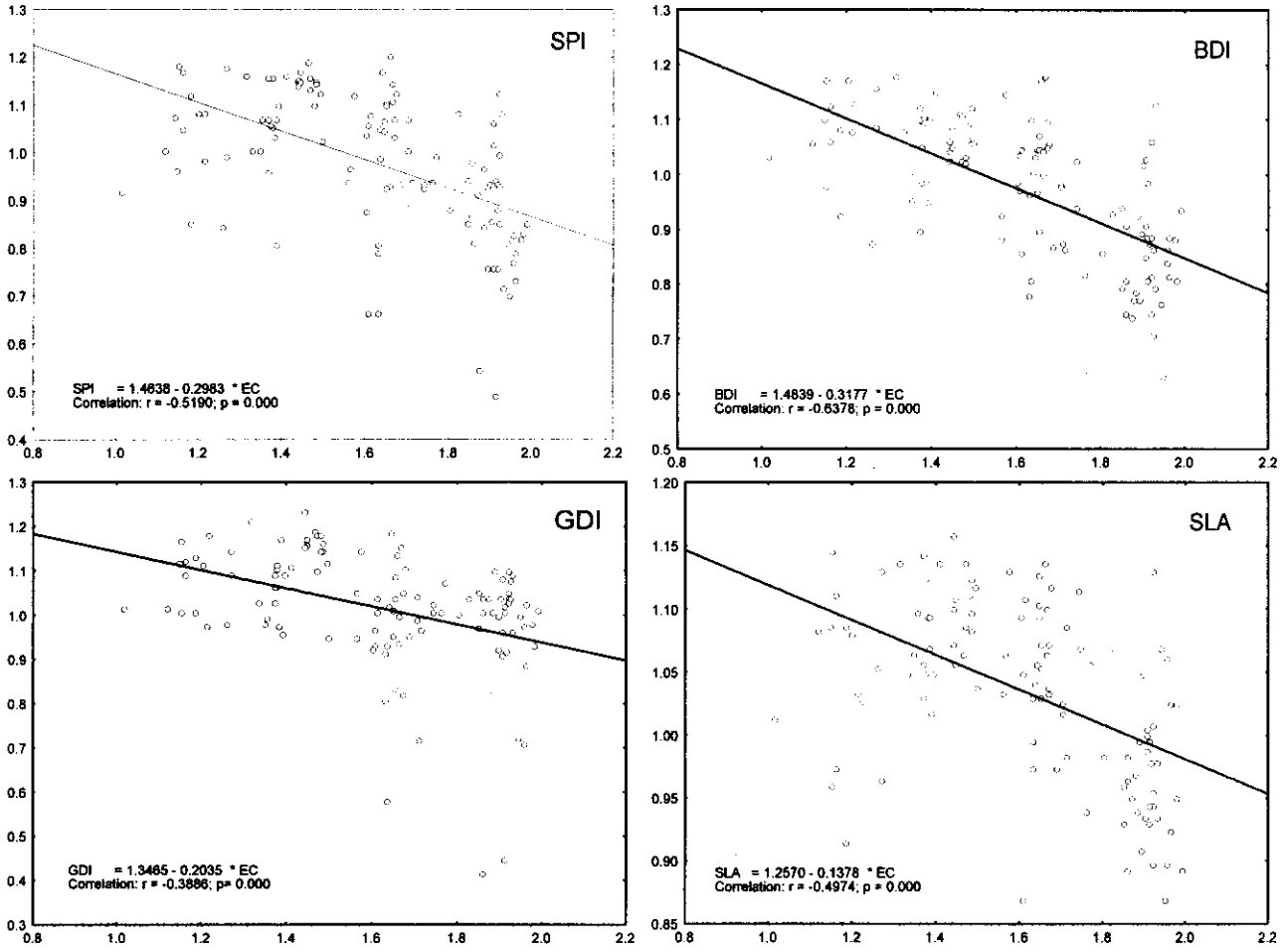

Figure 4.4: *Correlations between values for logEC and four diatom indices (SPI; Specific Pollution sensitivity Index, BDI; Biological diatom index, GDI; Generic Diatom Index, SLA; Sládeček's index of organic pollution) in the Vaal and Wilge Rivers (n = 113).*

X axis = logEC, Y axis = log diatom index score

4.3.3 Inter-correlation between diatom indices

Inter-correlations between the diatom indices tested in this study are presented in Table 4.6. All of the indices are significantly inter-correlated to varying degrees. Inter-correlation between the diatom indices has been reported by several authors (Prygiel & Coste, 1993b; Kelly *et al.*, 1995; Kwandrans *et al.*, 1998).

The highest correlation was found between SPI and CEC, SPI and LMI, SPI and BDI and SLA and LMI. It is important to note the correlation between SPI and the other diatom indices. SPI has the broadest species base and highest taxonomic resolution of all the indices, i.e. identification of taxa in some cases needs to be down to subspecies and form level. Other indices, such as CEC and BDI, have reduced species bases. The reduction of taxa necessary for the calculation of the index increases the usability of the index and does not necessarily detract from the accuracy of the final index score (Lenoir & Coste, 1996). Another important correlation to note is that between SPI and GDI (69 %). The calculation of the GDI score is based on identifications of taxa carried out to the genus level. Identification to genus level reduces the expertise needed by a technician for identification of the taxa encountered in a particular sample. However, this simplification is accompanied by a concomitant loss in the accuracy of the diatom index scores. Nevertheless, GDI is significantly correlated to both DIP and EC (see Table 4.3) and for practical purposes may prove useful as a monitoring tool.

TABLE 4.6

Pearson correlation coefficients between the calculated diatom indices at sites in the Vaal and Wilge Rivers Rivers (*index acronyms explained below)
Numerical values indicate significant correlations at $p < 0.01$ or higher
n=112 (Casewise deletion of missing data)

	SLA	DES	LMI	SHE	WAT	EPI	GDI	CEC	BDI	APDI
SPI	0.73	0.78	0.83	0.77	0.70	0.55	0.69	0.94	0.80	0.77
SLA		0.43	0.84	0.72	0.62	0.60	0.36	0.70	0.68	0.76
DES			0.61	0.69	0.51	0.24	0.53	0.74	0.46	0.44
LMI				0.75	0.54	0.43	0.59	0.76	0.69	0.81
SHE					0.70	0.47	0.43	0.67	0.56	0.60
WAT						0.69	0.37	0.72	0.64	0.55
EPI							0.30	0.58	0.49	0.39
GDI								0.64	0.56	0.49
CEC									0.81	0.72
BDI										0.74

*SPI; Specific Pollution sensitivity Index, SLA; Sládeček's index, DES; Descy's index, LMI; Leclercq & Maquet's Index, SHE; Schiefele and Schreiner's index, WAT; Watanabe's index, GDI; Generic Diatom Index, CEC; Council for European Communities index, BDI; Biological Diatom Index, APDI; Artois-Picardie Diatom Index, EPI; Eutrophication/Pollution Index.

Correlation between SPI and, for example, EPI is lower due to the fact that these two indices are used to reflect different aspects of water quality. SPI was designed to reflect general water quality (CEMAGREF, 1982), while EPI was designed only to reflect the effect of plant nutrients and ionic concentration on water quality (Dell'Uomo, 1996).

4.3.4 Comparing correlation results with those of European studies

Although diatom indices correlated well to the water quality variables measured in the present study it is necessary to compare these correlations to correlations demonstrated in similar studies in Europe. The comparison of correlation results is to determine the comparative level of success with which diatom indices may be applied as tools of bio-monitoring in South Africa.

TABLE 4.7

Pearson correlation coefficients between water quality variables and diatom indices at sites in the Vaal and Wilge Rivers, including additional comparative correlation data from European authors (*index acronyms explained below)
Numerical values indicate significant correlations at $p < 0.01$ or higher
Significantly different correlation coefficients highlighted in yellow

		See Table 4.3			See Table 4.2		
		pH	DIP	EC	DO_2	Temp.	Turb.
Present study *n* = 112	**SPI**	-0.29	-0.53	-0.52	..	-0.35	..
	SLA	-0.37	-0.51	-0.50	..	..	0.32
	DES	..	-0.36	-0.22	..	-0.27	..
	LMI	-0.34	-0.49	-0.50	..	..	0.26
	SHE	..	-0.37	-0.29	..	-0.29	..
	GDI	..	-0.32	-0.39	..	-0.29	..
	CEC	-0.34	-0.61	-0.59	..	-0.27	..
	BDI	-0.47	-0.43	-0.64	..	..	0.29
	APDI	-0.43	-0.33	-0.47	..	..	..
Kwandrans *et al.* (1998) *n* = 38	**SPI**	No data available	-0.51	-0.75	0.62	No data available	
	SLA		..	..	..		
	DES		-0.52	-0.75	0.60		
	LMI		-0.57	-0.69	0.62		
	SHE		..	-0.71	0.70		
	GDI		-0.51	-0.73	0.68		
	CEC		-0.62	-0.69	0.61		
	APDI		..	-0.56	0.50		
Prygiel & Coste (1993a) *n* = 355	**SPI**	0.15	0.54	0.17	0.38	0.36	..
	SLA	0.22	0.40	..	0.39	0.33	..
	DES	0.27	0.52	..	0.46	0.36	..
	LMI	0.22	0.52	..	0.44	0.35	0.14
	GDI	..	0.40	0.21	0.27	0.22	0.19
	CEC	0.16	0.50	0.19	0.37	0.35	0.14
Prygiel & Coste (1998) *n* = 188	**SPI**	0.17	0.64	0.56	0.62	No data available	
	BDI	0.15	0.62	0.61	0.62		
	APDI	0.22	0.62	0.54	0.63		

*SPI; Specific Pollution sensitivity Index, SLA; Sládeček's index, DES; Descy's index, LMI; Leclercq & Maquet's Index, SHE; Schiefele and Schreiner's index, GDI; Generic Diatom Index, CEC; Council for European Communities index, BDI; Biological Diatom Index, APDI; Artois-Picardie Diatom Index.

Table 4.7 is presented with the aim of indicating the degree with which the results obtained during different studies in Europe are comparable to the results obtained in the present study, which took place in South Africa.

Other than the Descy (DES) index, it can be seen that the correlation results between indices and water quality variables obtained in the present study are comparable and in some cases better than the correlations demonstrated in Europe. The same method used to evaluate the difference between the correlation coefficients in the previous section (4.3.1), was used to determine if there are any significant difference between correlation coefficients demonstrated by European authors and those demonstrated in the present study. The difference test takes into account the size of the samples (*n*), which is important when comparing correlation coefficients generated from samples of different sizes.

The only major exception in the similarity of the correlation coefficients generated in the present study and those generated by European authors (Table 4.7) can be seen in the correlation coefficients between DO and the diatom index scores. This may be because of the fact that a great variation in DO concentrations was not encountered in the present study. The median values for DO concentration ranged between 8 and 10 $mg.l^{-1}$. Levels of nutrients in the two rivers ensure high levels of photosynthesis and hence high levels of oxygen production. Although some extremes were noted in the levels of DO concentration, these may be because of external factors, such as oxygen depletion caused by a mat of water hyacinths, and the presence of major impoundments, rather than fluctuations in water quality (see discussion in Chapter 3, section 3.3.1).

Better correlation between pH and diatom index scores demonstrated in the present study (see Table 4.7) may have been because of the large variation in pH, due to pollution, encountered at the study sites. pH values recorded in this study ranged from a minimum of just above 7 to a maximum recorded pH of 9.35.

Kwandrans *et al.* (1998) demonstrated significantly higher correlation coefficients between electrical conductivity (EC) and the diatom indices DES, SHE and GDI than those demonstrated in the present study. The correlation analysis of Prygiel & Coste (1999) showed a significantly higher correlation between dissolved inorganic phosphate (DIP) and the ADPI than that demonstrated in Table 4.3.

In general it may be concluded that results obtained from the correlation analysis are comparable to those obtained in Europe. From this result it can, in turn be concluded firstly

that the diatom-based monitoring approach is useful in South Africa and secondly that certain diatom indices may be applied for use in the Vaal River, and later throughout South Africa after further testing in different parts of the country.

4.4 Regression analysis

The forward stepwise regression method takes the independent variable with the greatest contribution and adds it to the model first. Independent variables are then selected for inclusion based on their incremental contribution over the variable(s) already in the equation. Independent variables that are closely correlated in the correlation matrix may not all be included but rather other variables that also contribute to the variation in the index scores (Hair *et al.*, 1998). For this reason this method can give important additional information about the variables and conditions that influence the various diatom index scores over and above pure correlation. The results of the regressions are presented in graphs (Fig. 4.5) in which the predicted values for the combination of the environmental variables *vs.* a specific index are plotted against the observed (measured) variables. The multiple regression method has been used by Lenoir & Coste (1996) to demonstrate the relationship between several diatom indices and a combination of water quality variables.

For correlation analysis EC was chosen to represent the major ions (i.e. Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-}, K^{+} and SO_4^{2-}) and DIN was chosen to represent NH_4-N and the sum of NO_2-N + NO_3-N. Multiple regressions were performed on the data with two aims; firstly, to establish which of the component ions under the blanket terms of EC and DIN had any influence on the diatom index scores. Secondly regression analysis was used to determine which of the indices gave the best overall, i.e. integrative, reflection of general water quality. Multiple regression analysis was also performed on the Eutrophication/Pollution Index (EPI) to determine the degree of success with which this index reflects nutrient and ionic pollution. The regression method used is known as forward stepwise regression.

Fig. 4.5 demonstrates the levels of success of each of the diatom indices in indicating a suite of water quality variables. The degree of confidence in the indices is reflected by the r^2 value; the higher the value, the more accurate the indices are as integrative indicators of the measured water quality variables. The measured water quality variables account for 60% of the variation in the BDI scores, 48% of the variation in the SPI score, 40% of the variation in LMI scores, 35% of the variation in the GDI scores, 52% of the variation in the EPI scores and 46% of the variation of the SLA index scores. It can clearly be seen that the BDI index most successfully indicates general water quality. Other indices have stronger individual correlations to individual elements of water quality such as phosphate, for example EPI.

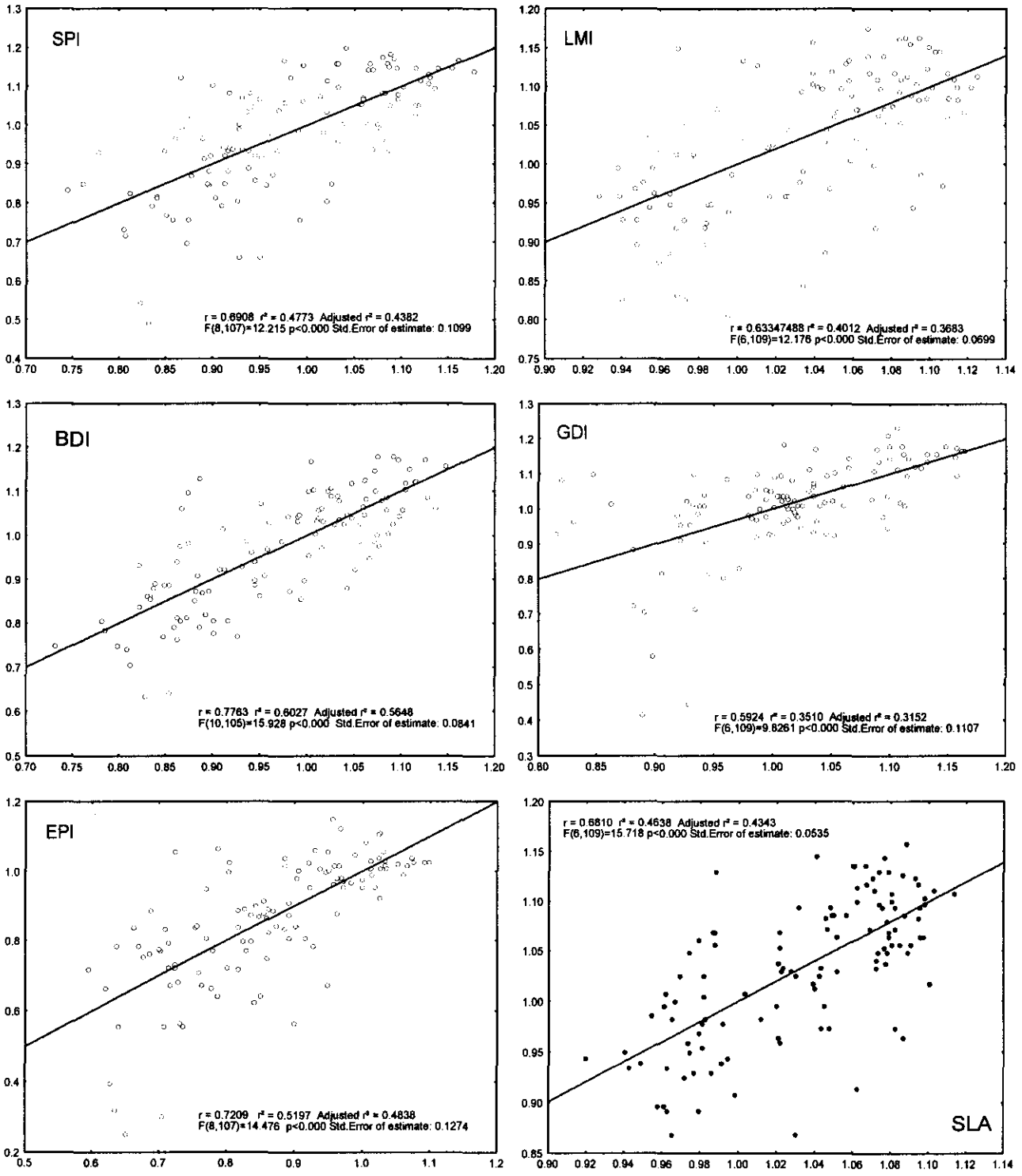

Figure 4.5: *Predicted vs. observed values for six diatom indices (SPI; Specific Pollution sensitivity Index, LMI; Leclercq & Maquet's Index BDI; Biological diatom index, GDI; Generic Diatom Index, EPI; Eutrophication/Pollution Index, SLA; Sládeček's index of organic pollution) in the Vaal and Wilge Rivers (n = 113).*

X axis = logPredicited diatom index values, Y axis = log observed diatom index values

Although GDI demonstrate the weakest relationship with general water quality, the relationship was highly significant ($p < 0.000$). With the exception of GDI, LMI demonstrated

the poorest performance of the six indices illustrated in Fig. 4.5. It can be concluded that the BDI is the most useful index for surveying general water quality, followed by the SPI. The GDI may be useful either for low-resolution studies or for introductory analyses when training technical staff in the use of diatom indices. The EPI should be used for monitoring of eutrophication rather than general water quality.

In France during the development of the Biological Diatom Index (BDI; Lenoir & Coste, 1996) the overall water quality analysis could explain 72% of the variation in the BDI, 53% in the SPI, 21% in the GDI, 22% in the SLA index, and 15% of the LMI. Lenoir & Coste (1996) believe that the limit for the performance of an index is obtained by using the BDI and that the additional 30% in the specific scoring of the index would need to be explained by the variability due to environmental factors not taken into account during the calculation of the index score. These factors include light, water flow (current speed), mineralisation and the nature of the substratum. From the results of the present study in which 60% of the variation in BDI scores can be explained by measured environmental variables, it can be concluded that this index is both applicable and useful for monitoring water quality in the Vaal and Wilge Rivers.

Six tables are presented below (Table 4.8-4.13) that summarise the regression results illustrated graphically in Fig. 4.5. Significant beta values are highlighted to demonstrate some of the additional factors that might have an influence on index scores.

TABLE 4.8

Regression Summary for Dependent Variable: **SPI**
$r = 0.6908$ $r^2 = 0.4773$ Adjusted $r^2 = 0.4382$
$F(8,107)=12.215$ $p<0.000$ Std.Error of estimate: 0.1099
Beta = standardised regression coefficient
B = non-standardised regression coefficient

	Beta	Std.Err.	B	Std.Err.	t(107)	p-level
Intercept			2.6591	0.5384	4.9392	0.0000
Ca^{2+}	**-1.1330**	**0.3588**	**-0.6442**	**0.2040**	**-3.1575**	**0.0021**
Temp	**-0.2492**	**0.0924**	**-0.2541**	**0.0943**	**-2.6956**	**0.0082**
Mg^{2+}	**0.9281**	**0.2639**	**0.5514**	**0.1568**	**3.5167**	**0.0006**
Turb	**-0.2119**	**0.0998**	**-0.0765**	**0.0360**	**-2.1240**	**0.0360**
DO	-0.0812	0.0778	-0.0698	0.0669	-1.0444	0.2987
DIN	0.1308	0.0905	0.0427	0.0295	1.4447	0.1515
pH	-0.1510	0.1187	-0.0829	0.0651	-1.2722	0.2061
Cl^-	-0.3439	0.3374	-0.1265	0.1241	-1.0195	0.3103

TABLE 4.9

Regression Summary for Dependent Variable: **LMI**
r = 0.6334 r^2 = 0.4012 Adjusted r^2 = 0.3683
F(6,109)=12.176 p<0.000 Std.Error of estimate: 0.0699
Beta = standardised regression coefficient
B = non-standardised regression coefficient

	Beta	Std.Err.	B	Std.Err.	t(109)	p-level
Intercept			0.9767	0.2029	4.8137	0.0000
Ca^{2+}	**-1.9930**	**0.4903**	**-0.6799**	**0.1673**	**-4.0647**	**0.0001**
Mg^{2+}	-0.0803	0.3812	-0.0286	0.1359	-0.2105	0.8336
TAL	**0.7607**	**0.3153**	**0.4482**	**0.1858**	**2.4124**	**0.0175**
Turb	-0.1516	0.0957	-0.0329	0.0207	-1.5850	0.1159
SO_4^{2-}	0.7712	0.4536	0.1497	0.0880	1.7002	0.0919
DIN	0.1193	0.0995	0.0234	0.0195	1.1991	0.2331

TABLE 4.10

Regression Summary for Dependent Variable: **BDI**
r = 0.7763 r^2 = 0.6027 Adjusted r^2 = 0.5648
F(10,105)=15.928 p<0.000 Std.Error of estimate: 0.0841
Beta = standardised regression coefficient
B = non-standardised regression coefficient

	Beta	Std.Err.	B	Std.Err.	t(105)	p-level
Intercept			3.1102	0.5204	5.9763	0.0000
Ca^{2+}	**-1.5361**	**0.2990**	**-0.7592**	**0.1478**	**-5.1372**	**0.0000**
Mg^{2+}	**1.1639**	**0.2437**	**0.6011**	**0.1258**	**4.7766**	**0.0000**
SiO_2Si	0.1815	0.1025	0.0575	0.0324	1.7709	0.0795
Turb	-0.1687	0.0933	-0.0530	0.0293	-1.8083	0.0734
NH_4-N	0.0295	0.0695	0.0145	0.0342	0.4253	0.6715
pH	**-0.2791**	**0.1129**	**-0.1331**	**0.0539**	**-2.4708**	**0.0151**
Temp	**-0.2452**	**0.0972**	**-0.2174**	**0.0862**	**-2.5222**	**0.0132**
DIP	**0.3182**	**0.1403**	**0.1116**	**0.0492**	**2.2674**	**0.0254**
DO	-0.1041	0.0688	-0.0778	0.0514	-1.5125	0.1334
K^+	-0.2305	0.1977	-0.0950	0.0815	-1.1655	0.2464

TABLE 4.11

Regression Summary for Dependent Variable: **GDI**
r = 0.5924 r^2 = 0.3510 Adjusted r^2= 0.3152
F(6,109)=9.8261 p<0.000 Std.Error of estimate: 0.1107
Beta = standardised regression coefficient
B = non-standardised regression coefficient

	Beta	Std.Err.	B	Std.Err.	t(109)	p-level
Intercept			1.6580	0.1459	11.3619	0.0000
K^+	**-0.6386**	**0.2277**	**-0.2762**	**0.0985**	**-2.8047**	**0.0060**
Turb	**-0.2962**	**0.1016**	**-0.0976**	**0.0335**	**-2.9149**	**0.0043**
NO_3-N+NO_2-N	**0.4154**	**0.1028**	**0.1054**	**0.0261**	**4.0400**	**0.0001**
SiO_2-Si	-0.1130	0.1114	-0.0375	0.0370	-1.0139	0.3129
Mg^{2+}	**0.7847**	**0.2624**	**0.4252**	**0.1422**	**2.9905**	**0.0034**
Ca^{2+}	**-0.9413**	**0.3255**	**-0.4882**	**0.1688**	**-2.8915**	**0.0046**

TABLE 4.12

Regression Summary for Dependent Variable: **EPI**
$r = 0.7209$ $r^2 = 0.5197$ Adjusted $r^2 = 0.4838$
$F(8,107)=14.476$ $p<0.000$ Std.Error of estimate: 0.1274
Beta = standardised regression coefficient
B = non-standardised regression coefficient

	Beta	Std.Err.	B	Std.Err.	t(107)	p-level
Intercept			-0.2147	0.5231	-0.4105	0.6823
DIP	**-0.3439**	**0.1104**	**-0.1678**	**0.0538**	**-3.1162**	**0.0024**
SO_4^{2-}	-0.1883	0.3789	-0.0736	0.1481	-0.4970	0.6202
pH	**0.2630**	**0.1038**	**0.1745**	**0.0689**	**2.5323**	**0.0128**
Turb	**-0.1878**	**0.0931**	**-0.0820**	**0.0406**	**-2.0174**	**0.0462**
NH_4-N	0.1344	0.0715	0.0920	0.0489	1.8799	0.0628
DO	-0.0846	0.0722	-0.0879	0.0751	-1.1714	0.2441
Cl^-	-0.7895	0.4094	-0.3511	0.1821	-1.9281	0.0565
Mg^{2+}	0.4163	0.2226	0.2990	0.1599	1.8706	0.0641

TABLE 4.13

Regression Summary for Dependent Variable: **SLA**
$r = 0.6810$ $r^2 = 0.4638$ Adjusted $r^2 = 0.4343$
$F(6,109)=15.718$ $p<0.000$ Std.Error of estimate: 0.0535
Beta = standardised regression coefficient
B = non-standardised regression coefficient

	Beta	Std.Err.	B	Std.Err.	t(109)	p-level
Intercept			1.1788	0.2783	4.2353	0.0000
SO_4^{2-}	0.5750	0.4384	0.0903	0.0689	1.3113	0.1925
NO_3-N+NO_2-N	0.0539	0.1013	0.0073	0.0137	0.5323	0.5956
Mg^{2+}	0.0873	0.3930	0.0252	0.1134	0.2222	0.8246
Ca^{2+}	**-1.8043**	**0.4732**	**-0.4982**	**0.1307**	**-3.8127**	**0.0002**
TAL	**0.8650**	**0.3008**	**0.4126**	**0.1435**	**2.8756**	**0.0049**
pH	-0.1890	0.1111	-0.0504	0.0296	-1.7011	0.0918

The information contained in Tables 4.8 to 4.13 can be summarised as follows: Of the major ions calcium and magnesium demonstrated a significant relationship to the different index scores and potassium demonstrated a significant relationship to the GDI index score. The Klip River, which carries effluent from the highly urbanised, industrialised and intensely mined areas of Southern Gauteng into the Vaal Barrage and in turn into the Vaal River, has high concentrations of the major ions calcium and magnesium.

Calcium is one of the major elements essential for living organisms for the maintenance of the structural and functional integrity of cell membranes (Wetzel, 1983). Calcium ions are often the major cations in inland waters but very little is known about the actual effects of changes in its concentration on aquatic biotas. Magnesium is an essential element, being found in chlorophyll and a number of enzymes. Potassium is involves in the ionic balance of all organisms and, because it occurs in much lower concentrations than, for example sodium, potassium can sometimes act as a nutrient the lack of which limits plant growth (Dallas & Day, 1993).

Turbidity also has an influence on some of the indices including SPI, GDI and EPI. Turbidity may influence diatom index scores in the manner discussed in section 4.3.1.1.

The Eutrophication/Pollution Index (EPI) is most significantly influenced by DIP as would be expected in a trophic index, additional influences come from pH and turbidity.

Alkalinity (TAL) has an influence on the index scores of both the LMI as well as the SLA index. Levels of TAL are elevated in industrial effluents and may also increase due to anthropogenic eutrophication. Temperature fluctuations influence both the SPI and BDI indices (see also Table 4.2). Temperature changes with the seasons, so at higher water temperature (i.e summer) index scores will show an increase in score due to the dilution of river water by rain water that increases general water quality. GDI is significantly influenced by the concentrations of nitrate + nitrite in the Vaal and Wilge Rivers. Plant nutrients in general affect indices such as the GDI as they are based on autotrophic organisms directly affected by nutrient levels in the surrounding environment.

4.5 Diatom index scores

In section 4.3 it was demonstrated that several indices showed a strong relationship to water quality (as reflected by the variables pH, total alkalinity (TAL), dissolved inorganic phosphate (DIP), dissolved inorganic nitrogen (DIN), electrical conductivity (EC), silicate silicon, dissolved oxygen, temperature and turbidity), comparable to results obtained in Europe (see Table 4.7). The diatom scores generated monthly for each site using the tested diatom indices may be found in Appendix 4. A selection of the tested diatom indices were chosen with the aim of representing the degree of aquatic pollution (both general, organic and trophic pollution) at each of the study sites in the Vaal and Wilge Rivers. The indices chosen for the representation of water quality are the Specific Pollution sensitivity Index (SPI), Leclercq & Maquet's Index (LMI), the Biological Diatom Index (BDI), the Generic Diatom Index (GDI), Sládeček's index (SLA) and the Eutrophication and Pollution Index (EPI). SPI was chosen as it has the broadest species base, LMI showed a good overall correlation to water quality, BDI showed the best overall correlation to water quality variables, GDI functions at a genus level of identification and is hence the simplest index to use. Of the two indices of organic pollution, the SLA index demonstrated the best overall correlation to water quality variables and the EPI showed the highest correlation to dissolved inorganic phosphate. These six different indices are presented in Fig. 4.6.

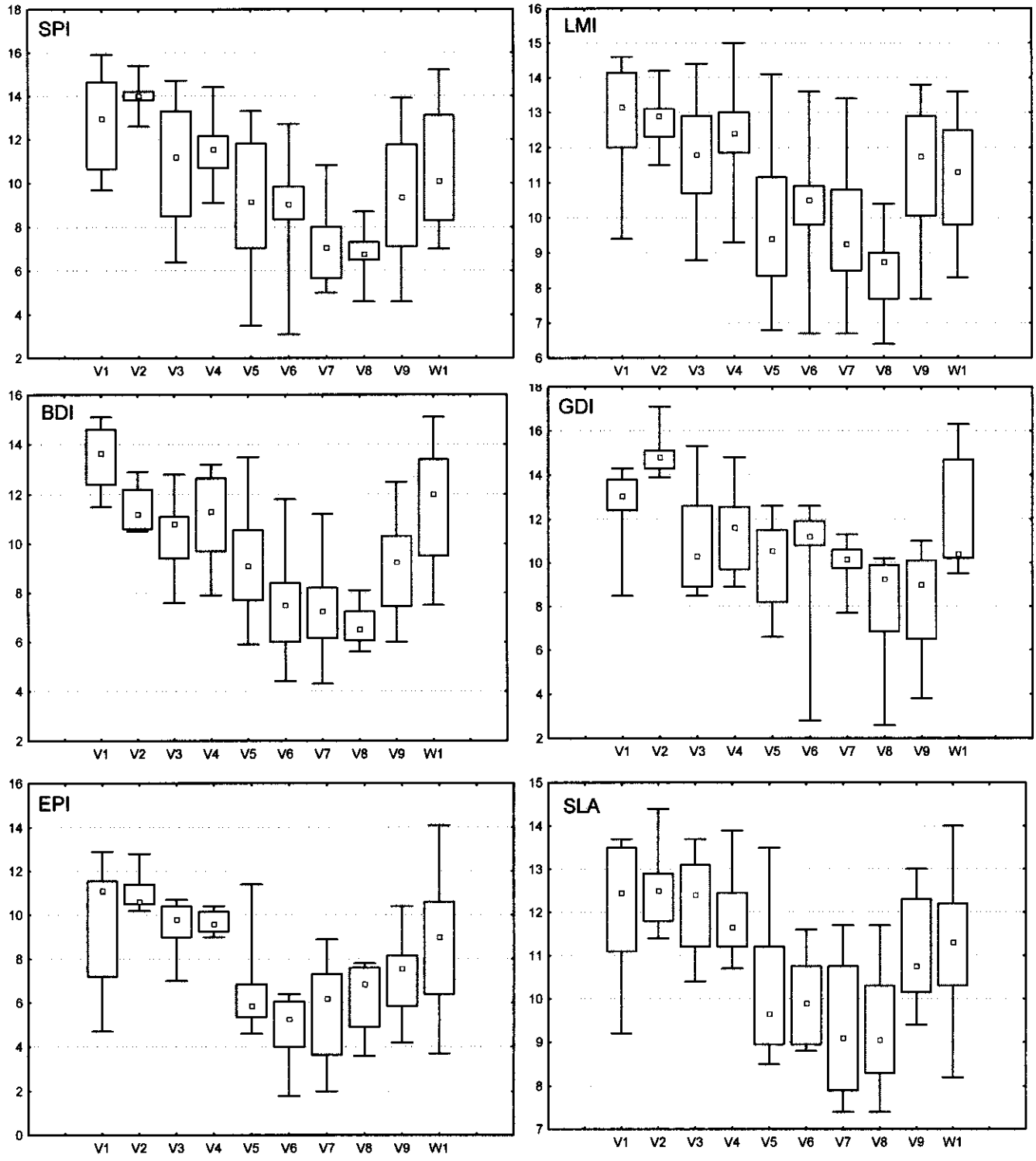

Figure 4.6: *Box plots of median annual values for six diatom indices in the Vaal and Wilge Rivers. (Boxes display the 25th to 75th percentiles of the variable, whiskers display the maximum and minimum values; V1 = Bloukop, V2 = Downstream of Grootdraai Dam, V3 = Gladdedrift, V4 = Downstream Vaal Dam, V5 = Goose Bay Estates downstream of Vaal Barrage, V6 = Schoemansdrift, V7 = Orkney, V8 = Kliplaatdrift, V9 = Downstream of Bloemhof Dam, W1 = Wilge River at Frankfort). X axis = Site, Y axis = Diatom index score*

The index scores range between 0 and 20, a decreasing score indicating an increasing level of pollution or eutrophication. Class limit values were imposed on diatom index scores for SPI and GDI to indicate levels of pollution in Finland (Eloranta & Soininen, 2002). These values are given in Table 4.14 and will be used in this study for the interpretation of the scores yielded by the various indices.

TABLE 4.14

Class limit values for diatom indices (Eloranta & Soininen, 2002)

Index score	Class	Trophy
>17	high quality	Oligotrophy
15 to 17	good quality	oligo-mesotrophy
12 to 15	moderate quality	Mesotrophy
9 to 12	poor quality	meso-eutrophy
<9	bad quality	Eutrophy

The six diatom indices presented in Fig. 4.6 include indices of general water quality (SPI, LMI, BDI, GDI), an index that integrates trophic status and ionic composition (EPI) and an index of organic pollution (SLA). All of the graphs in Fig 4.6, with the exception of the graph for EPI display a steady downward trend in water quality over distance up to point V8 at Balkfontein before the Vaal River flows into the Bloemhof Dam. This decline is interrupted at the sites of major dams. As discussed in section 3.2 water quality increases slightly, immediately downstream of large dams due to consumption and sedimentation of major nutrients and ions within the dam. These improvements are most noticeable at V4 below the Vaal Dam and V9 below Bloemhof Dam and in some indices at V2 below the Grootdraai Dam.

The EPI (Fig 4.6) can be seen to demonstrate a different trend than the other indices. A steady improvement can be seen from site V6 at Orkney to V8 at Kliplaatdrift in contrast to the other indices. This may be directly ascribed to the influence of plant nutrients (DIN and DIP), the concentration of which decreases from V6 to V8 and hence the concomitant increase in EPI score.

4.6 Overall evaluation of the diatom indices for use as a biomonitoring tool in South Africa

It has been demonstrated in the preceding sections that the diatom indices tested, especially the two general water quality indices, i.e. the Biological Diatom Index (BDI) and the Specific Pollution sensitivity Index (SPI), as well as a specific index, the Eutrophication/Pollution Index (EPI), can be used for water quality monitoring in the Vaal and Wilge River systems. It was one of the aims of this study to assess the effort needed to obtain results from the

various indices, i.e. how much taxonomic expertise is needed as well as how much time needs to be spent to gain a meaningful result. Both indices are based on a large number of species, and those determined from fewer taxa function successfully in the Vaal and Wilge Rivers.

SPI shows a good, significant correlation to DIP and other determinants used for assessing general water quality. The calculation of SPI depends on the correct identification of all taxa in the sample to species, variety and form level. Identification of diatom taxa to this level requires an in-depth knowledge of diatom taxonomy and cannot be used with success without this knowledge. Therefore only a person well trained in diatom taxonomy can use this index effectively. Such in-depth enumeration is also very time-consuming.

In contrast the calculation of BDI, which has a better relationship to general water quality only requires the identification of 209 key taxa. Although less taxa are used in the calculation, it still maintains a high level of integrity due to the long period and wide geographical range of testing. The reduced number of taxa in this index makes it easier for a non-diatom specialist to use than for example the Specific Pollution sensitivity Index (SPI).

An even simpler index is the GDI or Generic Diatom Index. As the name implies, this index is based only on the identification of diatom taxa up to genus level of 174 taxa. This index may be useful for the purposes of providing an initial indication that an aquatic system is polluted. However, the resolution of this index is considerably lower than the other two indices discussed above (SPI, BDI), which rely on species-level identifications.

The Eutrophication/Pollution Index (EPI) successfully reflects the levels of nutrient and ionic pollution in the Vaal and Wilge Rivers (see Table 4.3). EPI is based on 93 taxa with specific trophic and halobic affinities. The narrow species base, together with the high resolution of the results obtained, makes this index potentially useful for the trophic classification of other South African river systems.

Although the SLA index shows significant relationships to most of the elements of water quality measured in this study (see Table 4.3), the index was originally based on measurements for BOD_5. Unfortunately, no BOD_5 data was available for use in the present study and hence the SLA index would need to be tested against BOD_5 in either the Vaal River or other river systems.

4.7 Classification of the general water quality of the Vaal and Wilge Rivers

After the overall evaluation of the diatom indices a demonstration will be given below of the application of the Biological Diatom Index (BDI) for monitoring general water quality. The demonstration takes the form of a water quality map drawn for the month of June 2002, with various stretches coloured to give a reflection of different water classes determined with the aid of the BDI. The main purpose of the demonstration and the following discussion is to highlight the difference between chemical monitoring and biological monitoring of aquatic ecosystems. In addition, it is important to provide a visual realisation of water quality assessments, as it is this type of representation, which is most likely to be easily interpretable and have meaning for water management agencies such as DWAF. Prygiel & Coste (1993b) stated that when the environment is relatively stable, two or three diatom samples per year could be sufficient to estimate water quality using diatom indices. A single diatom sample taken in each season may provide an accurate overview of general water quality in South African rivers.

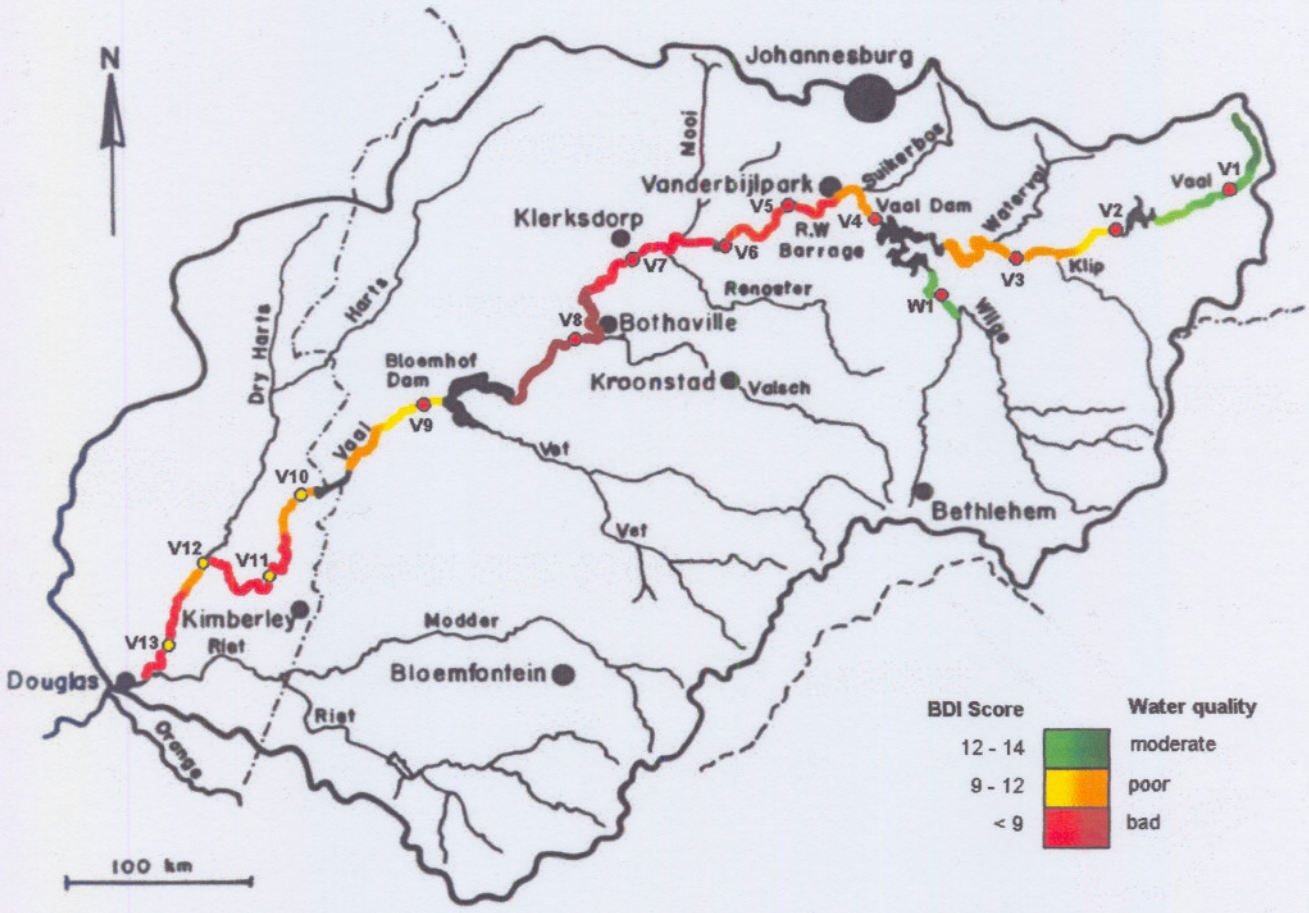

Figure 4.7: *General water quality classification using the Biological Diatom Index (BDI), for the month of June 2002 in the Vaal Catchment*

Long-term chemical monitoring programmes with a high frequency of sampling allow relatively accurate conclusions to be drawn about changing water quality. On the other hand, biological samples can be taken with less frequency as communities build up a "memory" of the prevailing chemical conditions in a river (see section 4.3.1.4). Diatom-based autecological indices can be particularly valuable in stream and river assessments because one-time assay of species composition of diatoms in streams could provide better characterisations of physical and chemical conditions than one-time measurement of those conditions (Stevenson & Pan, 1999).

The Specific Pollution sensitivity Index (SPI), the GDI (Generic Diatom Index) and the Council for European Communities index were created, as other existing diatom indices, upon more or less empirical ecological bases with limited geographic datasets. In spite of their suitability for the assessment of water quality in running waters, underlined by various authors (e.g. Whitton *et al.*, 1991; Eulin *et al.*, 1993; Prygiel, 1994; Kelly *et al.*, 1995), surveys performed in France (Lenoir & Coste, 1994) demonstrated that the performance of these indices was still open to improvement (Lenoir & Coste, 1996). With the aim of developing an improved index for water quality monitoring in France, the Biological Diatom Index (BDI) was created to be a truly practical but, nevertheless, not a simple diatom index capable of significantly improving the link between the index score and overall water quality. The BDI was established from a considerable data-base covering the whole of France, with reliable data on the diatom populations and water quality. Lenoir & Coste (1996) found that the variability of the physical and chemical parameters of the water served to explain 72% of the variability in the index scoring, which is twice that of the performances demonstrated in their study with existing diatom indices (maximum of 37% achieved by the SPI).

In the Vaal River the Biological Diatom Index (BDI) showed the closest correlation of all the tested indices to general water quality. The variability of the physical and chemical parameters of the water served to explain 60% of the variability in the BDI scores. Because of the strong correlation of the BDI index to all the measured variables of general water quality used in this study, this index was chosen to represent water quality in the Vaal and Wilge Rivers in the month of June 2002 (Fig. 4.7) to demonstrate the success of diatom indices in classifying a river system from a single sample.

If Fig. 4.7 is examined it can be seen that a once-off sampling of diatom communities gives an accurate reflection of the general water quality of the Vaal River. A steady decline in quality may be noted from site V1 at Bloukop near to the source of the Vaal River to site V8 at Klipplaatdrift before the Vaal River flows into the Bloemhof Dam. Water quality increases

after the Bloemhof Dam and again decreases, from V9 until V13 at Schmitsdrift just before the confluence of the Vaal with the Orange River. Water quality increases slightly at site V12 (Gamagara) after the confluence of the Harts River with the Vaal River. The classification of the Vaal River, based on diatom index scores, reflects known fluctuations in water quality in different regions of the Vaal River (see Chapter 3).

A summary of the general water quality of the Vaal River can be made from Braune & Rogers (1987). In general, the best quality waters of the Vaal Catchment are found in the catchment of the Vaal Dam. Water quality deteriorates downstream. Downstream of the Barrage the Vaal River quality is dominated by high sulphate loads with a corresponding smaller contribution of total alkalinity. This is due to high inputs from the tributaries draining the heartland of Gauteng, which are heavily contaminated by intensive mining and industrial activities. In the present study a similar trend in water quality was demonstrated (see Chapter 3). In the Vaal River downstream of Bloemhof Dam the upstream pollution effects are ameliorated to some extent by inflow of waters of low sulphate and high bicarbonate and chloride levels from the east (Braune & Rogers, 1987). However, irrigation return-flows contribute high TDS water rich in chlorides via the Harts River in the North (Braune & Rogers, 1987).

The value of diatom-based assessment techniques can be demonstrated by comparing results yielded by diatom index scores and other biological monitoring techniques. The South African Scoring System (SASS) is based on macroinvertebrate insects and is the backbone of most biomonitoring studies in South Africa. The SASS4 technique was used by Laas (2002) to assess the general water quality of the stretch of the Vaal River, which flows through the Vredefort Dome area (between points V6 and V7). It was found that in general the water quality was fair to excellent according to the SASS4 classification, dependant on the season. If the average concentrations of the chief chemical determinants used for the assessment of water quality are taken into account, this stretch of the Vaal River can by no means be classified as excellent or even fair. An inaccurate assessment of water quality was, therefore, yielded using SASS4 techniques. According to Prygiel & Coste (1993b) diatoms provide an interesting alternative or addition to invertebrate indices for the studying of large or canalised rivers. The two systems can be used together because diatom indices provide information mainly on water quality, including eutrophication and mineralisation, whereas invertebrate indices include information on sediment and streambed quality (Prygiel & Coste, 1993b).

4.8 Trophic classification of the Vaal and Wilge Rivers

A similar argument for using diatom as indicators of general water quality (see section 4.7) may be used for the use of diatoms for monitoring eutrophication. A single diatom sample per season could provide an accurate indication of levels of eutrophication in a river system. Diatoms, as primary producers, take up the nutrients implicated in eutrophication. Different diatom species have well defined limits to nutrients and thus the composition of any given diatom community provides a reflection of the concentrations of nutrients present in an aquatic ecosystem. The Eutrophication/Pollution Index (EPI) showed the closest correlation to measured levels of inorganic phosphate, and hence was chosen to represent the trophic status of the Vaal River in June 2002 (Fig. 4.8).

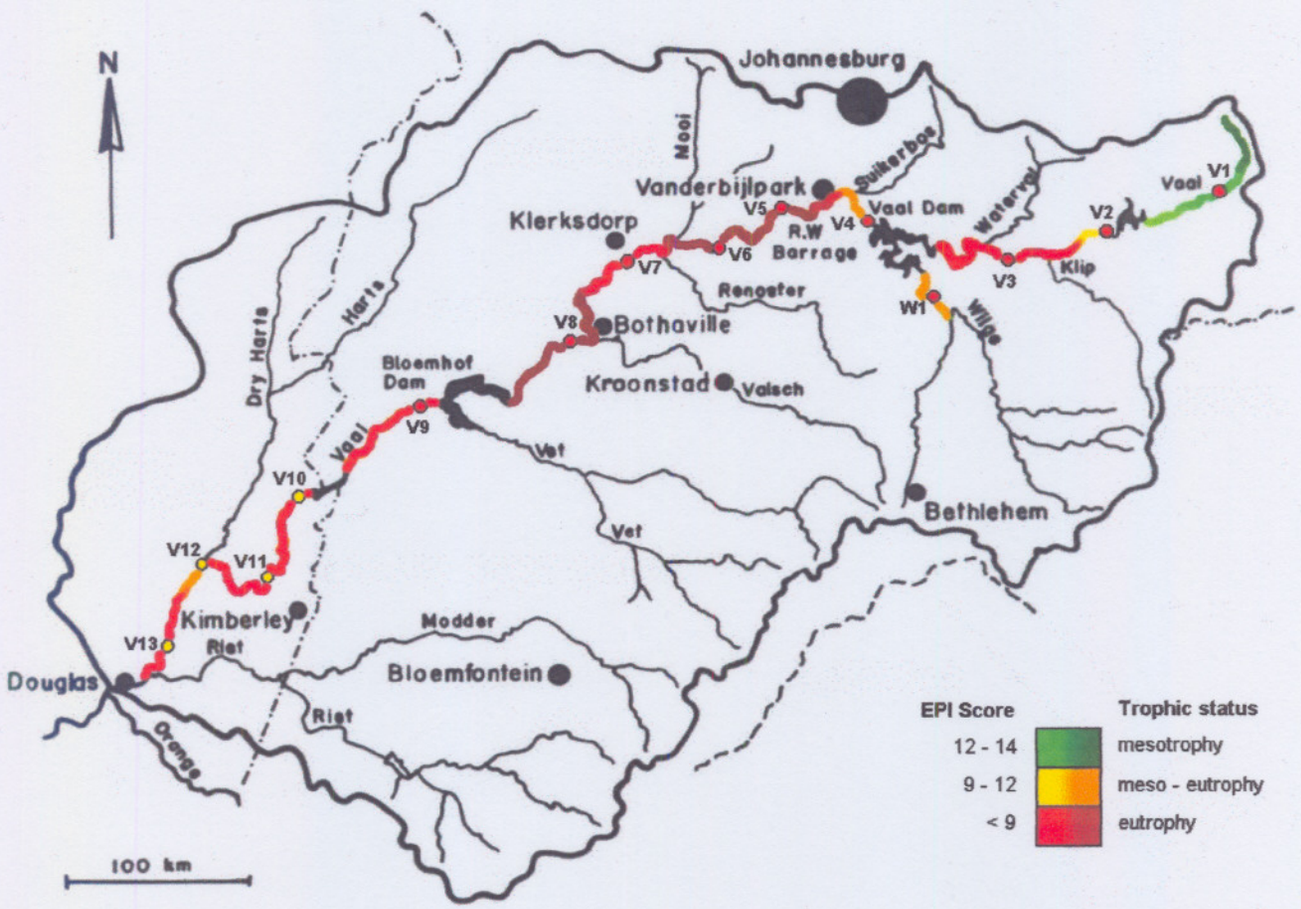

Figure 4.8: *Trophic status classification using the Eutrophication/Pollution Index (EPI), for the month of June 2002 in the Vaal Catchment*

The mid-Vaal has been classified as eutrophic (Janse van Vuuren, 2001). The rest of the Vaal River receives nutrient input from sources including agriculture, mining and industry, even the Wilge River, which has good general water quality has high ambient levels of nutrients probably originating from agricultural run-off. The EPI reflects levels of

eutrophication at the studied sites in the Vaal and Wilge Rivers as the index is correlated to levels of dissolved inorganic phosphate (DIP) in the Vaal and Wilge Rivers.

Criteria for assessing trophic status from total phosphate concentration are based on annual average values (OECD, 1982). Real time observations of ambient concentrations can lead to an incorrect understanding of the roles of nutrients such dissolved inorganic phosphate (DIP). It is, therefore, important to have a holistic ecosystem perspective of the situation (Walmsley, 2000). Walmsley (2000) is of the opinion that immediate national research should be directed at quantitatively assessing the eutrophication problem in terms of its extent and trends and the sources and levels of nutrients entering aquatic systems. Amongst other topics, Walmsley considers the development of national and catchment-based indicators for assessing eutrophication as vital.

Several diatom assessments per year could determine if an ecosystem is eutrophic, because the diatoms assimilate nutrients they provide an integrated picture of the recent past (up to six weeks in the Vaal and Wilge Rivers). Thus the number of samples needed to determine trophic status of a riverine ecosystem is less than for chemical monitoring. The Eutrophication/Pollution Index (EPI) or a similar diatom index needs to be tested and applied to eutrophication monitoring in South Africa.

4.9 Summary

The preceding sections of Chapter 4 may be summarised as follows: 98% of the diatom species encountered in the present study are cosmopolitan (i.e. occur in both Europe and South Africa). It was concluded in section 4.2 that outdated literature, in terms of taxonomical advances and nomenclature changes, has lead to the supposition by some authors that the diatom flora of South Africa is largely endemic.

When the relative abundance of diatom species composing the diatom communities sampled was used to calculate a diatom index score, the index scores generated showed significant correlation to different concentrations and levels of the measured water quality variables. The main water quality variables, which have an influence on diatom index scores in the Vaal River, are pH, total alkalinity (TAL), dissolved inorganic phosphate (DIP), electrical conductivity (EC) and turbidity. Diatom species composition changes seasonally as temperature changes, but the taxa forming the association remain indicative of a particular environmental condition.

Diatom community composition in the Vaal River provides an accurate indication of average water quality variables measured two weeks before sampling (Table 4.3) as well as average water quality variables measured six weeks before sampling (Table 4.5). Significant correlation was also demonstrated between once-off chemical samples and diatom indices (Table 4.2), however, the relationship between diatom indices and average water quality data is much stronger and from this it may be concluded that diatom provide an integrated reflection of general water quality confirming the previous postulation of authors such as Schoeman (1976).

Different diatom-based indices demonstrated different levels of correlation to different water quality variables. Of the diatom indices tested the Biological Diatom Index (BDI) demonstrated the best correlation to water quality variables and showed a consistent response over time (see Table 4.3 and 4.5). The Eutrophication/Pollution Index (EPI) showed the best relationship to measured concentrations of dissolved inorganic phosphate (DIP; Table 4.3) in the Vaal and Wilge Rivers and may, after further testing in South Africa, prove to be a useful indicator of the trophic status of riverine ecosystems.

In assessing the usefulness of the diatom indices, from the correlation results, for application as aquatic monitoring tools it is concluded that the Specific Pollution sensitivity Index (SPI) with its broad species base needs a knowledgeable diatom taxonomist to use the index

accurately. The Generic Diatom Index may prove useful for general low-resolution water quality assessments. The GDI needs a far less skilled diatom taxonomist to obtain a reliable result than either the SPI or BDI because of the reduced taxonomic base of the GDI. The BDI may prove useful as it has the closest relationship to water quality (demonstrated by correlation analysis) and is based on a limited number of species.

Correlation and regression results between diatom index scores and water quality variables were in most cases consistent (after similarity tests) between Europe and South Africa. Therefore, the tested diatom indices can be applied for water quality monitoring in South Africa in the Vaal and Wilge Rivers.

Regression analysis demonstrated a similar relationship between the Biological Diatom Index (BDI) and overall water quality, to the relationship demonstrated between the BDI and overall water quality in France. Again in the regression analysis BDI demonstrated the most significant relationship of all of the tested diatom indices to water quality.

When comparing the median diatom index scores (Fig. 4.6) for the period March 2002 to February 2003 to the median values for the water quality variables for the same period in the Vaal River (see Chapter 3), a similar decreasing trend in water quality downstream from the source can be noted. As in the median values for the water quality variables, the median diatom index scores show an increase in water quality downstream of major impoundments, coupled with less variation in water quality. The EPI index reflects (by an increasing score) the decreasing nutrient concentrations from V5 below the Vaal Barrage to V8 at Kliplaatdrift. The general water quality indices (SPI, GDI and BDI) show a decreasing water quality from V5 to V8 as pH and electrical conductivity increase.

The BDI was used to assess general water quality in the Vaal and Wilge Rivers, demonstrated by the map drawn to illustrate different water quality classes in different stretches of the Vaal River, as reflected by diatom index scores. The EPI was used to map the trophic status of the Vaal River for the month of June. It is important to provide a visual realisation of water quality assessments, as it is this type of representation, which is most likely to be easily interpreted and have meaning for water management agencies such as DWAF.

It is concluded, from the opinions of European authors as well as the results obtained during the present study, that diatom communities can build up "memory" of recent water quality conditions (up to six weeks in the Vaal and Wilge Rivers; see Table 4.5). The capacity of

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

In the previous chapters an attempt has been made to demonstrate the chemical and physical water quality of the Vaal River during the course of the present study (Chapter 3). Chapter 4 was set out with the intention of demonstrating a relationship between the structure of diatom communities (as reflected by diatom index scores) and the water quality variables discussed in Chapter 3. Several conclusions may be drawn based on the results found in both Chapter 3 and Chapter 4; an elaboration of these conclusions follows in the paragraphs below.

Several conclusions can be drawn based on Chapter 3. In general the chemical and physical quality of the Vaal River decreases downstream from the source until reaching the Bloemhof Dam. The median values for pH, total alkalinity and electrical conductivity all increase downstream in the Vaal River. Nutrient concentrations peak in the Vaal River after the Vaal Barrage at sampling site V5 and gradually decrease until the Vaal River enters the Bloemhof Dam. All of the study sites in the Vaal River, with the exception of site V2 just below the Grootdraai Dam, may be classified as eutrophic. In Chapter 3 major dams in the Vaal River (the Vaal, Bloemhof and Grootdraai) were shown to have increased water quality directly downstream of their walls in comparison to sites upstream of the dams. The downstream trend in water quality in the Vaal River observed in the present study was also documented by Braune & Rogers (1987). The Wilge River at site W1 was shown to have low median pH values and low median electrical conductivity concentration when compared to sites in the Vaal River. Median dissolved inorganic phosphate concentrations in the Wilge River at site W1 are elevated to the extent that the river may be classified as eutrophic (DWAF, 1995).

From the results presented in Chapter 4 it can be concluded that the tested diatom indices can be applied in water quality monitoring studies in the Vaal and Wilge Rivers and that the results yielded by diatom indices are consistent between the Northern and Southern Hemispheres (see Chapter 4, Table 4.7). These general conclusions as well as other conclusions and recommendations will be discussed below.

Of the tested diatom indices the Specific Pollution sensitivity Index (SPI), the Biological Diatom Index (BDI) and the Generic Diatom Index (GDI) are useful for monitoring general water quality in the Vaal and Wilge Rivers. The BDI most accurately integrates all the measured water quality variables and as such was the most successful index for indicating

general water quality (see Fig. 4.6). The GDI may be useful when only a general indication of water quality is required. The Eutrophication/Pollution index (EPI) accurately indicates the level of eutrophication in the Vaal and Wilge Rivers (see Chapter 4, Table 4.3 and section 4.3.2.1).

The Trophic diatom index (TDI) was less successful than the EPI in indicating levels of dissolved inorganic phosphate in the Vaal and Wilge Rivers. This may be because centric diatom species, which play a prominent part in eutrophication, are excluded from the TDI calculation.

When the median diatom index values presented in Chapter 4 are compared to the median values for the different water quality variables in Chapter 3, it can be seen that the diatom indices accurately reflect decreacing downstream trend in water quality of the Vaal River until it reaches the Bloemhof Dam. The increases in water quality (i.e decreace in the median levels or concentrations of water quality variables) just below the major dams in the Vaal River (Vaal, Bloemhof and Grootdraai) are also reflected by a concomitant increase in the median values of the diatom indices (see Fig. 4.6).

The overall conclusion after the testing of the diatom indices is that the SPI, BDI, GDI and EPI diatom indices are usable, accurate and valuable tools of bio-monitoring in the Vaal and Wilge Rivers, and may be used to quantify general aquatic pollution and levels of eutrophication in these rivers. After further testing, indices such as that of Sládeček (based on BOD_5 measurements) may be used to indicate organic pollution.

The results of this study show that not only do the diatom communities, when analysed, provide an accurate assessment of water quality, but these diatom communities also provide an integrated reflection of past water quality. The unique composite picture of ecosystem condition provided by the diatoms can only be replicated by intensive chemical monitoring studies. Diatom community composition in the Vaal River provides an accurate indication of average water quality variables measured two weeks before sampling (Table 4.3) as well as average water quality variables measured six weeks before sampling (Table 4.5). Significant correlation was also demonstrated between once-off chemical samples and diatom indices (Table 4.2), however, the relationship between diatom indices and average water quality data is much stronger and from this it may be concluded that diatom provide an integrated reflection of general water quality.

In general, the results of the present study presented in Chapter 4 show that diatom communities do not react to peaks in water quality variables, with the possible exception of pH. It is concluded that spikes in pH may determine diatom community composition rather than the average pH over a given period. However, the above-mentioned relationship between "spikes" of pH and diatom communities has not been demonstrated experimentally and thus should be the subject of further investigation.

J. Prygiel* (pers. comm.) on being appraised of the successful implementation of diatom indices in the Vaal and Wilge Rivers expressed the opinion that while most diatom taxa are cosmopolitan (i.e. present everywhere), making the use of European indices valuable to countries such as South Africa, Prygiel cautions that some taxa may be specific to the area (i.e. endemic) in question and are not taken into account in index calculations. This can lead to miss-assessments when these specific taxa are dominant (this is the case in French Guyana, French Antilles etc. – tropical regions). Miss-assessments could occur especially with indices such as BDI, which include a limited number of taxa. To rule out the possibility of erroneous water quality assessments, Prygiel recommends thorough testing of diatom indices before they are used for routine analysis.

The present study, carried out in the Vaal and Wilge Rivers, has completed only a part of what should be a nationwide testing of the diatom-based pollution indices. Based on the comments of Prygiel above, it has been assessed from the results of the present study that future research into the efficacy of diatom-indices as bio-monitoring tools should be undertaken in different physical and geographical regions of South Africa (e.g. the acidic streams of the Fynbos Biome in the Western Cape region). In addition, testing of the diatom indices is needed in rivers with different physical characteristics to the Vaal River. The Vaal River is generally broad and relatively slow flowing with several major and minor impoundments. It is recommended that the diatom indices tested in this study should also be tested in smaller shallower and fast flowing streams.

Besides the suitability of the diatom indices as tools of water quality monitoring in the Vaal River, the present study has demonstrated the application of the sub-cosmopolitan concept of Padisák (1998) at least for the Vaal and Wilge Rivers. This concept states that diatoms are "sub-cosmopolitan", i.e. they occur anywhere in the world where a certain set of environmental conditions exists. The term "sub-cosmopolitan" refers to two concepts, the first being that the majority of diatom species are "cosmopolitan" in their distribution, i.e. they

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occur globally. The second concept or the "sub" refers to the fact that although the majority of diatom species have the potential to have a global distribution, this distribution is limited or dictated by the specific environmental tolerances of the individual diatom species. The distribution of sub-cosmopolitan diatoms is directly related to the prevailing water quality conditions at any given site. Thus, the diatom community structure encountered in a particular water type with certain parameters in the Northern Hemisphere should be identical to the diatom community found in the water with the same quality parameters in the Southern Hemisphere.

The overall diatom species composition encountered in the Vaal and Wilge Rivers is 98% cosmopolitan (i.e. diatom species occur in both South Africa and Europe). The cosmopolitan occurrence of many diatom species is well known and has been demonstrated between many countries and continents e.g. Lange–Bertalot *in* Krammer (2000). Whether or not these cosmopolitan diatom species react in a similar way to water quality variables (i.e. can be classified as "sub-cosmopolitan" species) has been the subject of some debate (see discussion in Chapter 1).

The sub-cosmopolitan concept has been demonstrated in the present study. The present study shows consistent results generated from diatom indices, developed and tested in Europe, and the results yielded by the same diatom indices in South Africa (i.e. similar levels of correlation between specific water quality variables and diatom index scores; Table 4.7). The fact that index scores and the reactions of diatom communities to individual water quality variables are consistent between the hemispheres, leads to the conclusion that the majority of the diatom species encountered in the present study are not only cosmopolitan in their distribution, but also in their specific ecological tolerances, and hence the distribution of diatom species in the Vaal Catchment is indeed "sub-cosmopolitan".

Bate *et al.* (in press) did not consider the Biological Diatom Index (BDI) as suitable for use as a biomonitoring tool in the rivers and estuaries of South Africa. However, the authors do not present any experimental evidence for this opinion. The reasons cited were that as yet there is insufficient data in South Africa to determine which taxa are rare or not (BDI excludes rare diatom taxa from the index calculation). In addition, Bate and co-workers are also of the opinion that before an index approach can be attempted, there is a need to gather information on South African dominant species, their locations and the specific water quality tolerances of the species encountered.

In the author's opinion the application of previously developed and tested diatom indices negates the need for extensive research into the individual tolerances of diatom species occurring in South Africa. This information is already integrated into the various European diatom indices. If the sub-cosmopolitan concept holds true the distribution of diatoms within South Africa will change with changing water quality. For a sub-cosmopolitan group of species distribution data, though of scientific interest, is irrelevant to the testing of diatom indices. The main focus of future work in South Africa using diatoms as bio-indicators should be directed towards the further testing of existing index systems rather than initiating the process of index development in South Africa from scratch. Based on the results of further tests of diatom indices, the necessity or lack thereof to modify indices for general use in South Africa, or for monitoring specific effluents, may become clear.

After the European diatom indices have been more fully tested in South Africa, they may be simplified in terms of using only dominant taxa as recommended by some authors (e.g. Wu, 1999). Diatom based indices were, however, designed to play a slightly different role to that of the simpler, more rapid methods (like SASS). Diatom indices can be used to obtain a higher resolution of applicability when specific aspects of water quality (such as trophic status) need to be determined with the aid of biological monitoring agents. Diatoms as micro-algae are less dependant on the physical integrity of their habitat for survival than organisms such as aquatic macroinvertebrate fauna, which react not only to water quality but also to factors such as water quantity and have specific physical habitat requirements.

Attempts which have been made to find direct correlation between the macroinvertebrate index SASS4, and water quality variables have, so far, been unsuccessful (e.g. Vos *et al.*, 2002). Chutter (1998) states that SASS is less sensitive to increases in total dissolved solids than to other types of chemical change. There is, therefore, still a need for a biological indicator in addition to SASS (such as the diatom indices used in this study) that can be indicative of specific water quality variables.

The fact that diatom sampling methods have fewer restrictions in terms of habitat requirements than methods based upon the sampling aquatic macroinvertebrate fauna could facilitate the use of diatom indices in monitoring water quality in small tributaries, streams and industrial effluent. This conclusion is strengthened by Round's (2001) statement that "...river diatoms can colonize massive rivers but also "rivers" millimeters deep and centimeters wide..."

Finally, mention should be made of future research in which diatom community analysis may be used to indicate levels of aquatic pollution. Some diatom species can tolerate extremes of pH and salinity and may be found in effluent streams long after the macroinvertebrate fauna has disappeared (e.g. after a mine tailings spill; Sabater, 2000). Future studies should examine whether diatom community composition could give an accurate reflection of aquatic pollution by various water quality variables in mining and industrial effluent.

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APPENDIX 1 – SPECIES LIST AND UPDATED TAXONOMY

Diatom names in current use are followed by synonyms (indented), acronyms are from OMNIDIA v.3

Taxon	Acronym
**Achnanthidium exiguum* (Grunow) Czarnnecki	AEHE
Achnanthes exigua Grunow *in* Cleve & Grunow	AEXG
**Achnanthidium minutissimum* (Kützing) Czarnecki	ADMI
Achnanthes minutissima Kützing	AMIN
**Achnanthidium saprophila* (Kobayasi & Mayama) Round & Bukhtiyarova	ADSA
Achnanthes minutissima var.*saprophila* Kobayasi & Mayama	AMSA
Amphora castellata Giffen	AMCA
Amphora libyca Ehrenberg	ACOP
Amphora copulata (Kützing) Schoeman & Archibald	ALIB
**Amphora montana* Krasske	AMMO
Amphora submontana Hustedt	ASMO
Amphora ovalis (Kützing) Kützing	AOVA
**Amphora pediculus* (Kützing) Grunow	APED
Amphora subacutiuscula Schoeman	ASAC
Amphora veneta Kützing	AVEN
Asterionella formosa Hassall	AFOR
Aulacoseira distans (Ehrenberg) Simonsen	AUDI
Melosira distans (Eherenberg) Kützing	MDIS
**Aulacoseira granulata* (Ehrenberg) Ralfs	AUGR
Melosira granulata (Ehrenberg) Ralfs	MGRA
**Aulacoseira granulata* var. *angustissima* (O.Müller) Simonsen	AUGA
Melosira granulata var. *angustissima* O.Müller	MGAN
Aulacoseira muzzanensis (Meister) Krammer	AMUZ
Melosira granulata var. *muzzanensis* Bethge	MGMU
Caloneis bacillum (Grunow) Cleve	CBAC
Caloneis branderi (Hustedt) Krammer	CBRD
Stauroneis branderii Hustedt	SBRA
Caloneis molaris (Grunow) Krammer	CMOL
Caloneis schumanniana (Grunow) Cleve	CSHU
Caloneis silicula (Ehrenberg) Cleve	CSIL
**Cocconeis pediculus* Ehrenberg	CPED
**Cocconeis placentula* Ehrenberg	CPLA
Cocconeis placentula var. *euglypta* (Ehrenberg) Grunow	CPLE
Craticula accomoda (Hustedt) D.G. Mann in Round, Crawford & Mann	CRAC
Navicula accomoda Hustedt	NACO
Craticula halophila (Gunow *ex* Van Heurk) D.G. Mann in Round, Crawford & Mann	CHAL
Navicula halophila (Grunow) Cleve	NHAL
Navicula pseudohalophila Cholnoky	

**Craticula molestiformis* (Hustedt) Lange-Bertalot	
Navicula molestiformis Hustedt	NMLF
Navicula twymaniana Archibald	NTWY
Navicula haricola Cholnoky	
Craticula submolesta (Hustedt) Lange-Bertalot	CSBM
Navicula submolesta Hustedt	NSMO
Cyclostephanos damasi (Hustedt) Stoermer & Håkansson	CDAM
Stephanodiscus damasi Hustedt	SDAM
**Cyclostephanos dubius* (Fricke) Round	CDUB
**Cyclostephanos invisitatus* (M.H.Hohn & Hellerman) Theriot, Stoermer & Håkansson	CINV
Cyclotella atomus Hustedt	CATO
Cyclotella atomus var. *gracilis* Genkal & Kiss	CAGR
**Cyclotella meduanae* Germain	CMED
**Cyclotella meneghiniana* Kützing	CMEN
Cyclotella pseudostelligera Hustedt	CPST
Cymatopleura solea (Brébisson) W.Smith	CSOL
Cymatopleura librile (Ehenberg) Pantocsek	CLIB
**Cymbella affinis* Kützing	CAFF
Cymbella kolbei Hustedt	CKOL
**Cymbella tumida* (Brébisson) Van Heurck	CTUM
**Cymbella turgidula* Grunow	CTGL
Diadesmis contenta (Grunow *ex* Van Heurck) D.G. Mann *in* Round, Crawford & Mann	DCON
Navicula contenta Grunow	NCON
Diadesmis gallica var. *perpusilla* (Grunow) Lange-Bertalot	DGPE
Navicula gallica var. *perpusilla* (Grunow) Lange-Bertalot	NPEP
**Diatoma vulgare* Bory	DVUL
Diploneis elliptica (Kützing) Cleve	DELL
Diploneis oblongella (Naegeli) Cleve-Euler	DOBL
Encyonema caespitosum Kützing	ECAE
Cymbella caespitosa (Kützing) Brun	CCAE
Encyonema gracile Rabenhorst	ENNG
Cymbella gracilis (Ehrenberg) Kützing	CGRA
**Encyonema minutum* (Hilse *in* Rabenhorst) D.G.Mann *in* Round, Crawford & Mann	ENMI
Cymbella minuta Hilse *ex* Rabenhorst	CMIN
Cymbella ventricosa Kützing	CVEN
Encyonema perpusillium (Cleve-Euler) D.G.Mann *in* Round, Crawford & Mann	ENPE
Cymbella perpusilla Cleve-Euler	CPER
Encyonema silesiacum (Bleisch *in* Rabenhorst) D.G. Mann *in* Round, Crawford & Mann	ESLE
Cymbella silesiaca Bleisch *in* Rabenhorst	CSLE

**Encyonopsis microcephala* (Grunow) Krammer	ENCM
Cymbella microcephala Grunow	CMIC
**Eolimna minima* (Grunow) Lange-Bertalot	EOMI
Navicula minima Grunow	NMIN
**Eolimna subminuscula* (Manguin) Lange-Bertalot & Metzeltin	ESBM
Navicula subminuscula Manguin	NSBM
Navicula frugalis Hustedt	NFRU
**Epithemia adnata* (Kützing) Brébisson	EADN
**Epithemia sorex* Kützing	ESOR
Fallacia indifferens (Hustedt) D.G.Mann *in* Round, Crawford & Mann	FIND
Navicula indifferens Hustedt	NIDF
Fallacia insociabilis (Krasske) D.G. Mann *in* Round, Crawford & Mann	FINS
Navicula insociabilis Krasske	NINS
Fallacia monoculata (Hustedt) D.G.Mann *in* Round, Crawford & Mann	MMOC
Navicula monoculata Hustedt	NMOC
Fallacia tenera (Hustedt) D.G.Mann *in* Round, Crawford & Mann	FTNR
Navicula tenera Hustedt	NTNR
Fallacia tenera (Hustedt) D.G.Mann *in* Round, Crawford & Mann	FTNR
Navicula tenera Hustedt	NTNR
Fragilaria capucina Desmazières	FCAP
Synedra rumpens Kützing	SRUM
Fragilaria capucina var.*gracilis* (Østrup) Hustedt	FCGR
**Fragilaria capucina* var. *vaucheriae* (Kützing) Lange-Bertalot	FCVA
Fragilaria crotonensis Kitton	FCRO
Fragilaria miniscula (Grunow *in* Van Heurk) Williams & Round	FMIN
Synedra miniscula Grunow	SMIN
Fragilaria nanana Lange-Bertalot	FNAN
Synedra nana Meister	SYNA
**Fragilaria spec.* entspr. *Synedra acus* var. *angustissima* order var. *radians* sensu auct. nonnull. (See Krammer & Lange-Bertalot, 1991 ; Plate 114: Fig. 21*)	FUAN
Fragilaria tenera (W.Smith) Lange-Bertalot	FTEN
Synedra tenera W.Smith	SYNT
Frustulia vulgaris (Thwaites) De Toni	FVUL
Frustulifera pelliculosa (Brébisson) Lange-Bertalot	FPEL
Navicula pelliculosa (Brébisson *ex* Kützing) Hilse	NPEL
**Frustulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot	FSAP
Navicula saprophila Lange-Bertalot & Bonik	NSAP

* KRAMMER K and LANGE-BERTALOT H (1991) *Bacillariophyceae. Süßwasserflora von Mitteleuropa* 2 (3). Spektrum Akademischer Verlag, Heidelberg, Berlin.

Geissleria acceptata (Hustedt) Lange-Bertalot & Metzeltin	GACC
Navicula ignota var.*acceptata* (Hustedt) Lange-Bertalot	NIAC
*Geissleria decussis (*Østrup) Lange-Bertalot & Metzeltin	GDEC
Navicula decussis Østrup	NDEC
Gomphonema affine Kützing	GAFF
Gomphonema angustum Agardh	GANT
Gomphonema exilissimum (Grunow) Lange-Bertalot & Reichart	GEXL
Gomphonema parvulum var.*exilissimum* Grunow	GPXS
Gomphonema gracile Ehrenberg	GGRA
**Gomphonema minutum* (Agardh) Agardh	GMIN
**Gomphonema parvulum* Kützing	GPAR
**Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot	GPUM
Gomphonema intricatum var. *pumila* Grunow *in* Van Heurck	GIPU
Gomphonema truncatum Ehrenberg	GTRU
Gomphonema constrictum Ehrenberg	GCON
Gyrosigma acuminatum (Kützing) Rabenhorst	GYAC
Gyrosigma attenuatum (Kützing) Cleve	GYAT
Gyrosigma scalproides (Rabenhorst) Cleve	GSCA
Gyrosigma spencerii (Quekett) Griffith	GSPE
Hantzschia amphioxys (Ehrenberg) Grunow *in* Cleve & Grunow	HAMP
Hantzschia amphilepta (Grunow) Lange-Bertalot	HAPH
Hantzschia distinctepunctata Hustedt *in* Schmidt	HDIS
Hippodonta capitata (Ehrenberg) Lange-Bertalot & Metzeltin *in* Witkowski	HCAP
Navicula capitata Ehrenberg	NCAP
Hippodonta hungarica (Grunow) Lange-Bertalot & Metzeltin *in* Witkowski	HHUN
Navicula hungarica Grunow	NHUN
Lemnicola hungarica (Grunow) Round & Basson	LHUN
Achnanthes hungarica Grunow *in* Cleve & Grunow	AHUN
Luticola goeppertiana (Bleisch *in* Rabenhorst) D.G. Mann *in* Round, Crawford & Mann	LGOE
Navicula goeppertiana (Bleisch) H.L.Smith	NGOE
Luticola mutica (Kützing) D.G. Mann *in* Round, Crawford & Mann	LMUT
Navicula mutica Kützing	NMUT
Luticola nivalis (Ehrenberg) D.G. Mann *in* Round, Crawford & Mann	LNIV
Navicula nivalis Ehrenberg	NNIV
Luticola obligata (Hustedt) D.G.Mann *in* Round, Crawford & Mann	LOBG
Navicula obligata Hustedt	NOBG
Luticola ventricosa (Kützing) D.G.Mann *in* Round, Crawford & Mann	LVEN
Navicula mutica var.*ventricosa* Kützing	NMVE

Mayamaea agrestis (Hustedt) Lange-Bertalot	NAGR
Navicula agrestis Hustedt	
Mayamaea atomus (Kützing) Lange-Bertalot	MAAT
Navicula atomus (Kützing) Grunow	NATO
**Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot	MAPE
Navicula atomus var. *permitis* (Hustedt) Lange-Bertalot	NAPE
Mayamaea lacunolaciniata (Lange-Bertalot & Bonik) Lange-Bertalot	MLLC
Navicula lacunolaciniata Lange-Bertalot & Bonik	NLLC
**Melosira varians* Agardh	MVAR
Microcostatus kuelbsii (Lange-Bertalot) Lange-Bertalot	MKUE
Navicula kuelbsii Lange-Bertalot	NKUE
**Navicula adamantiformis* Archibald	NADF
Achnanthes adamantiformis Archibald	AADF
Navicula angusta Grunow	NAAN
**Navicula antonii* Lange-Bertalot	
Navicula menisculus var. *grunowii* Lange-Bertalot	NMEN
Navicula arvensis Hustedt	NARV
**Navicula capitatoradiata* Germain	NCPR
Navicula cari var. *linearis* (Østrup) Cleve-Euler	NCAL
Navicula concentrica Carter & Bailey-Watts	NCCT
Navicula constans Hustedt	NCST
Navicula cryptocephala Kützing	NCRY
**Navicula cryptotenella* Lange-Bertalot	NCTE
Navicula difficillima Hustedt	NDIF
**Navicula erifuga* Lange-Bertalot	NERI
Navicula festiva Krasske	NFES
Navicula vitrea (Østrup) Hustedt	NVIT
**Navicula germainii* Wallace	NGER
Navicula viridula var.*germainii* (Wallace) Lange-Bertalot	NVGE
**Navicula gregaria* Donkin	NGRE
Navicula integra (W.Smith) Ralfs	NITG
Navicula kotschyi Grunow	NKOT
Navicula leptostriata Jørgensen	
Navicula libonensis Schoeman	NLIB
Navicula microcari Lange-Bertalot	NMCA
Navicula microrhombus (Cholnoky) Schoeman & Archibald	NMCB
Fragilaria microrhombus Cholnoky	
Achananthes cogitata Archibald	
Navicula perminuta Grunow *in* Van Heurck	NPNU

Navicula phyllepta Kützing	NPHY
Navicula pseudolanceolata Lange-Bertalot	NPSL
Navicula radiosafallax Lange-Bertalot	NRFA
**Navicula recens* (Lange-Bertalot) Lange-Bertalot	NRCS
Navicula reichardtiana Lange-Bertalot	NRCH
Navicula rhynchocephala Kützing	NRHY
Navicula rostellata Kützing	NROS
Navicula viridula var.*rostellata* (Kützing) Cleve	NVRO
**Navicula schroeteri* Meister	NSHR
Navicula scoliopleura Schmidt	NSCO
**Navicula symmetrica* (Patrick)	NSYM
Navicula schroeteri var. *symmetrica* (Patrick) Lange-Bertalot	NSSY
Navicula tenelloides Hustedt	NTEN
**Navicula tripunctata* (O.Müller) Bory	NTPT
Navicula gracilis Eherenberg	NGRA
Navicula trivialis Lange-Bertalot	NTRV
Navicula vandamii Schoeman & Archibald	NVDA
Navicula veneta Kützing	NVEN
Navicula viridula (Kützing) Ehrenberg	NVIR
Neidium globiceps (Cleve-Euler) Cleve	NEGL
Nitzschia acicularioides Hustedt	NZCD
**Nitzschia acicularis* (Kützing) W.M.Smith	NACI
Nitzschia acula Hantzsch	NACU
Nitzschia amphibia Grunow	NAMP
Nitzschia amphibia fo. *rostrata* Hustedt	NZAR
**Nitzschia archibaldii* Lange-Bertalot	NIAR
Nitzschia aurariae Cholnoky	NAUR
Nitzschia bergii Cleve-Euler	NBRG
Nitzschia ardua Cholnoky	
**Nitzschia capitellata* Hustedt *in* A.Schmidt	NCPL
Nitzschia subcapitellata Hustedt	NSBC
Nitzschia communis Rabenhorst	NCOM
Nitzschia commutata Grunow *in* Cleve & Grunow	NICO
Nitzschia compressa var. *vexans* (Grunow) Lange-Bertalot	NZCV
Nitzschia siliqua Archibald	NSLQ
Nitzschia desertorum Hustedt	NDES
**Nitzschia dissipata* (Kützing) Grunow	NDIS
Nitzschia dissipata var. *media* (Hantszch) Grunow	NDME
Nitzschia eglei Lange-Bertalot	NIEG

**Nitzschia filiformis* (W.M.Smith) Van Heurk	NFIL
Nitzschia filiformis var. *conferta* (Richter) Lange-Bertalot	NFIC
Nitzschia flexoides Geitler	NFLX
**Nitzschia fonticola* Grunow *in* Cleve & Möller	NFON
Nitzschia fonticola var. *pelagica* Hustedt	NFPE
**Nitzschia frustulum* (Kützing)Grunow	NIFR
Nitzschia frustulum var. *bulnheimiana* (Rabenhorst) Grunow	NFBU
Nitzschia ganderheimiensisk Kützing	NGAN
Nitzschia calcicola Aleem & Hustedt	NZCC
Nitzschia graciliformis Lange-Bertalot	NIGF
Nitzschia gracilis Hantzsch	NIGR
Nitzschia inconspicua Grunow	NINC
Nitzschia intermedia Hantzsch *ex* Cleve &Grunow	NINT
Nitzschia intermedia var. *distans* Cleve-Euler	NIND
Nitzschia latens Hustedt	NLTE
**Nitzschia liebetruthii* Rabenhorst	NLBT
Nitzschia frustulum (Kützing) Grunow	NIFR
**Nitzschia linearis* (Agardh) W.M.Smith	NLIN
Nitzschia linearis var. *subtilis* (Grunow) Hustedt	NLSU
Nitzschia linearis var. *tenius* (W. Smith) Grunow	NZLT
Nitzschia microcephala Grunow *in* Cleve & Möller	NMIC
**Nitzschia palea* (Kützing) W.Smith	NPAL
**Nitzschia paleacea* (Grunow) Grunow *in* Van Heurk	NPAE
Nitzschia paleaeformis Hustedt	NIPF
Nitzschia parvula W.M.Smith	NPAR
Nitzschia perspicua Cholnoky	NPRP
Nitzschia pumila Hustedt	NPML
Nitzschia pura Hustedt	NIPR
**Nitzschia pusilla* (Kützing) Grunow	NIPU
Nitzschia recta Hantzsch *ex* Rabenhorst	NREC
Nitzschia reversa W.Smith	NREV
Nitzschia rostellata Hustedt	NIRO
Nitzschia sigma (Kützing) W.M.Smith	NSIG
Nitzschia sigmoidea (Nitzsch) W.M.Smith	NSIO
Nitzschia sinuata (Thwaites) Grunow	NSIN
Nitzschia solgensis Cleve-Euler	NSOL
Nitzschia sinuata var. *delgoni* (Grunow) Lange-Bertalot	NSDE
Nitzschia subacicularis Hustedt *in* A.Schmidt	NSUA
Nitzschia subtilis Grunow *in* Cleve Grunow	NISU

**Nitzschia supralitorea* Lange-Bertalot	NZSU
Nitzschia tropica Hustedt	NTRO
Nitzschia umbonata (Ehrenberg) Lange-Bertalot	NUMB
Nitzschia thermalis (Kützing) Auerswald *in* Rabenhorst	NTHM
Pinnularia borealis Ehrenberg	PBOR
Placoneis clementis (Grunow) Cox	PCLT
Navicula clementis Grunow	NCLE
Placoneis dicephala (W.Smith) Mereschkowsky	PDIC
Navicula dicephala Ehrenberg	NDIC
Placoneis elgenensis (Gregory) Cox	PELG
Navicula elginensis (Gregory) Ralfs *in* Pritchard	NELG
Planothidium daui (Foged) Lange-Bertalot	PDAU
Achnanthes daui Foged	ADAU
Planothidium delicatulum (Kützing) Round & Bukhtiyarova	PDEL
Achnanthes delicatula (Kützing) Grunow	ADEL
Planothidium ellipticum (Cleve) Round & Bukhitiyarova	PTEL
Achnanthes lanceolata var. elliptica Cleve	ALAE
**Planothidium frequentissimum* (Lange-Bertalot) Round & Bukhtiyarova	PLFR
Achnanthes lanceolata ssp. *frequentissima* Lange-Bertalot	ALFR
Planothidium haukianum (Grunow) Round & Bukhitiyarova	PTHA
Achnanthes delicatula ssp. *haukiana* Lange-Bertalot & Ruppel	ADHA
**Planothidium lanceolatum* (Brébisson) Round & Bukhitiyarova	PTLA
Achnanthes lanceolata (Brébisson)Grunow	ALAN
Planothidium rostratum (Østrup) Lange-Bertalot	PRST
Achnanthes lanceolata ssp. *rostrata* (Oestrup) Lange-Bertalot	ALAR
Psammodictyon constricta (Gregory) D.G.Mann *in* Round, Crawford & Mann	PCON
Nitzschia constricta (Gregory) Grunow	NZCO
Pseudostauraurosira brevistriata (Grunow *in* Van Heurk) Williams & Round	PSBR
Fragilaria brevistriata Grunow *in* Van Heurk	FBRE
**Reimeria sinuata* (Gregory) Kociolek & Stoermer	RSIN
Cymbella sinuata Gregory	CSIN
**Rhoicosphenia curvata* (Kützing) Grunow	RCUR
Rhoicosphenia abbreviata (C.Agardh) Lannge-Bertalot	RABB
Rhopalodia brebissonii Krammer	RBRE
Rhopalodia gibba (Ehrenberg) O.Müller	RGIB
Rhopalodia gibba var. *minuta* Krammer	RGMI
Rhopalodia gibberula (Ehrenberg) O.Müller	RGBL
Sellaphora nyassensis (O.Müller) D.G.Mann *in* Round, Crawford & Mann	SNYA
Navicula nyassensis O.Müller	NNYA
Sellaphora pupula (Kützing) Mereschowsky	SPUP

Navicula pupula Kützing	NPUP
Sellaphora pupula fo. *rostrata* (Hustedt) Bukhtiyarova	
Navicula pupula fo.*rostrata* Hustedt	NPUR
Sellaphora seminulum (Grunow) D.G.Mann	SSEM
Navicula seminulum Grunow	NSEM
Simonsenia delognei Lange-Bertalot	SIDE
Nitzschia delognei Grunow	NDLO
Nitzschia chasei Cholnoky	NCHS
Stauroneis brasiliensis (Zimmerman) Compere	STBR
Stauroneis crucicula Grunow	SZBR
Stauroneis obtusa Lagerstedt	SOBT
Staurosira construens Ehrenberg	SCON
Fragilaria construens (Ehrenberg) Grunow	FCON
Staurosira construens var. *venter* (Ehrenberg) Hamilton	SCVE
Fragilaria construens fo. *venter* (Ehrenberg) Hustedt	FCVE
Staurosirella pinnata (Ehrenberg) Williams & Round	SPIN
Fragilaria pinnata Ehrenberg	FPIN
**Stephanodiscus agassizensis* Hakansson & Kling	SAGA
**Stephanodiscus hantzschii* Grunow *in* Cleve & Grunow	SHAN
Stephanodiscus medius Håkansson	SMED
Stephanodiscus minutulus (Kützing) Cleve & Möller	STMI
Stephanodiscus rotula var. *minutula* (Kützing) Ross & Sims	SRMI
Stephanodiscus astrea var. *minutula* (Kützing) Grunow	
**Stephanodiscus parvus* Stoermer & Håkansson	SPAV
Surirella angusta Kützing	SANG
Surirella brebissonii Krammer & Lange-Bertalot	SBRE
Surirella brightwellii W.Smith	SBRI
Surirella splendida (Ehrenberg) Kützing	SSPL
Synedra acus Kützing	SACU
Fragilaria ulna var. *acus* (Kützing) Lange-Bertalot	FUAC
Synedra ulna (Nitzsch) Ehrenberg	SULN
Fragilaria ulna (Nitzsch) Lange-Bertalot	FULN
**Tabularia fasiculata* (C.Agardh) D.M. Williams & Round	TFAS
Fragilaria fasciculata (C. Agardh) Lange-Bertalot	FFAS
Thalassiosira duostra Pienaar	TDUO
**Thalassiosira pseudonana* Hasle & Heimdal	TPSN
Thalassiosira weissflogii (Grunow) Fryxell & Hasle	TWEI
Tryblionella angustata W.Smith	TANG
Tryblionella calida (Grunow) D.G.Mann *in* Round Crawford & Mann	TCAL
Nitzschia calida Grunow	NICA

Tryblionella coarctata (Grunow) D.G. Mann *in* Round, Crawford & Mann	TCOA
Nitzschia coarctata Grunow	NCOA
Tryblionella hungarica (Grunow) D.G.Mann	THUN
Nitzschia hungarica Grunow	NIHU
Tryblionella levidensis W.M.Smith	TLEV
Nitzschia levidensis (W.Smith) Grunow *in* Van Heurk	NLEV

APPENDIX 2 - PHOTOGRAPHIC PLATES ILLUSTRATING DOMINANT DIATOM SPECIES

Plate 1: (X 1250)

Fig. 1-2: *Melosira varians* C.Agardh

Fig. 3-4: *Aulacoseira granulata* (Ehrenberg) Ralfs

Fig. 5: *Aulacoseira granulata* var. *angustissima* (O.Müller) Simonsen

Fig. 6-9: *Cyclostephanos invisitatus* (M.H.Hohn & Hellerman) Theriot, Stoermer & Håkansson

Fig. 10-13: *Cyclostephanos dubius* (Fricke) Round *in* Theriot, Håkansson, Kociolek, Round & Stoermer

Fig. 14-15: *Cyclotella meneghiniana* Kützing

Fig. 16-18: *Cyclotella meduanae* Germain

Fig. 19-21: *Stephanodiscus agassizensis* Håkansson & Kling

Fig. 22-24: *Stephanodiscus parvus* Stoermer & Håkansson

Fig. 25-27: *Stephanodiscus hantzschii* Grunow

Fig. 28-29: *Thalassiosira pseudonana* Hasle & Heimdal

Fig. 30-31: *Diatoma vulgare* Bory

Fig. 32-38: *Fragilaria capucina* var. *vacheriae* (Kützing) Lange-Bertalot

Fig. 36-38: *Tabularia fasiculata* (C.Agardh) D.M. Williams & Round

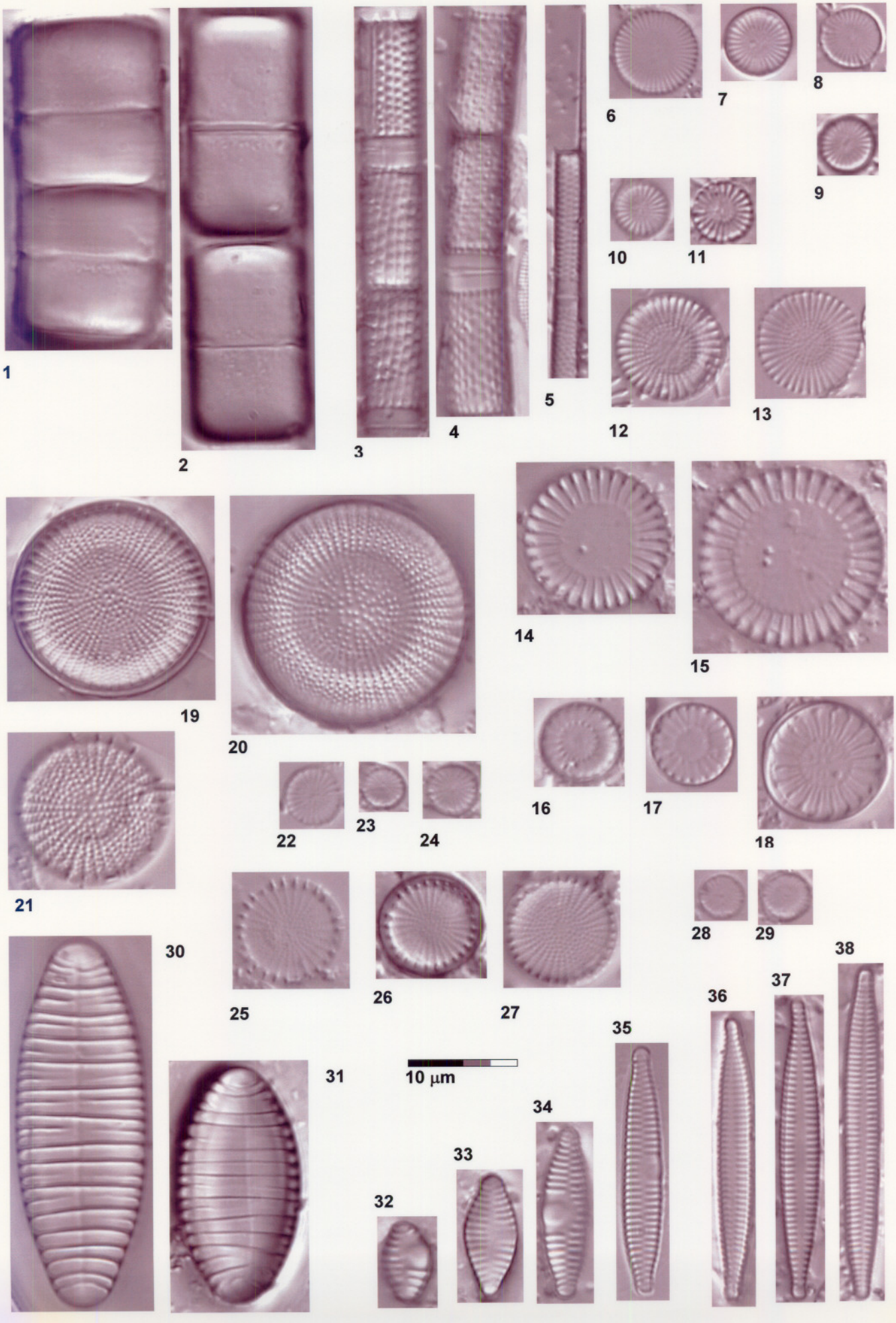
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Plate 2: (Fig. 1 X 500, Fig. 2 X 1250)

Fig. 1-2: *Fragilaria spec.* entspr. *Synedra acus* var. *angustissima* order var. *radians* sensu auct. nonnull. (See Krammer & Lange-Bertalot, 1991 ; Plate 114: Fig. 21*).

* KRAMMER K and LANGE-BERTALOT H (1991) *Bacillariophyceae. Süßwasserflora von Mitteleuropa 2* (3). Spektrum Akademischer Verlag, Heidelberg, Berlin.

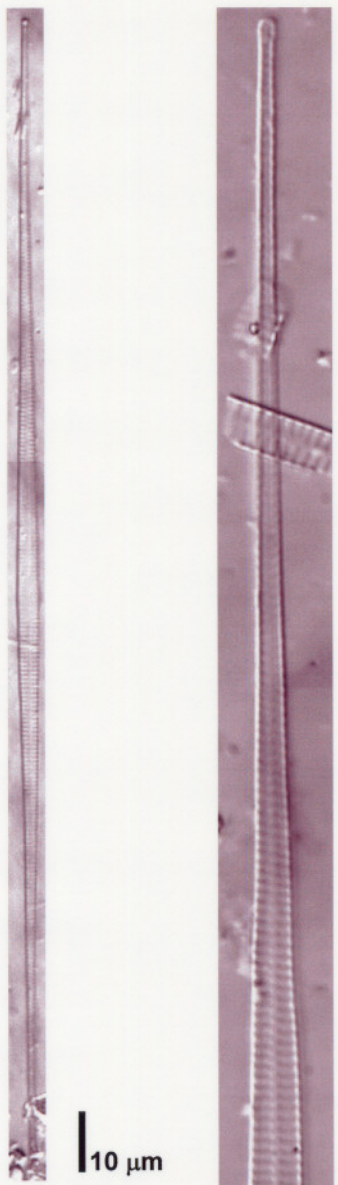
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Plate 3: (X 1250)

Fig. 1-6: *Achnanthidium minutissimum* (Kützing) Czarnecki

Fig. 7-13: *Achnanthidium saprophila* (Kobayasi & Mayama) Round & Bukhtiyarova

Fig. 14-18: *Achnanthidium exiguum* (Grunow) Czarnnecki

Fig. 19-21: *Panothidium lanceolatum* (Brébisson) Round & Bukhtiyarova

Fig. 22-26: *Panothidium frequentissimum* (Lange-Bertalot) Round & Bukhtiyarova

Fig. 27-30: *Cocconeis placentula* Ehrenberg

Fig. 31-33: *Cocconeis pediculus* Ehrenberg

Fig. 34-36: *Craticula molestiformis* (Hustedt) Mayama

Fig. 37-40: *Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot

Fig. 41-43: *Eolimna subminuscula* (Manguin) Lange-Bertalot & Metzeltin

Fig. 44-45: *Eolimna* sp.

Fig. 46-51: *Eolimna minima* (Grunow) Lange-Bertalot

Fig. 52-53: *Frustulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot

Fig. 54-55: *Navicula adamantiformis* Archibald

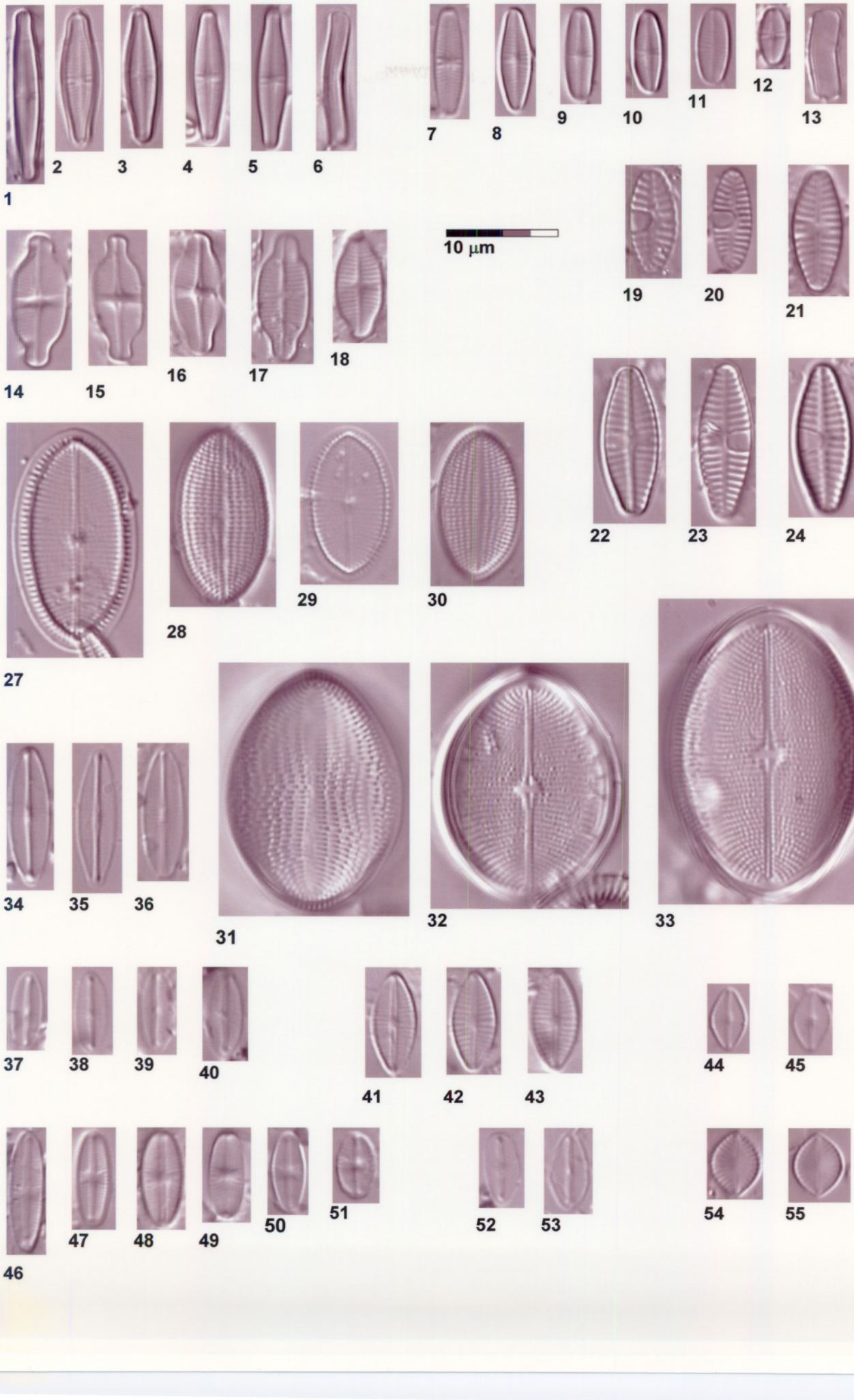
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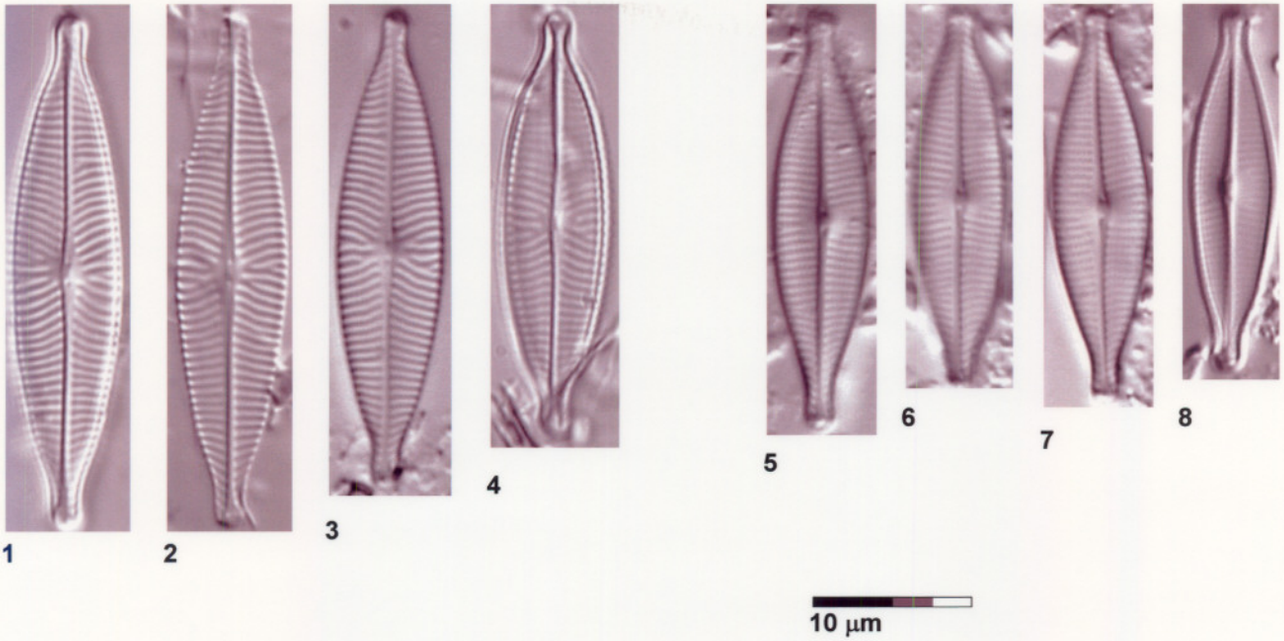
Plate 4: (X 1250)

Fig. 1-4: *Navicula capitatoradiata* Germain

Fig. 5-8: *Navicula gregaria* Donkin

Fig. 9-11: *Navicula germainii* Wallace

Fig. 12-15: *Navicula rostellata* Kützing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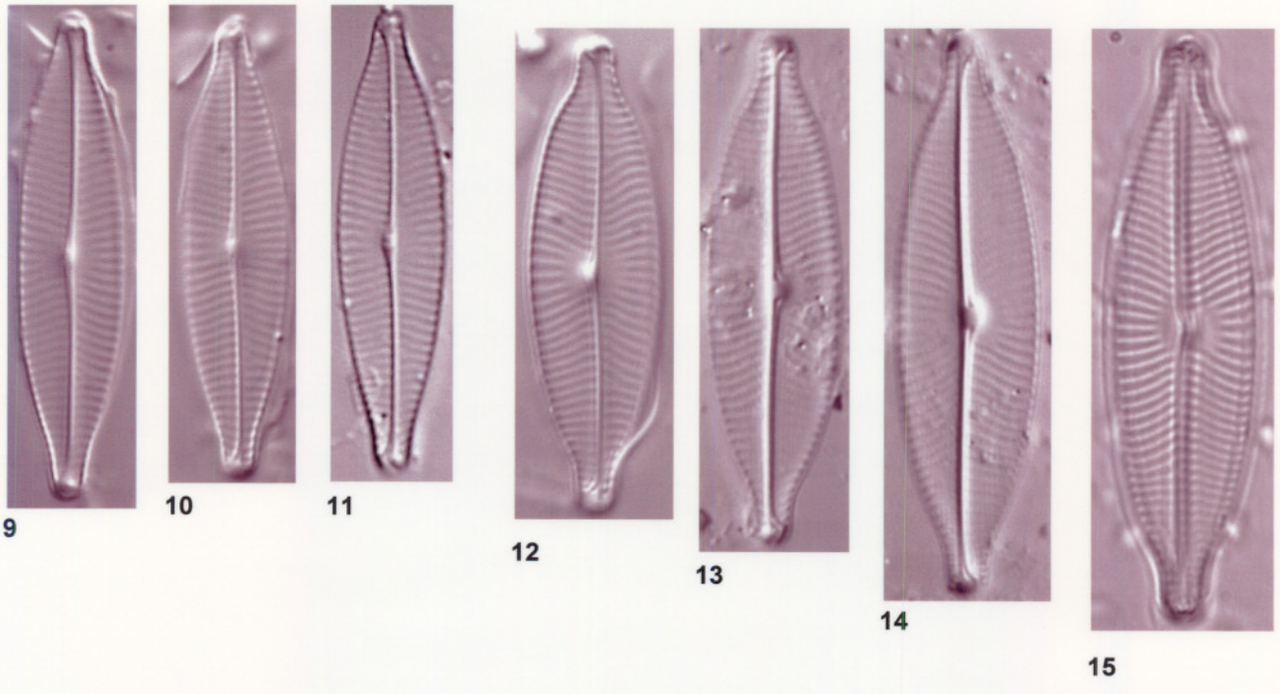
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Plate 5: (X 1250)

Fig. 1-3: *Navicula cryptotenella* Lange-Bertalot

Fig. 4-5: *Navicula tripunctata* (O.F.Müller) Bory

Fig. 6-8: *Navicula erifuga* Lange-Bertalot

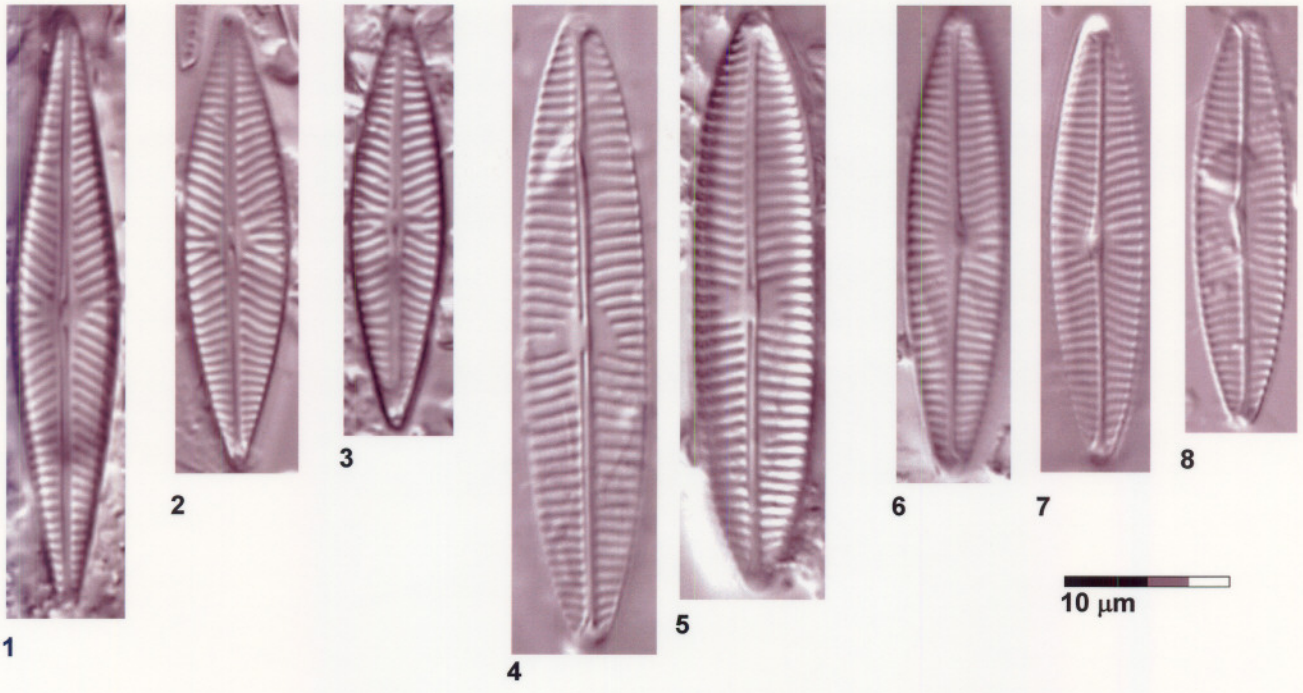
Fig. 9-11: *Navicula antonii* Lange-Bertalot

Fig. 12-16: *Navicula recens* (Lange-Bertalot) Lange-Bertalot

Fig. 17-18: *Navicula schroeteri* Meister

Fig. 19-21: *Navicula symmetrica* Patrick

Fig. 22-24: *Navicula veneta* Kützing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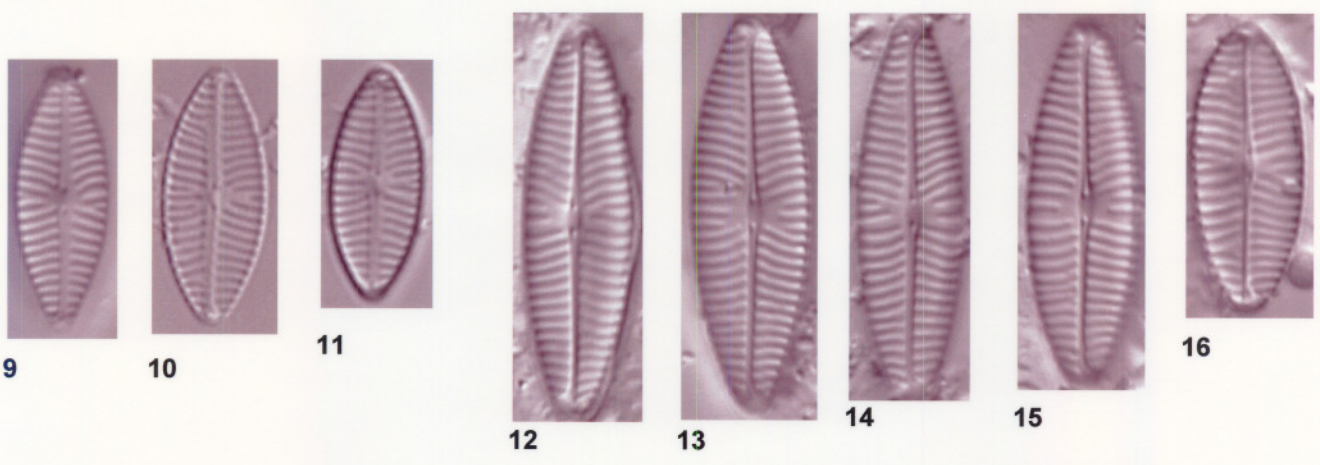
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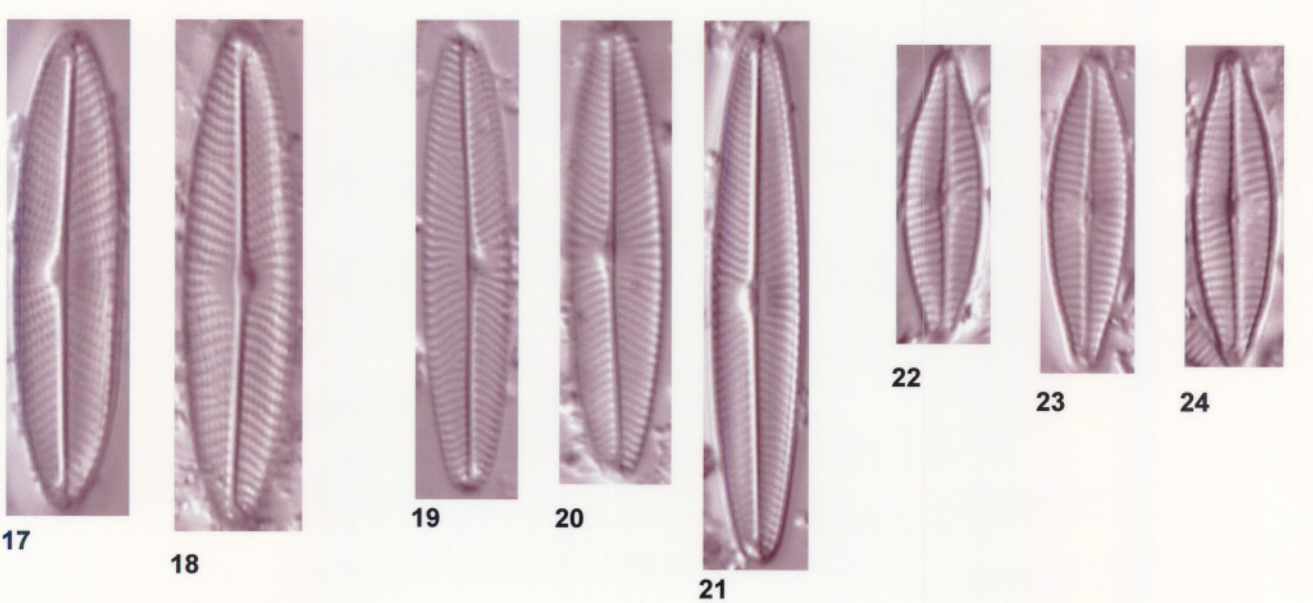
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Plate 6: (X 1250)

Fig. 1-4: *Amphora pediculus* (Kützing) Grunow

Fig. 5-8: *Amphora montana* Krasske

Fig. 9-13: *Gomphonema parvulum* (Kützing) Kützing

Fig. 14-17: *Gomphonema minutum* (Agardh) Agardh

Fig. 18-19: *Gomphonema pumilum* (Grunow) Lange-Bertalot & Reichart

Fig. 20-24: *Rhoicosphenia curvata* (Kützing) Grunow

Fig. 25-28: *Reimeria sinuata* (Gregory) Kociolek & Stoermer

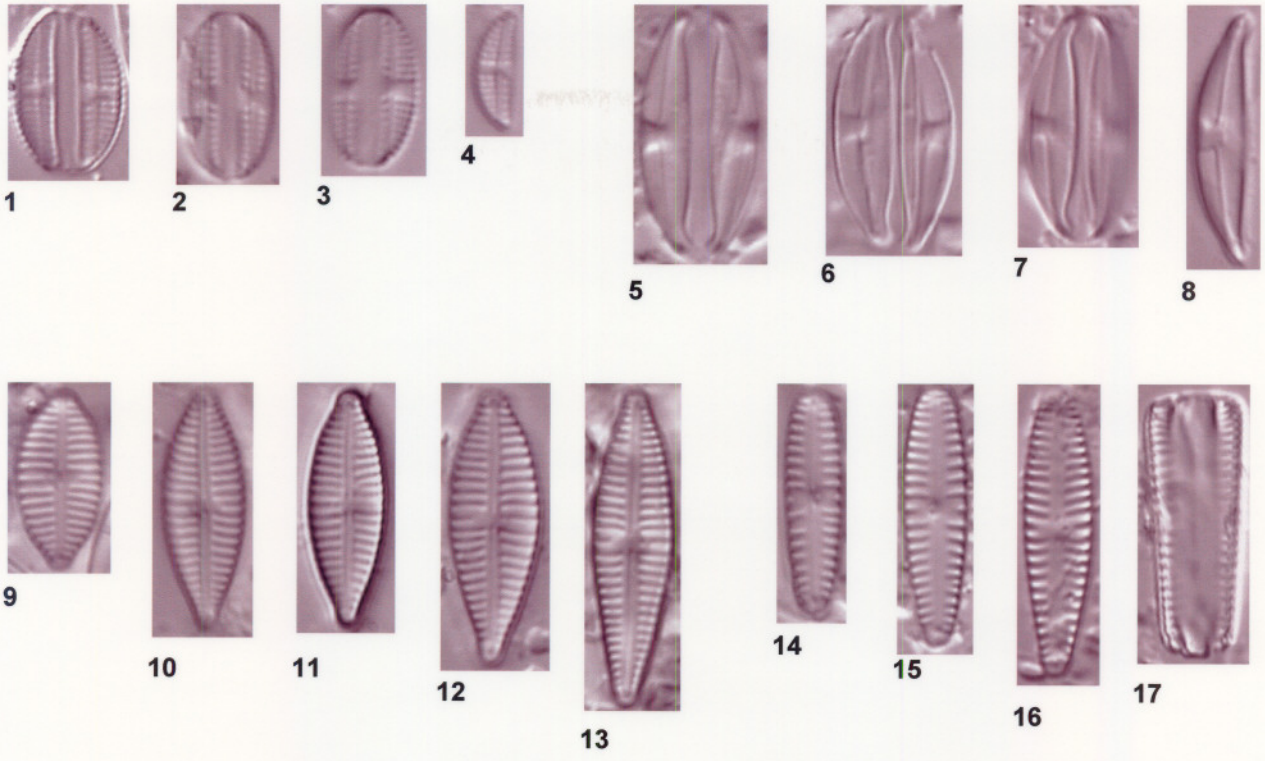
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Plate 7: (X 1250)

Fig. 1-6: *Cymbella affinis* Kützing *sensu* Krammer & Lange-Bertalot (1986)

Fig. 7-9: *Cymbella turgidula* Grunow

Fig. 10-11: *Cymbella tumida* (Bréb.) VanHeurck

Fig. 12-16: *Encyonema minutum* (Hilse *ex* Rabenhorst) D.G.Mann *in* Round, Crawford & Mann

Fig. 17-21: *Encyonopsis microcephala* (Grunow) Krammer


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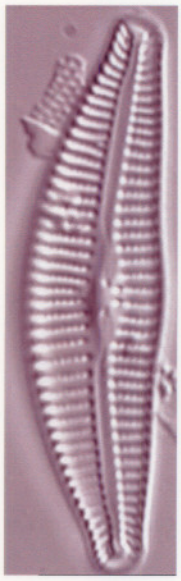
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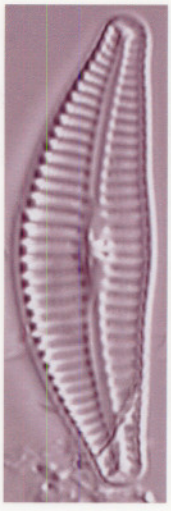
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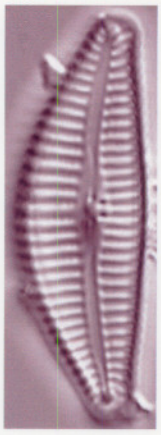
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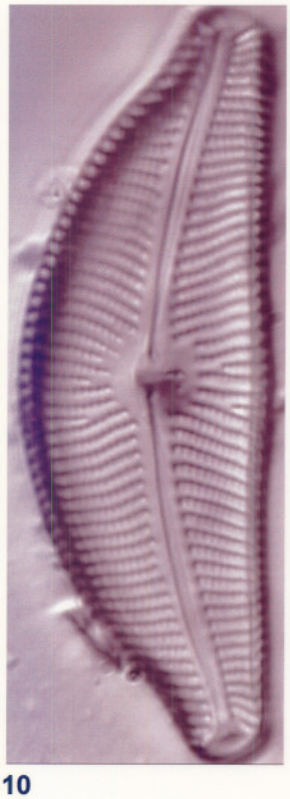
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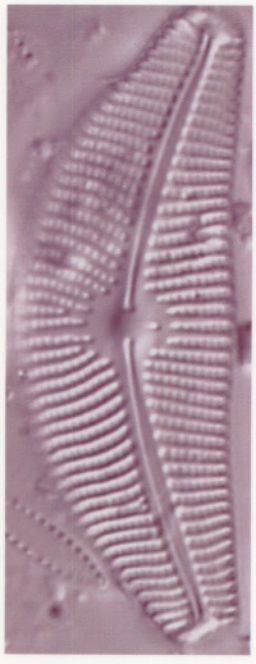
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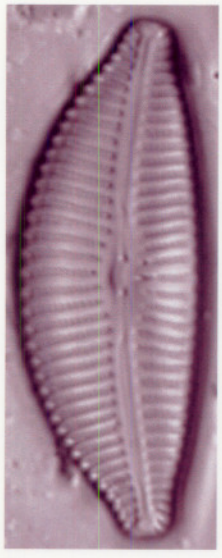
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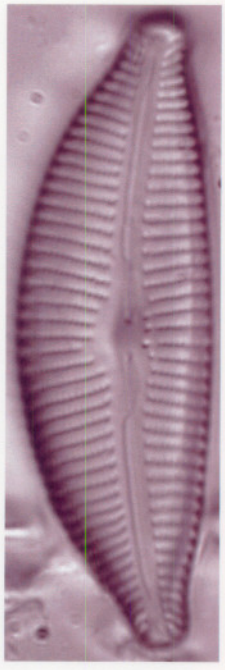
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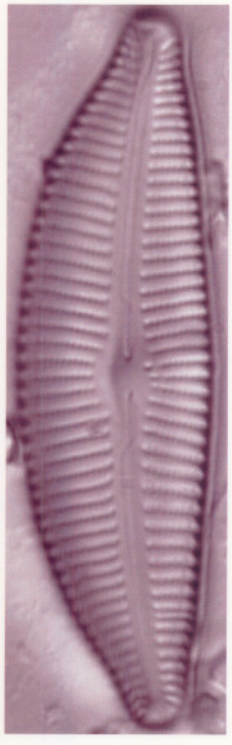
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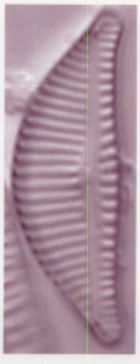
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Plate 8: (X 1250)

Fig. 1-4: *Epithemia adnata* (Kützing) Brébisson

Fig. 5-6: *Epithemia sorex* (Kützing)

Fig. 7-8: *Nitzschia linearis* (Agardh) W.Smith

Fig. 9-12: *Nitzschia acicularis* (Kützing) W.Smith

Fig. 13-15: *Nitzschia fonticola* Grunow

Fig. 16-18: *Nitzschia paleacea* Grunow

Fig. 19-23: *Nitzschia liebertruthii* Rabenhorst

Fig. 24-28: *Nitzschia frustulum* (Kützing) Grunow

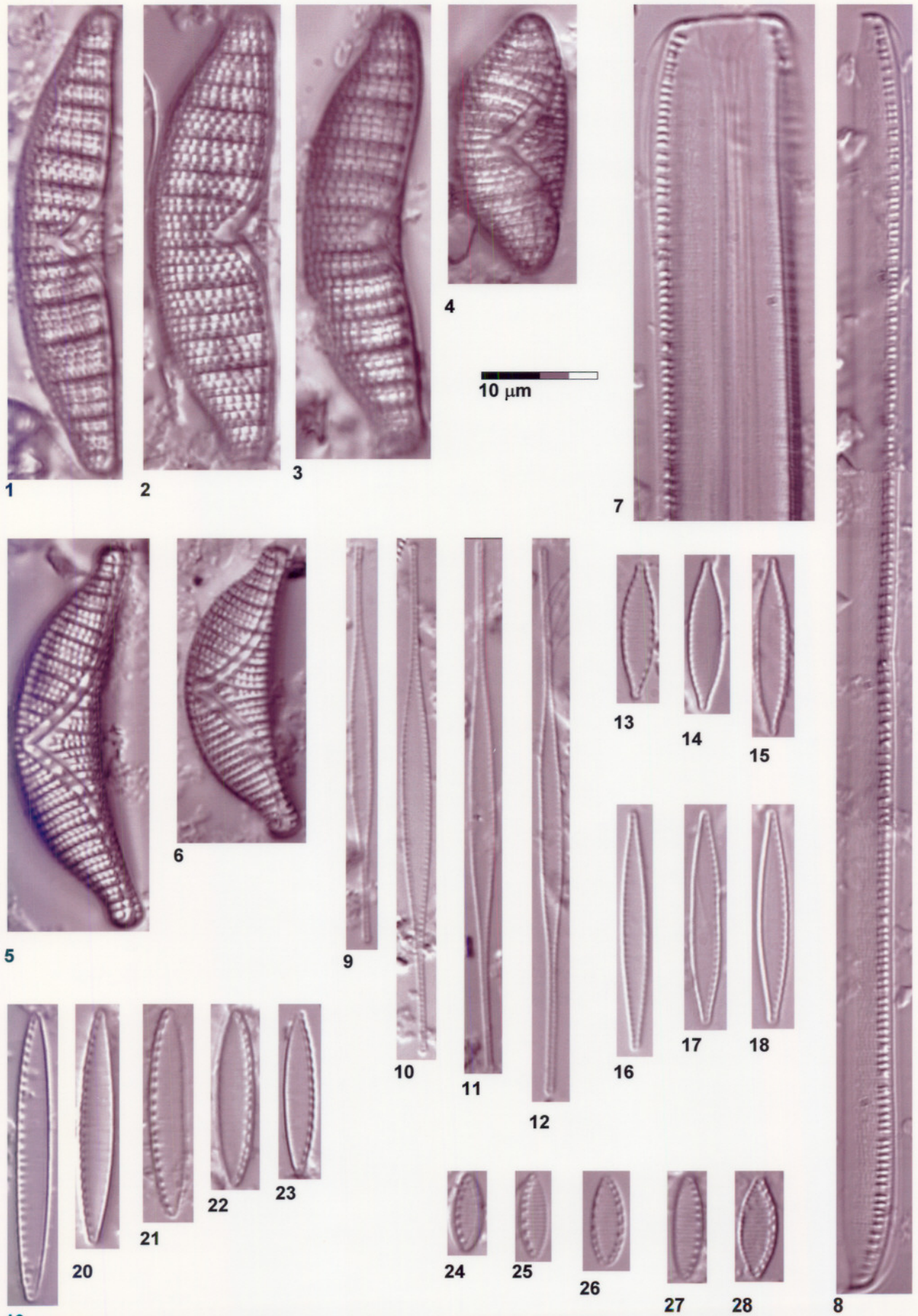
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Plate 9: (X 1250)

Fig. 1-6: *Nitzschia palea* (Kützing) W.Smith

Fig. 7-9: *Nitzschia capitellata* Hustedt

Fig. 10-12: *Nitzschia dissipata* (Kützing) Grunow

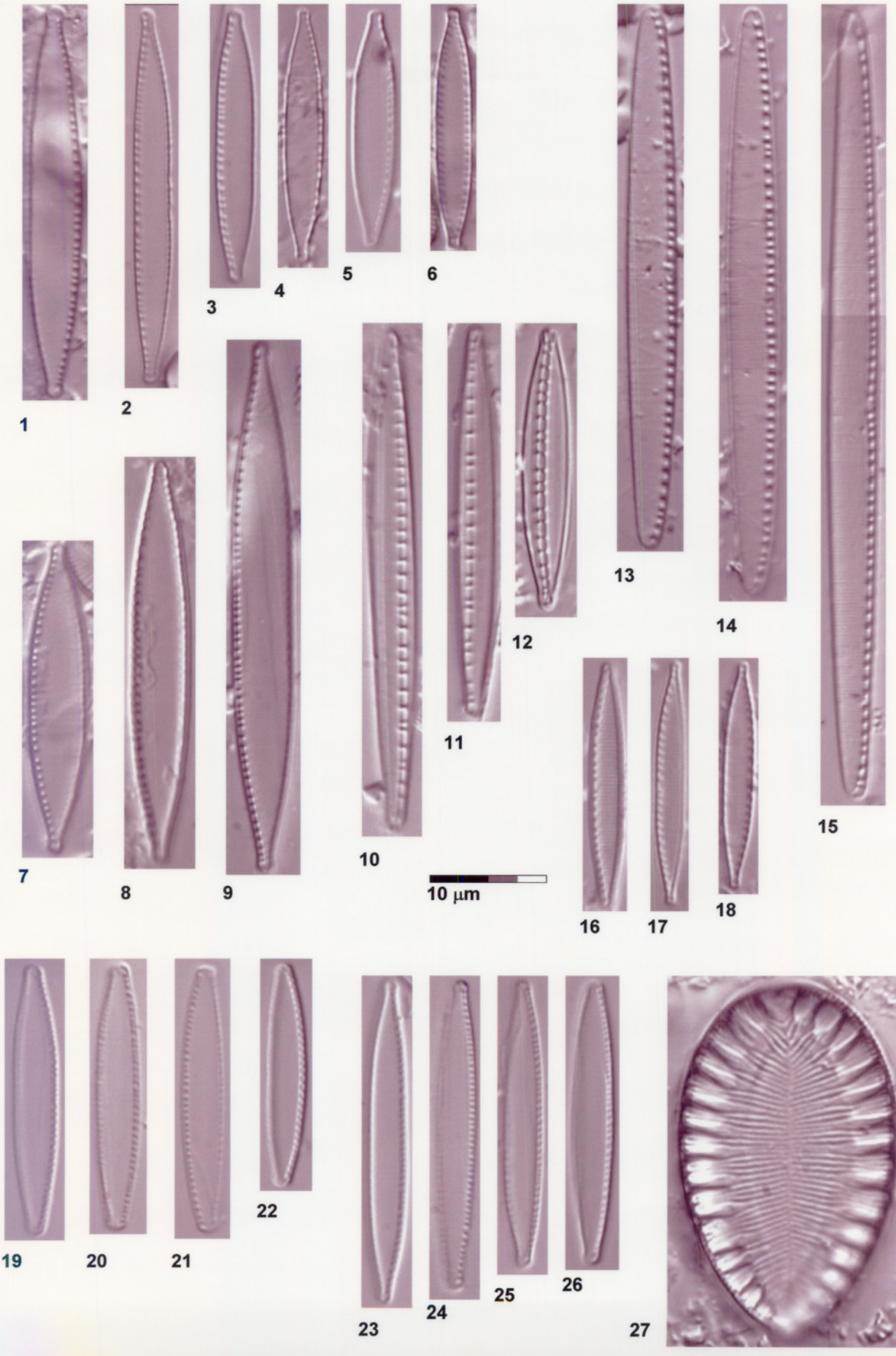
Fig. 13-15: *Nitzschia filiformis* (W.Smith) VanHeurck

Fig. 16-18: *Nitzschia supralitorea* Lange-Bertalot

Fig. 19-22: *Nitzschia pusilla* Grunow

Fig. 23-26: *Nitzschia archibaldii* Lange-Bertalot

Fig. 27: *Surirella brebissonii* Krammer & Lange-Bertalot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APPENDIX 3 - RELATIVE ABUNDANCE DATA

SITE V1 (VAAL RIVER AT BLOUKOP)
MARCH 2002 TO FEBRURAY 2003

Taxa	Month											
	M	A	M	J	J	A	S	O	N	D	J	F
Amphora ovalis (Kützing) Kützing				0.5						0.3		
Amphora pediculus (Kützing) Grunow				0.2					0.2			0.3
Amphora veneta Kützing										0.5		
Asterionella formosa Hassall					3.0			0.6				
Aulacoseira granulata (Ehrenberg) Ralfs				0.2							0.5	
Aulacoseira granulata var. *angustissima* (O.Müller) Simonsen				0.2					0.2	0.3		
Caloneis bacillum (Grunow) Cleve		1.5	0.9									
Caloneis branderi (Hustedt) Krammer				0.2								
Caloneis molaris (Grunow) Krammer												0.3
Caloneis schumanniana (Grunow) Cleve		0.3	1.2	2.2		2.0	0.5					
Cocconeis pediculus Ehrenberg				0.2		0.3				0.3		0.3
Cocconeis placentula Ehrenberg	46.5	49.4	26.5	3.0		6.0	1.6	6.5	32.7	40.1	49.1	13.4
Cocconeis placentula var.*euglypta* (Ehrenberg) Grunow												4.0
Craticula accomoda (Hustedt) D.G. Mann *in* Round, Crawford & Mann										0.8		
Craticula molestiformis (Hustedt) Lange-Bertalot	1.2		0.3				4.5	13.5	4.9			0.3
Craticula submolesta (Hustedt) Lange-Bertalot												0.3
Cyclostephanos dubius (Fricke) Round		0.3							0.2			
Cyclotella meneghiniana Kützing								0.3		0.8		
Cymbella affinis Kützing		1.2	6.0	41.1	11.9	7.2	1.9	1.1			0.5	
Cymbella tumida (Brébisson)Van Heurck			5.1	4.2	4.4	0.6			0.2			
Diatoma vulgare Bory			1.8	0.5	13.5	1.4	17.6					0.5
Diploneis elliptica (Kützing) Cleve	0.3	0.3	0.3	0.5								
Diploneis oblongella (Naegeli) Cleve-Euler		1.2	0.3									
Encyonema minutum (Hilse *in* Rabenhorst) D.G.Mann *in* Round, Crawford & Mann											0.5	
Encyonema perpusillium (Cleve-Euler) D.G.Mann *in* Round, Crawford & Mann												0.8
Encyonema silesiacum (Bleisch *in* Rabenhorst) D.G. Mann *in* Round, Crawford & Mann							0.5					
Encyonopsis microcephala (Grunow) Krammer											0.3	0.3
Eolimna subminuscula (Manguin) Lange-Bertalot & Metzeltin	6.3	0.3				0.3	8.8	26.2	30.6	1.6		0.5
Fragilaria capucina var.*vaucheriae* (Kützing) Lange-Bertalot					55.2			1.1				0.3
Frustulifera pelliculosa (Brébisson) Lange-Bertalot								2.8	0.2			
Frustulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	1.2								0.7			
Gomphonema gracile Ehrenberg										0.3		
Gomphonema minutum (Agardh) Agardh	3.0	1.5	23.2	5.7		31.3	0.5	11.3	12.1	3.0	1.4	1.6
Gomphonema parvulum Kützing	6.3	1.2	4.5	1.5	0.6	1.1	8.8	11.5	1.9		0.5	0.8
Gomphonema truncatum Ehrenberg								0.6				
Gyrosigma attenuatum (Kutz.)Cleve												0.3
Gyrosigma scalproides (Rabenhorst) Cleve												0.3
Hippodonta capitata (Ehrenberg) Lange-Bertalot & Metzeltin *in* Witkowski										0.3		
Luticola mutica (Kützing) D.G. Mann *in* Round, Crawford & Mann												0.3
Luticola nivalis (Ehrenberg) D.G. Mann *in* Round, Crawford & Mann												0.3
Mayamaea agrestis (Hustedt) Lange-Bertalot												0.3
Mayamaea atomus (Kützing) Lange-Bertalot								1.1			0.3	
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	3.9		0.3		0.6							
Melosira varians Agardh		0.3	0.9	0.5	3.6							
Navicua germainii Wallace	2.1						4.3	2.3	0.2	1.1		8.6
Navicula antonii Lange-Bertalot		0.3				0.3	2.4	2.3	0.5	2.5		
Navicula arvensis Hustedt											0.3	0.3
Navicula capitatoradiata Germain		0.3		1.0	1.1			0.6	0.2			
Navicula constans Hustedt										0.3		
Navicula cryptotenella Lange-Bertalot	0.9	2.6	3.0	4.5		0.9	1.6	1.7	0.7	0.8		0.3
Navicula erifuga Lange-Bertalot	2.4	1.2		0.2								4.8
Navicula gregaria Donkin									0.2	0.8		
Navicula microcari Lange-Bertalot											0.5	0.5
Navicula recens (Lange-Bertalot) Lange-Bertalot											0.3	
Navicula rostellata Kützing	0.3	0.9	0.6	1.0								4.3
Navicula schroeteri Meister							0.3	0.3				
Navicula symmetrica (Patrick)												0.3
Navicula tenelloides Hustedt							0.3		0.7	0.5		1.1
Navicula tripunctata (O.Müller) Bory		0.6										
Navicula vandamii Schoeman & Archibald											0.8	
Navicula veneta Kützing	0.9	0.3	0.3	1.0		0.3			0.2			1.3
Nitzschia acicularis (Kützing) W.M.Smith	0.9		0.6	0.5		0.3	0.3		0.7	0.3	0.3	
Nitzschia amphibia Grunow	0.3	0.9	0.6	0.7								0.3
Nitzschia capitellata Hustedt *in* A.Schmidt												0.3
Nitzschia compressa var. *vexans* (Grunow) Lange-Bertalot							0.3					
Nitzschia dissipata (Kützing) Grunow		0.6	0.9	0.7	2.8	23.9	3.5	2.5	0.9			
Nitzschia fonticola Grunow *in* Cleve & Möller	0.6						2.1	0.3		0.3		0.3
Nitzschia fonticola var.*pelagica* Hustedt	2.1	0.6	0.6				1.1			0.3		0.3
Nitzschia frustulum (Kützing)Grunow	0.6	3.2	0.3			0.9	0.8	0.3	1.9	1.6	1.4	1.1
Nitzschia gracilis Hantzsch	0.9	0.3			0.3	0.3	0.3			0.3		
Nitzschia inconspicua Grunow	0.3											
Nitzschia liebetruthii Rabenhorst											0.8	
Nitzschia linearis (Agardh) W.M.Smith	0.3		2.4	11.0	0.3	1.7						0.5
Nitzschia linearis var. *subtilis* (Grunow) Hustedt		0.6										
Nitzschia linearis var. *tenius* (W. Smith) Grunow												1.9
Nitzschia palea (Kützing) W.Smith	6.3	1.5	2.7	4.5	2.2	2.3	6.6	2.0	0.5	0.5	2.2	3.8
Nitzschia paleacea (Grunow) Grunow *in* Van Heurk							25.8	1.4	0.2	0.5		
Nitzschia paleaeformis Hustedt												0.5
Nitzschia pura Hustedt			0.3			0.3						
Nitzschia pusilla (Kützing)Grunow	3.9	0.3	0.6	0.2			0.5		0.2			
Nitzschia sinuata (Thwaites) Grunow				0.2								
Nitzschia sinuata var.*delognei* (Grunow) Lange-Bertalot									0.2			

Taxa	Month											
	M	A	M	J	J	A	S	O	N	D	J	F
Placoneis clementis (Grunow) Cox		0.3										
Placoneis elgenensis (Gregory) Cox										0.3		
Planothidium delicatulum (Kützing) Round & Bukhtiyarova									0.7			
Planothidium frequentissimum (Lange-Bertalot) Round & Bukhtiyarova											3.0	2.7
Planothidium haukianum (Grunow) Round & Bukhitiyarova							0.5		0.7	1.1		
Planothidium lanceolatum (Brébisson) Round & Bukhitiyarova									0.2	12.6	0.8	1.3
Psammodictyon constricta (Gregory) D.G.Mann *in* Round, Crawford & Mann			1.8	2.5		1.7				0.3		
Reimeria sinuata (Gregory) Kociolek & Stoermer	6.9	22.8	11.4	7.5		8.9	0.8	4.5	4.0	22.0	33.9	37.3
Rhoicosphenia curvata (Kützing) Grunow										0.3		
Rhopalodia gibba (Ehrenberg) O. Müller	0.3			0.2		0.3						0.3
Rhopalodia gibberula (Ehrenberg) O.Müller												0.5
Sellaphora pupula (Kützing) Mereschowsky												0.5
Sellaphora pupula fo. *rostrata* (Hustedt) Bukhtiyarova				0.2								
Stauroneis brasiliensis (Zimmerman) Compere		0.3										
Stephanodiscus hantzschii Grunow *in* Cleve &Grunow				0.5	0.3					0.3	0.3	0.3
Surirella angusta Kützing				0.2	0.3							
Surirella brebissonii Krammer & Lange-Bertalot										0.5		
Synedra acus Kützing							1.3			0.3		
Synedra ulna (Nitzsch) Ehrenberg	0.6		0.6				0.3				0.3	
Thalassiosira weissflogii (Grunow) Fryxell & Hasle		0.9										
Tryblionella calida (Grunow) D.G.Mann *in* Round Crawford and Mann										0.3		0.3
Tryblionella coarctata (Grunow) D.G. Mann *in* Round, Crawford & Mann			0.3									
Tryblionella levidensis W.M.Smith	0.6							0.3				

SITE V2 (VAAL RIVER DOWNSTREAM OF GROOTDRAAI DAM)
MARCH 2002 TO FEBRUARY 2003

Taxa	Month										
	A	M	J	J	A	S	O	N	D	J	F
Amphora pediculus (Kützing) Grunow	1.5	3.8	4.8	0.3		3.7	4.5	2.0	2.7	2.6	3.3
Asterionella formosa Hassall			0.2	2.8					0.2		
Aulacoseira granulata (Ehrenberg) Ralfs	0.3	1.3	4.4	3.3	0.3	1.6	1.1	2.6	1.7	1.2	0.9
Aulacoseira granulata var. *angustissima* (O.Müller) Simonsen	0.9	6.3	3.2	14.4	1.3	2.9	2.9	1.1	0.5	1.4	
Caloneis bacillum (Grunow) Cleve								1.1			
Cocconeis pediculus Ehrenberg	0.3	1.0	1.8	1.3		2.9	3.4	3.1	1.9	2.4	2.6
Cocconeis placentula Ehrenberg		19.9	11.5	0.8		2.1	1.1	8.2	0.5	1.4	7.0
Cyclostephanos dubius (Fricke) Round		0.5	0.9	0.8	0.3	0.8	0.4	0.6	0.7	0.8	
Cyclostephanos invisitatus (M.H.Hohn & Hellerman) Theriot, Stoermer & Håkansson											0.7
Cymbella affinis Kützing	2.9	5.3	3.4	3.6	2.1	0.3	0.2	1.1	0.5	0.6	3.7
Cymbella tumida (Brébisson)Van Heurck	0.3	0.3	0.2	1.0						0.2	0.5
Cymbella turgidula Grunow										0.4	
Diadesmis gallica var. *perpusilla* (Grunow) Lange-Bertalot									0.2		
Diatoma vulgare Bory	2.0	15.1	30.7	20.1	45.1	6.1	13.2	9.4	21.4	2.2	4.7
Encyonema caespitosum Kützing									0.5	2.6	1.4
Encyonema minutum (Hilse in Rabenhorst) D.G.Mann *in* Round, Crawford & Mann	0.3	2.3	2.1	3.3	2.6	8.2	1.6	6.3	2.2	3.6	1.4
Encyonema perpusillium (Cleve-Euler) D.G.Mann *in* Round, Crawford & Mann										0.6	
Encyonopsis microcephala (Grunow) Krammer	0.9	0.8	0.5	0.8	0.5		0.4			0.2	0.2
Epithemia sorex Kützing		0.3							0.2	0.4	
Fragilaria capucina Desmazières	1.2	0.8									0.5
Fragilaria capucina var.*gracilis* (Østrup) Hustedt										0.2	
Fragilaria capucina var.*vaucheriae* (Kützing) Lange-Bertalot			0.2	1.3	1.1	2.4	2.2	1.4	7.7	4.2	2.1
Gomphonema minutum (Agardh) Agardh			0.9			0.5	0.4			0.4	0.2
Gomphonema parvulum Kützing	0.3	1.5	0.7		0.3	1.9	0.0	0.3		1.2	0.2
Gomphonema exilissimum (Grunow) Lange-bertalot & Reichart										0.6	
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot										0.4	0.2
Gyrosigma acuminatum (Kützing) Rabenhorst										0.2	
Melosira varians Agardh		0.3	2.8	0.3							0.2
Hippodonta capitata (Ehrenberg) Lange-Bertalot & Metzeltin *in* Witkowski											0.2
Navicula capitatoradiata Germain		2.0	2.3	2.6	0.8	9.0	5.8	4.8	3.4	2.6	8.7
Navicula concentrica Carter & Bailey-Watts							1.3				
Navicula cryptocephala Kützing										0.2	
Navicula cryptotenella Lange-Bertalot		1.8		1.3		1.1	0.4	0.6	0.7	3.4	7.0
Geissleria decussis (Østrup) Lange-Bertalot & Metzeltin							0.2	0.3	0.2		
Navicula integra (W.Smith) Ralfs					0.3						
Navicula antonii Lange-Bertalot	0.6	2.5	1.8	0.5	1.3	2.9	1.6	3.4	3.4	1.8	2.8
Eolimna minima (Grunow) Lange-Bertalot											0.2
Craticula molestiformis (Hustedt) Lange-Bertalot								0.6			
Navicula rhynchocephala Kützing										0.2	
Frustulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot							0.4				0.2
Navicula symmetrica (Patrick)										0.2	
Navicula tenelloides Hustedt										0.8	1.2
Fallacia tenera (Hustedt) D.G.Mann *in* Round, Crawford & Mann						0.3					
Navicula tripunctata (O.Müller) Bory								0.9	1.2	5.2	4.0
Navicula trivialis Lange-Bertalot											0.2
Navicula vandamii Schoeman & Archibald										0.2	
Navicula veneta Kützing					0.5		0.2	0.9			0.5
Navicula rostellata Kützing	0.6		0.2							1.2	0.5
Navicula viridula (Kützing) Ehrenberg									0.2	0.2	
Nitzschia acicularis (Kützing) W.M.Smith					0.3						
Nitzschia amphibia Grunow								0.6			
Nitzschia capitellata Hustedt *in* A.Schmidt											0.2
Nitzschia dissipata (Kützing) Grunow		0.5		4.1	5.3	2.9	1.8	4.5	7.2	2.6	0.7
Nitzschia filiformis (W.M.Smith) Van Heurk			0.2					0.3	0.2		
Nitzschia fonticola Grunow in Cleve & Möller									0.2		0.2
Nitzschia frustulum (Kützing)Grunow		0.3						1.4		1.6	3.0
Nitzschia inconspicua Grunow	0.3	0.3							0.7		
Nitzschia liebetruthii Rabenhorst											1.6
Nitzschia linearis var. *subtilis* (Grunow) Hustedt			0.2						0.2	0.4	
Nitzschia linearis var. *tenius* (W. Smith) Grunow											0.2
Nitzschia palea (Kützing) W.Smith		0.5	0.5	0.3		0.3		1.1	2.7	0.8	1.2
Nitzschia paleacea (Grunow) Grunow in Van Heurk											0.2
Nitzschia pura Hustedt				1.8	0.3	1.9	1.3	0.0	1.0		
Planothidium delicatulum (Kützing) Round & Bukhtiyarova											0.2
Planothidium lanceolatum (Brébisson) Round & Bukhitiyarova			0.2								
Reimeria sinuata (Gregory) Kociolek & Stoermer		0.3	0.9		1.1	2.1	0.9	4.0	0.2	0.4	0.7
Rhoicosphenia curvata (Kützing) Grunow		0.8	0.5		0.3			0.3	0.2	0.4	0.2
Rhopalodia gibba (Ehrenberg) O. Müller		0.5									
Staurosira construens var. *venter* (Ehrenberg) Hamilton		0.0	0.2								
Stephanodiscus agassizensis Hakansson & Kling		0.5	5.5	19.0	0.5	1.1	2.9	5.4	4.6	2.0	0.9
Stephanodiscus hantzschii Grunow in Cleve &Grunow									0.2		
Synedra acus Kützing	0.3								0.2		
Synedra ulna (Nitzsch) Ehrenberg				0.3							

SITE V3 (VAAL RIVER AT GLADDEDRIFT)
MARCH 2002 TO FEBRUARY 2003

Taxa	Month										
	M	A	M	J	J	A	S	O	N	D	F
Amphora montana Krasske	0.6		0.3	0.3	0.3	0.8				0.3	0.5
Amphora ovalis (Kützing) Kützing						0.3					
Amphora pediculus (Kützing) Grunow					0.3				0.3	1.7	
Asterionella formosa Hassall				0.3	0.6	0.3		0.3			
Aulacoseira granulata (Ehrenberg) Ralfs	5.9	1.4	1.9	1.5	1.1	0.3	1.7	0.8			0.5
Aulacoseira granulata var. *angustissima* (O.Müller) Simonsen	0.0	0.3	1.6	0.9	0.0	0.3		0.6		0.6	
Caloneis bacillum (Grunow) Cleve	0.6	0.0	2.2	0.9	1.1	1.4		0.3	0.8	0.3	
Caloneis molaris (Grunow) Krammer											0.5
Cocconeis pediculus Ehrenberg				0.6				0.8	1.6		
Cocconeis placentula Ehrenberg	2.2	73.8	8.1	8.9	11.2	4.7	14.3	1.4	25.3	7.2	6.7
Craticula molestiformis (Hustedt) Lange-Bertalot	5.6	0.8	1.6	0.6	0.0	0.3	0.3				0.2
Craticula submolesta (Hustedt) Lange-Bertalot					0.3						
Cyclostephanos dubius (Fricke) Round	0.3	0.3	26.2	6.0	9.0	1.4	0.3			0.3	
Cyclotella meneghiniana Kützing	0.3			1.2	1.1	0.3	0.8	0.3			
Cymbella affinis Kützing				0.0	1.1						
Cymbella tumida (Brébisson)Van Heurck				0.6							
Diatoma vulgare Bory					0.8						
Diploneis elliptica (Kützing) Cleve									0.0	0.3	0.5
Encyonema minutum (Hilse *in* Rabenhorst) D.G.Mann *in* Round, Crawford & Mann	4.4	0.5	0.3								1.2
Eolimna sp.											0.2
Eolimna subminuscula (Manguin) Lange-Bertalot & Metzeltin	5.9	0.3				0.3	0.6	1.7	0.5	0.8	1.2
Epithemia adnata (Kützing) Brébisson			0.8	0.9	3.1	0.3	5.6	0.3	1.3	3.6	
Epithemia sorex Kützing				0.9	5.0	0.8	2.8	26.5	16.2	2.5	
Fragilaria capucina var.*gracilis* (Østrup) Hustedt					0.3						
Fragilaria capucina var.*vaucheriae* (Kützing) Lange-Bertalot		0.5			0.6	0.3	0.8		0.3		
Fragilaria miniscula (Grunow in Van Heurk) Williams & Round											0.5
Frustulia vulgaris (Thwaites) De Toni						0.6					
Frustulifera pelliculosa (Brébisson) Lange-Bertalot	0.3	0.3	0.5				0.3		0.8		
Frustulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	0.3										
Gomphonema affine Kützing					0.6						
Gomphonema minutum (Agardh) Agardh	1.6	1.4	1.4	0.9	4.8	4.1	16.8	1.1	8.2	7.2	10.2
Gomphonema parvulum Kützing	4.4	1.1	5.1	2.7	0.6	1.7	0.8	0.6	2.1	1.1	12.7
Gomphonema truncatum Ehrenberg					0.3						
Gyrosigma scalproides (Rabenhorst) Cleve	2.2				0.3	3.3	0.8				
Hippodonta capitata (Ehrenberg) Lange-Bertalot & Metzeltin *in* Witkowski						0.3				0.3	
Lemnicola hungarica (Grunow) Round & Basson								0.3			
Luticola mutica (Kützing) D.G. Mann *in* Round, Crawford & Mann			0.3								
Luticola obligata (Hustedt) D.G.Mann *in* Round, Crawford & Mann										0.3	
Mayamaea atomus (Kützing) Lange-Bertalot						0.8				0.0	0.7
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	3.7	1.1	0.5						1.6	0.8	
Melosira varians Agardh					0.3			0.3			
Navicua germainii Wallace	1.2			0.6	0.8						
Navicula accomoda Hustedt									0.3		0.5
Navicula adamantiformis Archibald		0.3			0.3						
Navicula antonii Lange-Bertalot	0.6			0.3	0.0	0.8		1.7	0.8	0.6	
Navicula arvensis Hustedt					0.3						
Navicula capitatoradiata Germain			0.3	2.1	0.3			1.7	0.3		
Navicula concentrica Carter & Bailey-Watts					0.3	0.3		0.3			
Navicula cryptocephala Kützing							0.3			0.3	
Navicula cryptotenella Lange-Bertalot	1.9	0.8	8.6	4.8	3.6	5.2	3.9	4.5	0.8	5.0	10.2
Navicula erifuga Lange-Bertalot											0.2
Navicula festiva Krasske						0.8					
Navicula gregaria Donkin	0.6					0.8			0.3	0.8	
Navicula ignota var.*acceptata* (Hustedt) Lange-Bertalot	0.9										
Navicula perminuta Grunow *in* Van Heurck	0.3		0.5								
Navicula rostellata Kützing	1.9					1.1	1.7			0.3	0.5
Navicula schroeteri Meister	9.3					0.6				1.4	
Navicula symmetrica (Patrick)		0.5	0.8	1.2	1.4	4.4	1.1		0.3	2.2	4.0
Navicula tenelloides Hustedt	1.6	0.8	0.5	1.8	0.6	1.4	1.1	0.8		2.5	3.0
Navicula tripunctata (O.Müller) Bory					0.8		0.3			0.6	
Navicula vandamii Schoeman & Archibald											0.2
Navicula veneta Kützing	0.9		0.5		1.7	5.5	0.8		0.5	2.5	0.2
Navicula viridula (Kützing) Ehrenberg	0.3									0.3	
Nitzschia acicularis (Kützing) W.M.Smith	2.5	0.8	1.1	14.6	7.3	1.4	0.8				
Nitzschia capitellata Hustedt *in* A.Schmidt	2.5	0.8	4.3	0.6	0.3	1.1	0.3				1.0
Nitzschia commutata Grunow *in* Cleve & Grunow				0.3							
Nitzschia compressa var. vexans (Grunow) Lange-Bertalot	0.6		0.3							0.3	
Nitzschia dissipata (Kützing) Grunow			0.3	10.1	0.8	1.7	2.0	12.8	0.5	0.3	0.2
Nitzschia filiformis (W.M.Smith) Van Heurck	0.3		0.8		0.6						
Nitzschia flexoides Geitler						0.6					
Nitzschia fonticola Grunow *in* Cleve & Möller	0.3					0.8	0.3	0.6			0.2
Nitzschia fonticola var.*pelagica* Hustedt									0.5		
Nitzschia frustulum (Kützing)Grunow	2.2	1.6	1.4	0.6	3.6	4.1	14.0	1.7	11.2	20.8	11.7
Nitzschia frustulum var.*bulnheimiana* (Rabenhorst) Grunow	0.6										3.5
Nitzschia graciliformis Lange-Bertalot & Simonsen					4.5	1.4	0.3				
Nitzschia gracilis Hantzsch			0.5	0.3	0.6	0.3					0.2
Nitzschia inconspicua Grunow	0.3										
Nitzschia liebetruthii Rabenhorst											1.5
Nitzschia linearis (Agardh) W.M.Smith										0.3	
Nitzschia linearis var. *tenius* (W. Smith) Grunow				1.5	0.6		0.6	0.3			
Nitzschia palea (Kützing) W.Smith	14.6	0.0	5.1	2.1	2.5	9.9	2.5	0.3	0.3	2.2	15.6
Nitzschia paleacea (Grunow) Grunow *in* Van Heurk	3.1	1.1	1.4	14.9	3.6	14.0	0.6	0.8	2.9		
Nitzschia pumila Hustedt		0.3				0.8					
Nitzschia pusilla (Kützing)Grunow	0.6						0.6				
Nitzschia rostellata Hustedt			1.6								
Nitzschia sigma (Kützing)W.M.Smith	0.9										

Taxa	Month										
	M	A	M	J	J	A	S	O	N	D	F
Nitzschia subacicularis Hustedt *in* A.Schmidt				1.2							
Placoneis elgenensis (Gregory) Cox						0.6					
Planothidium daui (Foged) Lange-Bertalot											0.5
Planothidium ellipticum (Cleve) Round & Bukhitiyarova									0.5		
Planothidium frequentissimum (Lange-Bertalot) Round & Bukhtiyarova										11.6	0.7
Planothidium haukianum (Grunow) Round & Bukhitiyarova					0.6		0.3	0.3		0.6	
Planothidium lanceolatum (Brébisson) Round & Bukhitiyarova	1.9	0.3		0.3	0.0	1.7	0.6	1.7		0.3	
Planothidium rostratum (Østrup) Lange-Bertalot		0.0	0.5		1.7	3.0	0.8	1.4	0.5		
Psammodictyon constricta (Gregory) D.G.Mann *in* Round, Crawford & Mann					0.3	0.3					
Pseudostaurosira brevistriata (Grunow *in* Van Heurk) Williams & Round											2.5
Reimeria sinuata (Gregory) Kociolek & Stoermer	0.3	9.3	2.4	2.4	5.0	5.8	4.8		8.5	16.1	3.5
Rhoicosphenia curvata (Kützing) Grunow					0.8	0.6	3.6				
Rhopalodia brebissonii Krammer			0.8	0.9		1.9		0.6			
Rhopalodia gibba (Ehrenberg) O. Müller			0.3	4.8	0.8	0.6	0.3	1.4			0.2
Rhopalodia gibberula (Ehrenberg) O.Müller				0.6		0.6		0.3			0.5
Sellaphora pupula (Kützing) Mereschowsky	0.3										
Stephanodiscus agassizensis Håkansson & Kling	7.8	0.8	3.0	0.3	8.7	0.6	3.6	1.7	0.3	0.8	1.0
Stephanodiscus hantzschii Grunow *in* Cleve & Grunow			1.1	2.4	0.8					0.8	
Surirella angusta Kützing							0.3				
Surirella brebissonii Krammer & Lange-Bertalot						1.9	1.4	0.3			
Surirella brightwellii W.Smith	0.6						0.3				
Surirella splendida (Ehrenberg) Kützing			0.3			0.3					
Synedra acus Kützing											0.2
Synedra ulna (Nitzsch) Ehrenberg			0.5								1.5
Tabularia fasiculata (C.Agardh) D.M. Williams & Round			9.5	2.7			0.3				
Thalassiosira duostra Pienaar				0.6			0.6				
Thalassiosira weissflogii (Grunow) Fryxell & Hasle	0.9					0.3					
Tryblionella calida (Grunow) D.G.Mann *in* Round Crawford and Mann	0.6									0.3	
Tryblionella levidensis W.M.Smith	0.3					0.3		0.3	0.3	0.6	0.5

SITE V4 (VAAL RIVER BELOW VAAL DAM)
MARCH 2002 TO FEBRUARY 2003

Taxa	Month											
	M	A	M	J	J	A	S	O	N	D	J	F
Achnanthidium saprophila (Kobayasi & Mayama) Round & Bukhtiyarova	6.9	2.1	5.8	14.1	0.4	1.0	48.2	21.4	3.4	6.6	2.9	0.3
Amphora montana Krasske	2.5			0.6		0.7				0.3		0.3
Amphora pediculus (Kützing) Grunow	5.0	1.3	0.3	0.9			0.2	0.2	0.9	4.6	0.8	0.8
Amphora veneta Kützing	0.6	0.8									0.3	
Aulacoseira granulata (Ehrenberg) Ralfs	2.2	3.4	0.6	0.3	0.2	0.5			0.3	0.3		0.3
Aulacoseira granulata var. *angustissima* (O.Müller) Simonsen	0.8	0.5	0.3		0.4	0.7			0.6	1.1	0.3	1.0
Caloneis molaris (Grunow) Krammer			0.3						0.3			
Cocconeis pediculus Ehrenberg			0.3							0.3		
Cocconeis placentula Ehrenberg	15.2	2.9	1.2	0.6	0.2				1.1	28.7	65.8	15.1
Craticula molestiformis (Hustedt) Lange-Bertalot	2.5	0.3		0.6	1.6		0.2	1.3	0.3			
Cyclostephanos dubius (Fricke) Round	0.3	0.8	0.6									0.3
Cyclostephanos invisitatus (Hohn & Hellerman) Theriot, Stoermer & Hakansson												0.8
Cyclotella meneghiniana Kützing	0.6	0.3										
Cymatopleura solea (Brébisson) W.Smith								0.2				
Cymbella tumida (Brébisson)Van Heurck		1.8	0.3		0.4		0.4	1.1			0.5	
Cymbella turgidula Grunow *in* A.Schmidt	1.1	30.1	26.4	13.8	5.4	15.9	5.2	10.4	7.4		0.8	
Diatoma vulgare Bory				0.3	8.2	8.2	0.8	1.7	2.0	0.3		
Diploneis elliptica (Kützing) Cleve		0.3								0.3		
Encyonema minutum (Hilse *in* Rabenhorst) D.G.Mann *in* Round, Crawford & Mann	0.6						0.4	0.2	0.3			
Encyonema perpusillium (Cleve-Euler) D.G.Mann *in* Round, Crawford & Mann		1.6	1.4					0.2				
Eolimna subminuscula (Manguin) Lange-Bertalot & Metzeltin	1.9	0.5	0.3		0.4		0.2	0.4	1.1	0.3	0.5	1.5
Fragilaria capucina var.*vaucheriae* (Kützing) Lange-Bertalot		0.3	0.9	0.3	13.6	29.1	19.5	30.7	44.9	0.9	0.3	0.5
Frustulifera pelliculosa (Brébisson) Lange-Bertalot	0.6				0.8							
Frustulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot				0.3			0.2					0.3
Gomphonema affine Kützing	0.3											0.0
Gomphonema angustum Agardh												0.5
Gomphonema minutum (Agardh) Agardh	3.6	3.1	4.9	14.4	1.0	0.0	0.2	0.8	0.0	43.7	5.7	4.3
Gomphonema parvulum Kützing	3.0	1.6	2.9	18.6	10.8	4.2	1.0	4.2	3.7	0.0	0.5	0.5
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot												1.0
Gomphonema truncatum Ehrenberg		1.0			0.4	0.2						
Mayamaea atomus (Kützing) Lange-Bertalot												1.3
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	3.0				1.2			0.4	0.3	0.3		0.3
Melosira varians Agardh				1.2	3.0	4.0	1.7	0.8	0.3		0.3	
Navicula adamantiformis Archibald		1.3	0.3	0.6								
Navicula antonii Lange-Bertalot	5.0	1.8	2.3	0.3	8.0	7.7	4.0	1.7	6.6	2.0	3.1	3.3
Navicula capitatoradiata Germain	0.3			0.3	3.4	5.7	3.7	3.4	5.7	0.3		
Navicula cryptocephala Kützing	0.3											
Navicula cryptotenella Lange-Bertalot	0.3	1.0		0.6	2.6	0.2			0.3			0.3
Navicula erifuga Lange-Bertalot												1.3
Navicula lacunolaciniata Lange-Bertalot & Bonik												0.3
Navicula recens (Lange-Bertalot) Lange-Bertalot												0.8
Navicula schroeteri Meister	0.3											
Navicula symmetrica (Patrick)	0.8	0.3	0.3							0.3	0.3	
Navicula tenelloides Hustedt	1.4											0.3
Navicula tripunctata (O.Müller) Bory	0.0	0.3										0.3
Navicula vandamii Schoeman & Archibald	0.6				0.2	0.2						
Navicula veneta Kützing									0.3		0.3	
Navicula viridula (Kützing) Ehrenberg				0.6								
Nitzschia acicularis (Kützing) W.M.Smith				0.3								
Nitzschia capitellata Hustedt *in* A.Schmidt					0.4				0.3			1.5
Nitzschia desertorum Hustedt												1.5
Nitzschia dissipata (Kützing) Grunow				0.6	1.0	0.5	0.2					0.3
Nitzschia filiformis (W.M.Smith) Van Heurck												0.3
Nitzschia fonticola Grunow *in* Cleve & Möller									0.3			1.5
Nitzschia fonticola var.*pelagica* Hustedt												0.5
Nitzschia frustulum (Kützing)Grunow	4.4	0.3	0.3	0.6			0.2			0.3	1.8	3.1
Nitzschia frustulum var.*bulnheimiana* (Rabenhorst) Grunow												0.8
Nitzschia inconspicua Grunow										0.3		
Nitzschia liebetruthii Rabenhorst	15.5	15.4	41.4	19.8	4.4	8.0	7.1	16.9	14.6	1.7	5.0	12.0
Nitzschia linearis (Agardh) W.M.Smith				1.8								
Nitzschia linearis var.*subtilis* (Grunow) Hustedt					23.6	3.7	0.2					
Nitzschia microcephala Grunow *in* Cleve & Möller												0.8
Nitzschia palea (Kützing) W.Smith	4.4	18.6		0.3	3.6	0.7			0.3	0.6	0.0	6.6
Nitzschia paleacea (Grunow) Grunow *in* Van Heurk		0.3	0.3		2.6	1.7						
Nitzschia paleaeformis Hustedt		0.5										
Nitzschia pusilla (Kützing)Grunow		1.0										
Planothidium daui (Foged) Lange-Bertalot	0.3											0.5
Planothidium frequentissimum (Lange-Bertalot) Round & Bukhtiyarova	6.9	0.5	0.9			0.2		0.2	0.3	0.3	0.8	
Planothidium haukianum (Grunow) Round & Bukhitiyarova	0.3											
Planothidium lanceolatum (Brébisson) Round & Bukhitiyarova					0.8							0.8
Reimeria sinuata (Gregory) Kociolek & Stoermer	5.5	0.5	2.3	0.3	0.4	0.5	0.2			4.9	9.1	31.4
Rhoicosphenia curvata (Kützing) Grunow	0.8	1.3	1.2			1.0			0.3	0.9	0.3	1.3
Sellaphora nyassensis (O.Müller) D.G.Mann *in* Round, Crawford & Mann										0.3		
Simonsenia delognei Lange-Bertalot												0.3
Stephanodiscus agassizensis Hakansson & Kling	0.6	2.4	0.6	0.9		2.0	0.8	0.2	0.3	0.6	0.8	1.0
Surirella angusta Kützing					0.8	2.0						
Synedra ulna (Nitzsch) Ehrenberg		0.3		0.3		0.2	1.2	1.3				0.3

SITE V5 (VAAL RIVER AT GOOSE BAY ESTATES DOWNSTREM OF VAAL BARRAGE)
MARCH 2002 TO FEBRUARY 2003

Taxa	Month											
	M	A	M	J	J	A	S	O	N	D	J	F
Amphora castellata Giffen											0.8	
Amphora montana Krasske	2.0			0.3	0.5		0.4	0.3	2.2	0.5	0.8	2.8
Amphora ovalis (Kützing) Kützing					0.7	0.5	0.6		0.3			
Amphora pediculus (Kützing) Grunow	1.7		2.2	10.9	0.7	0.2	0.4	4.7			0.5	0.3
Amphora veneta Kützing			0.4	1.2	0.2			0.5				
Asterionella formosa Hassall										0.3		0.3
Aulacoseira granulata (Ehrenberg) Ralfs	3.6	3.5	10.8	5.9	9.4	0.7	2.3	1.3	12.5	7.4	4.6	2.8
Aulacoseira granulata var. *angustissima* (O.Müller) Simonsen	1.4	2.7	1.2	1.5							0.3	
Caloneis bacillum (Grunow) Cleve					0.2				0.3		0.5	
Cocconeis pediculus Ehrenberg	0.3	0.2	0.2		0.5		1.0	0.3	0.6			
Cocconeis placentula Ehrenberg	6.2	32.8	16.6	4.4	6.1	0.7	0.8	2.3	6.0	76.5	3.8	1.6
Craticula accomoda (Hustedt) D.G. Mann *in* Round, Crawford & Mann				0.6		0.5	2.7	1.8	0.9			
Craticula molestiformis (Hustedt) Lange-Bertalot	0.6						0.2	0.3				1.6
Craticula submolesta (Hustedt) Lange-Bertalot												
Craticula submolesta (Hustedt) Lange-Bertalot								0.3				
Cyclostephanos dubius (Fricke) Round	0.3				0.2		0.4	0.8				
Cyclotella meduanae Germain			0.2									
Cyclotella meneghiniana Kützing		0.2	0.2	1.5	1.5	0.7	0.6		2.5			0.8
Cymbella affinis Kützing			0.2		0.2							
Diatoma vulgare Bory	0.6		0.4	1.5	12.3	12.1	18.4	1.8	0.3			
Diploneis elliptica (Kützing) Cleve				0.9						0.3		
Eolimna minima (Grunow) Lange-Bertalot												0.8
Eolimna subminuscula (Manguin) Lange-Bertalot & Metzeltin	12.6	0.5	0.2	13.6	0.5	0.5	4.6	13.6	5.6		0.8	5.4
Frustulifera pelliculosa (Brébisson) Lange-Bertalot	0.8											
Frustulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	0.6			1.2		0.2	2.7	5.0				
Gomphonema minutum (Agardh) Agardh			0.8		0.2							
Gomphonema parvulum Kützing	1.1	0.5		0.9				2.3	1.6	0.3	2.3	6.2
Gomphonema parvulum var.*exilissimum* Grunow	2.8	1.7										
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot								0.5				
Gyrosigma acuminatum (Kützing) Rabenhorst					0.2							
Hantzschia amphioxys (Ehrenberg) Grunow *in* Cleve & Grunow												
Hanzschia amphilepta (Grunow) Lange-Bertalot										0.3		
Hippodonta capitata (Ehrenberg) Lange-Bertalot & Metzeltin *in* Witkowski			0.4	4.1	2.9			2.6	1.3	0.3		0.3
Lemnicola hungarica (Grunow) Round & Basson					0.2		0.2					
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot				0.3			0.2	0.5				2.3
Melosira varians Agardh	0.3			0.3	0.7	1.7	0.8	1.3				
Navicula adamantiformis Archibald	0.3		0.2	0.3								
Navicula antonii Lange-Bertalot				3.6	7.0	3.2	1.3	2.9	1.9	2.1	0.8	0.5
Navicula capitatoradiata Germain	0.3		0.6		1.7	1.9	0.2		0.6			0.3
Navicula cryptotenella Lange-Bertalot	0.3	0.2	0.4	0.9	0.7	0.2	1.0	0.3	1.9	1.1	0.5	0.5
Navicula erifuga Lange-Bertalot												2.8
Navicula gregaria Donkin				4.7	26.9	60.4	35.6	29.8	13.2	1.1		
Navicula kotschyi Grunow									3.4	0.8	0.5	
Navicula recens (Lange-Bertalot) Lange-Bertalot	2.2	5.2	5.1	10.9	6.1		0.2	0.8	6.3	2.6	31.7	31.0
Navicula rostellata Kützing			0.8	0.9			0.4	0.3	0.3	0.3	1.0	0.8
Navicula schroeteri Meister	1.1	1.0					0.2			0.5		0.8
Navicula scoliopleura Schmidt											0.3	
Navicula symmetrica (Patrick)			0.4	1.2	0.2			0.3	0.9		0.3	0.5
Navicula tenelloides Hustedt												
Navicula tripunctata (O.Müller) Bory								0.3				0.8
Navicula vandamii Schoeman & Archibald				0.3	1.2			0.5				
Navicula veneta Kützing		0.2	0.2	0.9	0.7	0.2		0.3	0.9	1.1	1.8	2.6
Nitzschia acicularis (Kützing) W.M.Smith	0.3											
Nitzschia amphibia Grunow		0.0						0.8	0.3			
Nitzschia capitellata Hustedt *in* A.Schmidt	0.3	0.2		0.9			9.9	4.2	2.8	0.3	0.3	1.0
Nitzschia compressa var. *vexans* (Grunow) Lange-Bertalot											0.3	
Nitzschia desertorum Hustedt												1.0
Nitzschia filiformis (W.M.Smith) Van Heurck									0.3			
Nitzschia fonticola Grunow *in* Cleve & Möller								0.3	1.9		0.3	6.5
Nitzschia frustulum (Kützing)Grunow	20.4	0.2	0.4	1.8				2.3	0.3		0.3	3.4
Nitzschia inconspicua Grunow	1.1							1.3				0.3
Nitzschia levidensis (W.Smith) Grunow *in* Van Heurck					0.2							
Nitzschia liebetruthii Rabenhorst	19.9	28.8	11.0	1.8	1.2		0.6	0.5	0.3	0.3	1.0	4.9
Nitzschia linearis (Agardh) W.M.Smith						0.2					0.3	
Nitzschia microcephala Grunow *in* Cleve & Möller									0.3			
Nitzschia palea (Kützing) W.Smith	0.8	0.5	0.2	0.6	0.7	1.2	0.8	1.3	22.3	0.5	43.1	5.4
Nitzschia paleacea (Grunow) Grunow *in* Van Heurk							0.2	0.3				4.1
Nitzschia pusilla (Kützing)Grunow	0.3							1.3	0.6			
Nitzschia sigma (Kützing) W.M.Smith									0.6	0.3		
Nitzschia supralitorea Lange-Bertalot												6.5
Placoneis dicephala (W.Smith) Mereschkowsky												0.3
Placoneis elgenensis (Gregory) Cox			0.2	0.6	0.2		0.4	0.5		0.5		
Planothidium frequentissimum (Lange-Bertalot) Round & Bukhtiyarova	3.6			0.9	0.2	0.5	1.5	0.3	0.3	0.3	0.8	
Planothidium haukianum (Grunow) Round & Bukhitiyarova								0.8				
Psammodictyon constricta (Gregory) D.G.Mann *in* Round, Crawford & Mann		0.2		3.6	2.9	0.2	0.4	1.3	0.3	0.3		
Reimeria sinuata (Gregory) Kociolek & Stoermer		2.0	0.4	0.3	0.2	0.2						

Rhoicosphenia curvata (Kützing) Grunow	7.6	17.6	42.9	11.2	4.8	1.5	0.8	4.2	0.3			
Taxa						**Month**						
Rhopalodia gibba (Ehrenberg) O. Müller											0.3	
Sellaphora nyassensis (O.Müller) D.G.Mann *in* Round, Crawford & Mann		0.2										
Sellaphora pupula (Kützing) Mereschowsky		0.5		1.5			0.4	0.8	2.2	0.3	0.8	
Stephanodiscus agassizensis Håkansson & Kling	1.4	0.5	1.0	3.6	6.1	10.4	2.7	1.8	2.8	1.9	1.0	0.8
Stephanodiscus parvus Stoermer & Håkansson												0.3
Surirella angusta Kützing			0.4									
Surirella brebissonii Krammer & Lange-Bertalot					0.5	1.7	0.6	0.3				
Synedra ulna (Nitzsch) Ehrenberg							7.1	0.3				
Tabularia fasiculata (C.Agardh) D.M. Williams & Round	0.3											
Thalassiosira weissflogii (Grunow) Fryxell & Hasle					0.5							
Tryblionella calida (Grunow) D.G.Mann *in* Round Crawford and Mann			0.4							0.3	0.3	

SITE V6 (VAAL RIVER AT SCHOEMANSDRIFT)
MARCH 2002 TO FEBRUARY 2003

Taxa	Month											
	M	A	M	J	J	A	S	O	N	D	J	F
Amphora pediculus (Kützing) Grunow	0.6	0.8					1.0	0.5	0.3	1.5	6.6	0.2
Amphora veneta Kützing	0.3					0.2						
Aulacoseira granulata (Ehrenberg) Ralfs	0.9	17.5	40.0	8.5	10.4	6.7	3.5	6.6	7.6	17.9	3.2	1.2
Aulacoseira granulata var. *angustissima* (O.Müller) Simonsen		0.5	1.0	0.4		0.2					0.2	0.5
Cocconeis pediculus Ehrenberg			0.5	0.4	0.5	2.2	1.2	2.6	2.8	1.2		
Cocconeis placentula Ehrenberg	71.6	27.6	5.6	15.1	5.4	3.0	3.0	4.3	16.5	15.5	9.8	
Craticula molestiformis (Hustedt) Lange-Bertalot	0.9	0.3		0.2		0.2						
Cyclostephanos dubius (Fricke) Round		0.5	0.3	0.2	4.8	6.5	1.5	0.7				
Cyclotella meduanae Germain					0.2							
Cyclotella meneghiniana Kützing		0.3	1.0	4.9	5.7	4.2	1.7	3.1	32.2	13.2	1.2	0.5
Cymbella affinis Kützing		0.3										
Diatoma vulgaris Bory							0.2					
Diploneis elliptica (Kützing) Cleve		0.3	0.3									
Elolimna sp.		4.6	0.3									
Eolimna subminuscula (Manguin) Lange-Bertalot & Metzeltin	13.9	2.2	1.3	20.9	5.4	9.0	2.0	3.8	6.1	5.9	18.8	0.2
Frustulifera pelliculosa (Brébisson) Lange-Bertalot					0.2							
Frustulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	0.6	0.3	0.8	1.5		1.5	2.0	1.2		0.3	1.0	
Gomphonema parvulum Kützing	0.3	1.4		0.2					0.3		1.0	1.0
Hippodonta capitata (Ehrenberg) Lange-Bertalot & Metzeltin *in* Witkowski		0.8				0.2			0.3			
Hippodonta hungarica (Grunow) Lange-Bertalot & Metzeltin *in* Witkowski					0.2							
Lemnicola hungarica (Grunow) Round & Basson									0.3			
Luticola ventricosa (Kützing) D.G.Mann *in* Round, Crawford & Mann										0.3		
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	2.8			0.4		0.7	0.2	0.2			2.9	
Melosira varians Agardh			0.3									
Navicula adamantiformis Archibald	0.3	9.0	2.3	0.2								
Navicula angusta Grunow									0.5			
Navicula antonii Lange-Bertalot	0.3			1.7	0.2	1.0	4.2	0.5	0.3	1.5	1.0	
Navicula capitatoradiata Germain			0.3			0.5	0.2	0.2			0.2	
Navicula cryptocephala Kützing				1.1	0.2							
Navicula cryptotenella Lange-Bertalot		1.1		0.6	0.2	0.2		0.2		2.1	1.2	
Navicula recens (Lange-Bertalot) Lange-Bertalot	1.1	3.0	0.5	0.9		0.2		0.2		5.6	2.9	8.0
Navicula rostellata Kützing		0.5	0.5								0.2	0.5
Navicula scoliopleura Schmidt							0.5				0.2	
Navicula symmetrica (Patrick)		2.2		0.4	0.0	0.2			1.0	5.0	0.7	2.2
Navicula tenelloides Hustedt												0.2
Navicula tripunctata (O.Müller) Bory			0.3							0.3		
Navicula vandamii Schoeman & Archibald				0.2								
Navicula veneta Kützing	0.3	0.5		0.2					0.8	2.9	4.4	0.2
Nitzschia acicularis (Kützing) W.M.Smith		0.3				0.2						
Nitzschia amphibia Grunow												0.2
Nitzschia capitellata Hustedt *in* A.Schmidt		2.7	0.5	0.6	0.2	0.2	0.0	0.5	1.5	0.3	0.2	0.5
Nitzschia dissipata (Kützing) Grunow			0.3						0.0	0.3	0.0	0.0
Nitzschia filiformis (W.M.Smith) Van Heurck		0.3	0.3						0.3	0.6	0.2	1.0
Nitzschia fonticola Grunow *in* Cleve & Möller											0.2	1.0
Nitzschia frustulum (Kützing)Grunow	1.7	0.5		0.4			0.2	0.5	0.8	1.2	4.6	0.5
Nitzschia gracilis Hantzsch				0.4								
Nitzschia intermedia Hantzsch *in* Cleve & Grunow												0.7
Nitzschia liebetruthii Rabenhorst	0.9	3.3										38.0
Nitzschia microcephala Grunow *in* Cleve & Möller									0.3			
Nitzschia palea (Kützing) W.Smith	0.6	3.3	1.5	1.9	1.4	1.0	0.7	0.5	4.6	1.5	2.7	37.8
Nitzschia paleacea (Grunow) Grunow *in* Van Heurk									0.5		1.2	
Nitzschia pusilla (Kützing)Grunow									0.3			0.2
Nitzschia sigma (Kützing)W.M.Smith											0.2	
Nitzschia umbonata (Ehrenberg) Lange-Bertalot									0.3			
Placoneis elgenensis (Gregory) Cox					0.2							
Planothidium delicatulum (Kützing) Round & Bukhtiyarova					0.2	0.2						
Planothidium frequentissimum (Lange-Bertalot) Round & Bukhtiyarova		0.3					0.2	0.2	0.0	0.3	0.2	
Psammodictyon constricta (Gregory) D.G.Mann *in* Round, Crawford & Mann			0.3	3.6	1.8	0.2		0.2	0.8	0.9		
Reimeria sinuata (Gregory) Kociolek & Stoermer	0.3	0.5	0.3	0.2							1.5	
Rhoicosphenia curvata (Kützing) Grunow	0.9	2.5	0.3	0.6		0.2			0.5	1.5		
Stephanodiscus agassizensis Hakansson & Kling	2.0	10.7	40.8	29.6	44.6	53.6	74.3	66.4	19.5	16.4	2.7	2.9
Stephanodiscus hantzschii Grunow *in* Cleve &Grunow					14.3	5.7	1.5	5.5	0.8	0.3		0.2
Surirella brebissonii Krammer & Lange-Bertalot			0.5	4.1	3.6	1.2	1.0	1.7	0.3	0.3		
Synedra acus Kützing									0.3			1.9
Thalassiosira weissflogii (Grunow) Fryxell & Hasle			0.3						0.3	0.9		
Tryblionella calida (Grunow) D.G.Mann *in* Round Crawford and Mann					0.2							
Tryblionella levidensis W.M.Smith		0.3										

SITE V7 (VAAL RIVER AT ORKNEY)
MARCH 2002 TO FEBRUARY 2003

Taxa	Month											
	M	A	M	J	J	A	S	O	N	D	J	F
Amphora montana Krasske		0.3		0.8		0.6		0.2	0.4	0.5	0.2	
Amphora pediculus (Kützing) Grunow			0.3								0.2	
Amphora subacutiuscula Schoeman											0.7	2.7
Aulacoseira granulata (Ehrenberg) Ralfs			0.3	2.1	2.0	1.8	0.2	0.0	0.8	2.5	2.3	0.5
Aulacoseira granulata var. *angustissima* (O.Müller) Simonsen				0.3								0.5
Cocconeis pediculus Ehrenberg	0.0	0.3					0.6	6.5	1.4	0.3		
Cocconeis placentula Ehrenberg	62.5	11.6	7.1	2.6	1.4	1.8	0.4	4.7	1.8	17.3	7.9	3.2
Craticula molestiformis (Hustedt) Lange-Bertalot			0.3	0.3			0.2	1.5		0.8	0.5	0.3
Cyclosephanos damasi (Hustedt) Stoermer & Håkansson							1.8		0.6	0.5	0.2	
Cyclostephanos dubius (Fricke) Round	0.3	1.6	9.5	2.1	2.8	3.6	0.2	0.4				0.3
Cyclostephanos invisitatus (M.H.Hohn & Hellerman) Theriot, Stoermer & Håkansson						1.2	2.5	0.6	0.2		0.5	0.3
Cyclotella atomus Hustedt					0.6		0.8				0.0	0.3
Cyclotella meduanae Germain					3.1						1.8	0.5
Cyclotella meneghiniana Kützing			3.3	0.5	6.0	16.0	9.4	3.2	5.0	1.0	0.7	0.5
Encyonopsis microcephala (Grunow) Krammer						0.3						
Eolimna minima (Grunow) Lange-Bertalot								0.2		0.8		
Eolimna sp.	1.3	2.7	3.3	0.5	0.6			0.4	2.6	1.0		14.6
Eolimna subminuscula (Manguin) Lange-Bertalot & Metzeltin	17.6	2.7	0.9	0.3	0.9	0.6	0.4	3.0	12.6	29.4	4.1	0.5
Frustulifera pelliculosa (Brébisson) Lange-Bertalot							0.2			0.3		
Frustulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	1.3		0.6	1.0	0.9	0.0	0.6			0.8	0.5	
Gomphonema minutum (Agardh) Agardh	0.3			0.3								
Gomphonema parvulum Kützing	0.5	0.3		0.5			0.2		2.0	0.5	1.8	3.0
Hippodonta capitata (Ehrenberg) Lange-Bertalot & Metzeltin *in* Witkowski					0.3							
Mayamaea agrestis (Hustedt) Lange-Bertalot	0.3							0.4				
Mayamaea atomus (Kützing) Lange-Bertalot									0.6	0.5	0.2	
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot							0.2	6.5	4.8	11.2	0.2	
Melosira varians Agardh									0.4			
Microcostatus kuelbsii (Lange-Bertalot) Lange-Bertalot												0.3
Navicua germainii Wallace				0.3								
Navicula adamantiformis Archibald		16.7	10.4	1.0	1.4	0.3	0.8					
Navicula antonii Lange-Bertalot			0.3	1.6	1.1	7.9	0.6	1.9	1.4	0.8		
Navicula arvensis Hustedt										0.3		
Navicula capitatoradiata Germain								0.2	1.0		0.2	
Navicula cari var.*linearis* (Østrup) Cleve-Euler												0.3
Navicula cryptocephala Kützing	0.3			0.3								
Navicula cryptotenella Lange-Bertalot		1.6		0.3				0.6	0.8		0.2	
Navicula erifuga Lange-Bertalot								3.9	8.6	2.8	4.5	3.0
Navicula gregaria Donkin							0.2	0.2				
Navicula halophila (Grunow) Cleve					0.6							
Navicula microrhombus (Cholnoky) Schoeman & Archibald		0.3										
Navicula recens (Lange-Bertalot) Lange-Bertalot	3.4	2.7							18.2	5.6	43.7	27.8
Navicula rostellata Kützing	0.0	0.5	0.3	0.3	0.3			0.2	0.6		0.5	1.3
Navicula schroeteri Meister	0.5	0.5		0.3	0.3							
Navicula symmetrica (Patrick)						0.3	0.2	2.8	4.4	0.5		0.5
Navicula tenelloides Hustedt												0.3
Navicula tripunctata (O.Müller) Bory							0.6	1.3	2.2	0.3		
Navicula veneta Kützing	0.3					0.3	2.5	12.3	5.2	3.0	1.1	0.5
Neidium globiceps (Cleve-Euler) Cleve											0.2	
Nitzschia acicularis (Kützing) W.M.Smith					0.9							1.6
Nitzschia amphibia Grunow			0.0	0.5								
Nitzschia capitellata Hustedt *in* A.Schmidt			0.3					0.9				2.7
Nitzschia compressa var. *vexans* (Grunow) Lange-Bertalot												0.3
Nitzschia desertorum Hustedt								0.6	0.4	0.3	0.7	0.5
Nitzschia eglei Lange-Bertalot									0.2			
Nitzschia filiformis (W.M.Smith) Van Heurck											0.7	0.3
Nitzschia fonticola Grunow *in* Cleve & Möller								0.4	0.0	0.8	0.2	10.5
Nitzschia frustulum (Kützing)Grunow	2.4	1.1		0.3	0.6		0.2	0.9	4.0	5.1	3.8	1.3
Nitzschia inconspicua Grunow									2.0	1.8	0.7	
Nitzschia intermedia Hantzsch *in* Cleve & Grunow											0.2	0.3
Nitzschia liebetruthii Rabenhorst	3.1	0.3		0.3					0.2	2.3	2.9	1.9
Nitzschia linearis var. *subtilis* (Grunow) Hustedt												1.9
Nitzschia palea (Kützing) W.Smith	3.9	4.9	5.3	2.9	2.3	1.5	4.5	9.1	0.8	2.8	12.7	13.2
Nitzschia paleacea (Grunow) Grunow *in* Van Heurk					0.8	0.3	0.2	1.3	0.4			
Nitzschia paleaeformis Hustedt										0.5		0.5
Nitzschia parvula W.M.Smith												0.3
Nitzschia pusilla (Kutzing) Grunow				0.3	0.6			0.4				
Nitzschia recta Hantzsch *in* Rabenhorst				0.3								
Nitzschia sigma (Kutzing) W.M.Smith		0.3										0.8
Nitzschia subcapitellata Hustedt									2.2		1.8	0.5
Nitzschia supralitorea Lange-Bertalot										0.5		
Nitzschia tropica Hustedt								0.9				
Psammodictyon constricta (Gregory) D.G.Mann *in* Round, Crawford & Mann				0.3	0.0	0.3	0.0	1.9	1.2	0.5	0.5	0.3
Pseudostaurosira brevistriata (Grunow *in* Van Heurk) Williams & Round							0.6					
Reimeria sinuata (Gregory) Kociolek & Stoermer	1.3	3.0									0.7	
Rhoicosphenia curvata (Kützing) Grunow		0.5							0.2			
Sellaphora pupula (Kützing) Mereschowsky												0.8
Staurosira construens Ehrenberg									0.2			
Staurosirella pinnata (Ehrenberg) Williams & Round					0.6							
Stephanodiscus agassizensis Håkansson & Kling	0.8	47.4	56.7	73.8	58.8	26.0	45.5	23.7	7.2	2.8	2.5	0.3
Stephanodiscus hantzschii Grunow *in* Cleve &Grunow			0.3	0.0	9.7	34.7	25.8	1.5	0.4	0.3	0.2	
Stephanodiscus minutulus (Kützing) Cleve & Möller						0.9						
Stephanodiscus parvus Stoermer & Håkansson								6.5				
Surirella angusta Kützing			0.3									

Taxa	Month										
	M	A	M	J	J	A	S	O	N	D	J	F
Surirella brebissonii Krammer & Lange-Bertalot			0.3	6.3	3.4	1.5	0.2	0.2				
Synedra acus Kützing												0.5
Synedra ulna (Nitzsch) Ehrenberg									1.0			
Thalassiosira pseudonana Hasle & Heimdal							0.2		3.8	1.5	0.2	0.3
Tryblionella hungarica (Grunow) D.G.Mann								0.4				
Tryblionella calida (Grunow) D.G.Mann *in* Round Crawford and Mann		0.3								0.3		
Tryblionella levidensis W.M.Smith		0.0	0.3									

SITE V8 (VAAL RIVER AT KLIPLAATDRIFT)
MARCH 2002 TO FEBRUARY 2003

Taxa	Month											
	M	A	M	J	J	A	S	O	N	D	J	F
Amphora ovalis (Kützing) Kützing						0.3						0.0
Amphora pediculus (Kützing) Grunow		0.2	0.2	0.2				9.4	1.3	0.9		2.1
Amphora veneta Kützing			0.2			0.3	0.2			0.3		
Aulacoseira distans (Ehrenberg) Simonsen						0.3						
Aulacoseira granulata (Ehrenberg) Ralfs	0.2		0.5			0.6			0.4	14.3	1.2	1.9
Aulacoseira granulata var. *angustissima* (O.Müller) Simonsen		0.2					0.2			0.3		0.4
Cocconeis pediculus Ehrenberg					0.3	0.6	1.0	0.8	2.1			
Cocconeis placentula Ehrenberg	1.3	0.9	1.9	2.9	4.1	1.7	0.2	0.0	0.0	0.9		
Craticula molestiformis (Hustedt) Lange-Bertalot	0.2	0.2	0.7	0.9	0.8			0.2	0.6			0.2
Cyclosephanos damasi (Hustedt) Stoermer & Håkansson			0.2	1.3	0.8							
Cyclostephanos dubius (Fricke) Round	0.2	0.4	1.0	2.0	4.9	3.1	0.5		0.2		0.2	
Cyclostephanos invisitatus (M.H.Hohn & Hellerman) Theriot, Stoermer & Håkansson	0.2	0.2	6.4	6.1	3.3	1.7	0.7	2.0	0.0	6.9		2.1
Cyclotella atomus Hustedt		2.2	1.2	1.8	1.6	0.3	1.7		0.2			0.4
Cyclotella meduanae Germain		0.7	1.2	1.1	0.5		0.7	0.4	10.9	23.7	0.5	1.9
Cyclotella meneghiniana Kützing	0.4	0.7	1.0	4.3	11.4	9.3	11.0	1.2	2.6	1.4		0.4
Cyclotella pseudostelligera Hustedt					0.3							
Cymbella tumida (Brébisson)Van Heurck											0.5	
Diadesmis contenta (Grunow ex Van Heurck) D.G. Mann *in* Round, Crawford & Mann							0.2					
Diatoma vulgare Bory						0.8	0.2					
Encyonema minutum (Hilse *in* Rabenhorst) D.G.Mann *in* Round, Crawford & Mann						0.6	0.2					
Encyonopsis microcephala (Grunow) Krammer						0.0	2.4					
Eolimna minima (Grunow) Lange-Bertalot			0.7			1.1		2.4	13.7	1.4		1.5
Eolimna sp.	47.7	2.4	1.9	6.3	3.0	0.6		0.2	3.0	2.0		1.9
Eolimna subminuscula (Manguin) Lange-Bertalot & Metzeltin	9.2	1.5		4.5	2.4	0.6	0.2	1.6	0.2	1.4		0.6
Fallacia indifferens (Hustedt) D.G.Mann *in* Round, Crawford & Mann			0.2									
Fallacia monoculata (Hustedt) D.G.Mann *in* Round, Crawford & Mann									0.4			
Fragilaria nanana Lange-Bertalot									0.4			
Frustulifera pelliculosa (Brébisson) Lange-Bertalot	0.2			0.2	0.8				0.0			
Frustulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	0.7			3.8	0.8	0.3		0.4	0.4			
Gomphonema minutum (Agardh) Agardh				0.0				0.4				
Gomphonema parvulum Kützing	3.3	0.2	1.2	1.6			0.5	1.6	1.3	0.3	3.6	0.6
Gomphonema truncatum Ehrenberg							0.2	0.4	0.2			0.2
Gyrosigma scalproides (Rabenhorst) Cleve					0.3	0.3				0.3		
Hantzschia amphioxys (Ehrenberg) Grunow *in* Cleve & Grunow							0.2					
Lemnicola hungarica (Grunow) Round & Basson					0.3		0.2		0.4	0.3		
Luticola goeppertiana (Bleisch *in* Rabenhorst) D.G. Mann *in* Round, Crawford & Mann							0.2					
Luticola goeppertiana (Bleisch *in* Rabenhorst) D.G. Mann *in* Round, Crawford & Mann							0.2					
Luticola mutica (Kützing) D.G. Mann *in* Round, Crawford & Mann							0.5					
Luticola nivalis (Ehrenberg) D.G. Mann *in* Round, Crawford & Mann					0.0	0.3	0.2					
Mayamaea agrestis (Hustedt) Lange-Bertalot		0.2										
Mayamaea atomus (Kützing) Lange-Bertalot			0.2					1.2	0.2			0.2
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	7.0		0.7	1.1	2.7	0.3		2.4	1.3	1.1		0.6
Melosira varians Agardh										0.3		
Navicula adamantiformis Archibald	2.6	1.8	2.9	3.1	2.7	0.3			0.2			
Navicula antonii Lange-Bertalot				0.4					0.2			0.2
Navicula arvensis Hustedt	0.2		0.2	0.2	0.3							0.0
Navicula cryptotenella Lange-Bertalot				0.2	0.5	0.3	0.7	1.2			0.2	1.0
Navicula erifuga Lange-Bertalot	0.7		0.2	2.0	0.5	1.1		1.6	0.8	0.6	0.2	7.3
Navicula germainii Wallace				0.2				0.2				
Navicula libonensis Schoeman			0.2									
Navicula microrhombus (Cholnoky) Schoeman & Archibald		0.2										
Navicula perminuta Grunow *in* Van Heurck						1.7						
Navicula phyllepta Kützing				0.2								
Navicula recens (Lange-Bertalot) Lange-Bertalot	4.2	1.5	6.7	2.5	1.1	0.3	0.5	4.4	0.9	2.9	6.2	12.9
Navicula reichardtiana Lange-Bertalot					0.3							
Navicula rostellata Kützing				0.2				0.2				0.2
Navicula schroeteri Meister										0.3		
Navicula symmetrica (Patrick)	0.7		0.7	2.5	2.7	2.8	1.2	1.2	0.6		0.7	1.7
Navicula tenelloides Hustedt			0.0	0.0	0.5	0.3				0.3		0.4
Navicula vandamii Schoeman & Archibald	0.2		0.0	0.2								
Navicula veneta Kützing	1.3		2.1	4.3	9.8	6.5	1.5	2.8	9.8	1.4	1.2	2.3
Navicula viridula (Kützing) Ehrenberg					0.3							
Nitzschia acicularioides Hustedt												0.2
Nitzschia acicularis (Kützing) W.M.Smith				0.2		0.3					0.2	0.0
Nitzschia amphibia Grunow	0.2							0.4	0.6			0.4
Nitzschia archibaldii Lange-Bertalot						2.8						
Nitzschia capitellata Hustedt *in* A.Schmidt			0.2			1.1	1.2	0.2	0.6	0.0	0.2	0.2
Nitzschia communis Rabenhorst							1.0					
Nitzschia desertorum Hustedt		0.2										
Nitzschia filiformis (W.M.Smith) Van Heurck									1.7	0.3	16.2	3.1
Nitzschia filiformis var.*conferta* (Richter) Lange-Bertalot												2.5
Nitzschia fonticola Grunow *in* Cleve & Möller			0.2	0.9	0.5		0.2				2.1	0.6
Nitzschia frustulum (Kützing)Grunow	2.2	0.9	1.2	7.4	4.1	1.7	0.5	39.8	4.9	23.1	2.1	47.9
Nitzschia frustulum var. *bulnheimiana* (Rabenhorst) Grunow		0.9				0.3						
Nitzschia gracilis Hantzsch			0.2	0.2								
Nitzschia inconspicua Grunow	1.1	0.7	0.7	0.9	1.1			4.8	0.4	0.6		
Nitzschia intermedia var.*distans* Cleve-Euler				0.2								
Nitzschia latens Hustedt							1.2					
Nitzschia liebetruthii Rabenhorst	2.9	1.8	0.5	0.4							58.2	
Nitzschia linearis var. *subtilis* (Grunow) Hustedt									0.8	0.3		0.6
Nitzschia palea (Kützing) W.Smith	9.5	2.4	0.2	2.2	2.4	9.3	1.2	0.8	1.5	0.6	0.2	0.4
Nitzschia paleacea (Grunow) Grunow *in* Van Heurk						5.1	6.8		0.4		1.7	0.6
Nitzschia paleaeformis Hustedt			0.5			0.8	0.7	0.2				

Taxa	Month											
	M	A	M	J	J	A	S	O	N	D	J	F
Nitzschia pusilla (Kützing) Grunow											0.2	
Nitzschia sigmoidea (Nitzsch) W.M.Smith						0.3						
Nitzschia subcapitellata Hustedt										0.3		
Nitzschia supralitorea Lange-Bertalot	0.2							6.8	5.1	2.6	2.9	
Pinnularia borealis Ehrenberg	0.2											
Psammodictyon constricta (Gregory) D.G.Mann *in* Round, Crawford & Mann			0.2	0.7	0.5	0.6	0.5		0.2			0.2
Pseudostauraurosira brevistriata (Grunow *in* Van Heurk) Williams & Round									0.2	0.3	0.7	0.2
Reimeria sinuata (Gregory) Kociolek & Stoermer						0.3	0.2					
Rhoicosphenia curvata (Kützing) Grunow		0.2	0.0	0.4								
Rhopalodia gibba (Ehrenberg) O. Müller												0.2
Rhopalodia gibberula (Ehrenberg) O.Müller										0.3		
Sellaphora pupula (Kützing) Mereschowsky										0.3		
Sellaphora seminulum (Grunow) D.G.Mann	0.2											
Stauroneis obtusa Lagerstedt							0.2					
Stephanodiscus agassizensis Hakansson & Kling		7.9	7.4	4.9	10.3	11.9	56.0	5.2	3.9	2.6	0.2	0.2
Stephanodiscus hantzschii Grunow *in* Cleve &Grunow			0.2		5.7	20.1	2.7		0.6	1.1		
Stephanodiscus minutulus (Kützing) Cleve & Möller	1.3	2.0	1.2	0.2	1.4	0.6	0.0	0.2	0.2	1.4		
Stephanodiscus parvus Stoermer & Håkansson		61.1	50.8	19.3	11.4	4.2	0.5	4.4	25.9	0.9		0.6
Surirella brebissonii Krammer & Lange-Bertalot				0.4	0.3	0.6						
Synedra ulna (Nitzsch) Ehrenberg									0.8			
Thalassiosira duostra Pienaar										0.3		
Thalassiosira pseudonana Hasle & Heimdal	1.1	7.5	0.0	1.3	2.7	1.7	0.0	0.2		0.6	0.2	0.2
Thalassiosira weissflogii (Grunow) Fryxell & Hasle												0.2
Tryblionella levidensis W.M.Smith			0.2							0.3		

SITE V9 (VAAL RIVER DOWNSTREAM OF BLOEMHOFF DAM WEIR)
MARCH 2002 TO FEBRURAY 2003

Taxa	Month											
	M	A	M	J	J	A	S	O	N	D	J	F
Amphora ovalis (Kützing) Kützing	0.2											
Amphora pediculus (Kützing) Grunow	0.2	0.2	39.0	25.6	39.6		0.5	0.2	1.5	0.5	0.5	
Amphora veneta Kützing		0.2		0.2				0.2				
Aulacoseira ambigua (Grunow) Simonsen									0.7			
Aulacoseira granulata (Ehrenberg) Ralfs	0.4	0.2	1.7	1.2	0.9	1.2	1.2	0.5	3.5	0.2	0.7	0.3
Aulacoseira granulata var. *angustissima* (O.Müller) Simonsen	0.2	0.2	2.4	0.5		0.4	0.2	0.7	0.7		0.2	0.5
Aulacoseira muzzanensis (Meister) Krammer				1.0		0.2			1.0		0.2	
Caloneis molaris (Grunow) Krammer									0.2			
Cocconeis pediculus Ehrenberg			4.8	3.7	1.9	0.6	0.5	1.9	0.7	0.2	1.6	0.3
Cocconeis placentula Ehrenberg	0.2	0.5	1.3	3.2	26.2	0.4	1.0	9.3	2.0	3.4	21.7	9.4
Craticula molestiformis (Hustedt) Lange-Bertalot	0.2			0.2	0.2	0.2				0.7		
Cyclostephanos dubius (Fricke) Round		0.7	0.2	0.2	0.4	0.4	0.5	0.2	1.0			0.5
Cyclostephanos invisitatus (M.H.Hohn & Hellerman) Theriot, Stoermer & Håkansson			0.2		0.4	0.2	6.2	3.7	0.7	1.0	0.2	0.3
Cyclotella atomus Hustedt										0.2		0.3
Cyclotella meneghiniana Kützing		0.5			0.2		0.2		0.5			
Cymbella kolbei Hustedt			0.2									
Diatoma vulgare Bory			3.7	20.7	3.0	15.8	2.5	0.2				
Diploneis elliptica (Kützing) Cleve			0.2	0.2	0.2		0.5		2.2	2.0	0.7	0.5
Eolimna minima (Grunow) Lange-Bertalot	0.4		0.7	1.2						0.2		
Eolimna sp.	0.2											
Eolimna subminuscula (Manguin) Lange-Bertalot & Metzeltin	3.8	0.2	0.4		2.6		0.5			0.2		
Fallacia tenera (Hustedt) D.G.Mann *in* Round, Crawford & Mann										0.2		
Fragilaria capucina var.*vaucheriae* (Kützing) Lange-Bertalot						0.2					1.4	
Fragilaria tenera (W.Smith) Lange-Bertalot												0.3
Frustulifera pelliculosa (Brébisson) Lange-Bertalot							0.5					
Frustulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot			0.2		0.2			0.2				
Gomphonema minutum (Agardh) Agardh				0.2		0.2						
Gomphonema parvulum Kützing	1.6	2.1					0.5			0.7	0.9	0.8
Gyrosigma acuminatum (Kützing) Rabenhorst			0.2									
Hippodonta capitata (Ehrenberg) Lange-Bertalot & Metzeltin *in* Witkowski				0.5	0.2				1.2	0.5	0.5	0.5
Mayamaea atomus (Kützing) Lange-Bertalot					0.2		0.2					0.3
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	0.9	0.7	0.4	0.7	0.2	0.4		0.9	0.5	0.2		
Melosira varians Agardh	1.6	0.7	4.6	9.3	1.3	0.2						
Navicula antonii Lange-Bertalot	0.7	0.2	1.1	1.2	3.9	0.2	10.1	27.0	36.4	9.5	5.8	1.8
Navicula capitatoradiata Germain				1.2	0.4	0.2		2.3		0.7	0.2	
Navicula cari var.*linearis* (Østrup) Cleve-Euler												0.5
Navicula cryptocephala Kützing				0.2			0.2	1.4		0.2		
Navicula cryptotenella Lange-Bertalot		0.7	2.8	7.8	1.1	0.4	1.7	3.0	2.0	1.0	3.7	
Navicula erifuga Lange-Bertalot	0.2	0.2	0.2	1.0			0.2	0.2	2.0	1.5	5.1	8.7
Navicula germainii Wallace				0.2			0.7	0.2	0.2	0.2	0.5	0.5
Navicula gregaria Donkin				0.2			2.5	2.1	2.7	0.7		
Navicula radiosafallax Lange-Bertalot		0.2										
Navicula recens (Lange-Bertalot) Lange-Bertalot	14.0	51.8	11.5	5.4	1.3	0.2	9.2	5.1	3.2	2.7	31.1	39.9
Navicula rhynchocephala Kützing			0.2									
Navicula rostellata Kützing		2.9	1.5	1.7	0.6	0.2	0.5	0.7	2.5	1.5	1.6	7.1
Navicula schroeteri Meister		0.5										
Navicula symmetrica (Patrick)										0.5		
Navicula tenelloides Hustedt		0.2						0.2	0.7	2.0	1.9	0.8
Navicula tripunctata (O.Müller) Bory			0.4	0.5	1.1							
Navicula vandamii Schoeman & Archibald				0.2		1.2	0.2	1.4	3.7	1.0	0.7	0.8
Navicula veneta Kützing			0.2			0.2	3.0	0.7	2.0	0.5		0.3
Nitzschia acicularis (Kützing) W.M.Smith							0.2					
Nitzschia archibaldii Lange-Bertalot						27.7	0.2		0.2			0.3
Nitzschia aurariae Cholnoky						0.2	0.2			0.2		
Nitzschia capitellata Hustedt *in* A.Schmidt						0.2	0.5	0.5		0.7	0.7	0.8
Nitzschia desertorum Hustedt												1.3
Nitzschia dissipata (Kützing) Grunow				0.7								
Nitzschia dissipata var. media (Hantzsch) Grunow									0.2			
Nitzschia filiformis (W.M.Smith) Van Heurck	0.9	1.0			0.4		0.5	0.5			0.2	0.5
Nitzschia filiformis var.*conferta* (Richter) Lange-Bertalot	0.4		0.2									
Nitzschia flexoides Geitler								1.4				
Nitzschia fonticola Grunow *in* Cleve & Möller	0.2	0.5		0.2	0.2	1.3	0.2	1.6	1.0	5.4		1.3
Nitzschia fonticola var. *pelagica* Hustedt					0.4							
Nitzschia frustulum (Kützing)Grunow	4.7	1.2	3.3	0.7	3.7	2.9	3.2	2.6	3.2	25.2	10.7	3.1
Nitzschia frustulum var.*bulnheimiana* (Rabenhorst) Grunow				0.2						1.2		1.0
Nitzschia inconspicua Grunow	1.6		3.9		1.3	0.2				3.9		
Nitzschia liebetruthii Rabenhorst	38.1	26.5	5.2	1.0	0.4	0.6	6.7	2.1	2.2	9.8	3.7	4.5
Nitzschia linearis (Agardh) W.M.Smith				0.2								
Nitzschia linearis var. *subtilis* (Grunow) Hustedt					0.2		1.2	0.5				
Nitzschia palea (Kützing) W.Smith	0.2	0.2	0.4	2.0	0.2	1.0	2.0	4.7	0.2	7.3	1.2	6.0
Nitzschia paleacea (Grunow) Grunow *in* Van Heurk	0.2	0.2				3.5	0.5	0.2	0.2	0.5		1.8
Nitzschia paleaeformis Hustedt				0.2		1.0		0.2	0.7	0.5	0.2	
Nitzschia perspicua Cholnoky							0.2					
Nitzschia pumila Hustedt				0.7								
Nitzschia pusilla (Kützing)Grunow	24.9	2.6	1.1	0.2		0.2	0.5	0.5		1.7	0.5	
Nitzschia recta Hantzsch *in* Rabenhorst				0.7								
Nitzschia subtilis Grunow *in* Cleve & Grunow	0.2											
Nitzschia supralitorea Lange-Bertalot									0.2			
Placoneis clementis (Grunow) Cox	0.4	0.2	0.4		0.2			0.2	0.2	1.2	1.2	0.5

Taxa	Month											
	M	A	M	J	J	A	S	O	N	D	J	F
Planothidium delicatulum (Kützing) Round & Bukhtiyarova					0.2			0.2				
Planothidium frequentissimum (Lange-Bertalot) Round & Bukhtiyarova			0.2				0.2				0.5	0.3
Psammodictyon constricta (Gregory) D.G.Mann *in* Round, Crawford & Mann			0.7	2.0	0.6			0.9	0.5		0.0	0.3
Reimeria sinuata (Gregory) Kociolek & Stoermer					1.5					0.5	0.0	0.3
Rhoicosphenia curvata (Kützing) Grunow		0.2	2.2	0.7	1.1						0.5	
Sellaphora pupula (Kützing) Mereschowsky			0.2						0.2			
Sellaphora seminulum (Grunow) D.G.Mann								0.2				
Staurosira construens Ehrenberg												0.3
Stephanodiscus agassizensis Håkansson & Kling	2.0	2.1	2.2	1.0	2.4	11.4	8.2	4.4	12.5	0.5	0.2	0.3
Stephanodiscus medius Håkansson			0.2		0.2							
Stephanodiscus minutulus (Kützing) Cleve & Möller						0.2	0.2	0.5				
Stephanodiscus parvus Stoermer & Håkansson						19.1	24.0	10.9	0.2			
Surirella angusta Kützing				0.5	0.2			0.2	0.2			
Surirella brebissonii Krammer & Lange-Bertalot						0.2	0.5	0.2	0.2			
Synedra acus Kützing						0.2						
Synedra ulna (Nitzsch) Ehrenberg	0.4	1.7	0.9	0.2	0.2							
Thalassiosira duostra Pienaar									0.2			
Thalassiosira pseudonana Hasle & Heimdal						7.3	3.0	1.4				
Thalassiosira weissflogii (Grunow) Fryxell & Hasle		0.2							0.2			
Tryblionella calida (Grunow) D.G.Mann *in* Round Crawford and Mann			0.2									

SITE V10 - V13 (JUNE 2002)				
Taxon	Site			
	V10* June	V11** June	V12*** June	V13**** June
Achnanthidium minutissimum (Kützing) Czarnecki			18.2	0.3
Achnanthidium saprophila (Kobayasi & Mayama) Round & Bukhtiyarova	0.5		2.4	0.3
Amphora montana Krasske			1.5	
Amphora ovalis (Kützing) Kützing				0.3
Amphora pediculus (Kützing) Grunow		1.1	0.5	
Amphora veneta Kützing			0.2	1.9
Aulacoseira granulata (Ehrenberg) Ralfs			0.2	
Aulacoseira granulata var. *angustissima* (O.Müller) Simonsen	1.5	0.4		0.8
Caloneis molaris (Grunow) Krammer	1.0			
Cocconeis pediculus Ehrenberg		5.3		7.5
Cocconeis placentula Ehrenberg	4.6	5.1	0.5	5.2
Craticula molestiformis (Hustedt) Lange-Bertalot	0.5	0.2	0.7	
Cyclostephanos dubius (Fricke) Round	6.9	4.7		2.2
Cyclostephanos invisitatus (M.H.Hohn & Hellerman) Theriot, Stoermer & Håkansson	2.6	2.7		1.1
Cyclotella atomus Hustedt		0.2		
Cyclotella meneghiniana Kützing	1.0	0.8	0.2	0.6
Cymbella tumida (Brébisson)Van Heurck	0.3			0.3
Diatoma vulgare Bory	0.3	8.7		1.7
Diploneis elliptica (Kützing) Cleve	0.3	0.2		
Encyonopsis microcephala (Grunow) Krammer	0.3		6.8	3.0
Eolimna sp.		0.2	0.2	
Epithemia adnata (Kützing) Brébisson	0.8			
Epithemia sorex Kützing				0.6
Fragilaria capucina var.*vaucheriae* (Kützing) Lange-Bertalot	0.3		0.5	
Fragilaria spec. entspr. *Synedra acus* var. *angustissima* order var. *radians* sensu auct. nonnull.			0.2	13.8
Frustulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot			0.2	
Gomphonema affine Kützing				0.6
Gomphonema parvulum Kützing	2.1	0.4	6.8	9.9
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	0.3		2.7	
Gomphonema truncatum Ehrenberg			0.2	0.8
Mayamaea agrestis (Hustedt) Lange-Bertalot	0.3			
Mayamaea atomus (Kützing) Lange-Bertalot	0.3			
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	1.0		2.4	
Navicula antonii Lange-Bertalot			0.5	1.4
Navicula capitatoradiata Germain			0.2	0.3
Navicula cryptocephala Kützing	0.3			
Navicula cryptotenella Lange-Bertalot	5.9	2.3	1.7	4.4
Navicula enfuga Lange-Bertalot		1.1	5.3	1.1
Navicula germainii Wallace			0.5	0.6
Navicula libonensis Schoeman	0.3			
Navicula recens (Lange-Bertalot) Lange-Bertalot	0.5	7.4	1.9	3.3
Navicula rostellata Kützing	0.3	0.2	1.0	0.6
Navicula symmetrica (Patrick)			0.2	
Navicula tenelloides Hustedt	0.5	0.2	0.5	1.1
Navicula vandamii Schoeman & Archibald			1.7	0.3
Navicula veneta Kützing			11.4	
Nitzschia acicularis (Kützing) W.M.Smith	20.8		0.7	0.6
Nitzschia archibaldii Lange-Bertalot			1.2	1.1
Nitzschia capitellata Hustedt *in* A.Schmidt				
Nitzschia dissipata (Kützing) Grunow	4.6	0.4		
Nitzschia fonticola Grunow *in* Cleve & Möller	0.3		0.2	
Nitzschia frustulum (Kützing)Grunow	0.5	2.5	6.1	6.1
Nitzschia graciliformis Lange-Bertalot & Simonsen	1.5			
Nitzschia intermedia Hantzsch *in* Cleve & Grunow	0.5			
Nitzschia liebetruthii Rabenhorst	0.3	2.3	7.0	0.3
Nitzschia linearis (Agardh) W.M.Smith	0.3			
Nitzschia palea (Kützing) W.Smith	1.0		0.5	1.7
Nitzschia paleacea (Grunow) Grunow *in* Van Heurk	12.9		2.7	0.8
Nitzschia paleaeformis Hustedt		0.2	0.2	
Nitzschia pumila Hustedt			1.2	1.4
Nitzschia pura Hustedt	0.3			
Nitzschia pusilla (Kützing)Grunow	0.5		0.5	0.6
Nitzschia reversa W.Smith			0.0	0.6
Nitzschia supralitorea Lange-Bertalot	0.3		0.2	
Planothidium daui (Foged) Lange-Bertalot	0.5			0.3
Planothidium frequentissimum (Lange-Bertalot) Round & Bukhtiyarova	0.3			
Planothidium lanceolatum (Brébisson) Round & Bukhtiyarova			0.2	
Psammodictyon constricta (Gregory) D.G.Mann *in* Round, Crawford & Mann			1.7	0.6
Reimeria sinuata (Gregory) Kociolek & Stoermer	1.8	0.6	0.2	1.7
Rhoicosphenia curvata (Kützing) Grunow	0.3	3.8	1.9	
Rhopalodia gibba (Ehrenberg) O.Müller	0.8			
Rhopalodia gibberula (Ehrenberg) O.Müller	0.5			
Stephanodiscus agassizensis Hakansson & Kling	1.0	37.0	5.6	14.4
Stephanodiscus hantzschii Grunow *in* Cleve &Grunow	1.3			0.6
Stephanodiscus minutulus (Kützing) Cleve & Möller		3.6		0.6
Stephanodiscus parvus Stoermer & Håkansson	14.7	6.1		2.5
Synedra acus Kützing		1.7		
Synedra ulna (Nitzsch) Ehrenberg	1.5	0.2		1.9
Tabularia fasiculata (C.Agardh) D.M. Williams & Round	0.5			0.8
Thalassiosira pseudonana Hasle & Heimdal	0.3	0.2		
Tryblionella angustata W.Smith	0.5			

*V10; Vaal River below Vaalharts Dam Weir
**V11; Vaal River at DeHoop
***V12; Vaal River at Gamagara
****V13; Vaal River at Schmitsdrift

SITE W1 (WILGE RIVER AT FRANKFORT)
MARCH 2002 TO FEBRURY 2003

Taxa	Month										
	M	A	M	J	J	A	S	O	N	J	F
Achnanthidium minutissimum (Kützing) Czarnecki					12.4	0.7	1.2	10.6	3.2	0.2	0.3
Achnanthidium saprophila (Kobayasi & Mayama) Round & Bukhtiyarova	0.3	0.7		0.3	4.7	0.7	0.2	16.1	0.9	0.4	0.0
Amphora montana Krasske	10.4	0.2	10.3				0.2		0.2	2.5	0.3
Amphora ovalis (Kützing) Kützing	0.3										
Amphora pediculus (Kützing) Grunow		1.8	0.2	0.3	0.2	0.0	0.0	0.6	0.5	0.6	0.3
Amphora veneta Kützing								0.2			
Asterionella formosa Hassall						2.6		3.5	0.9		
Aulacoseira distans (Ehrenberg) Simonsen			0.2								0.6
Aulacoseira granulata var. *angustissima* (O.Müller) Simonsen									0.2		0.3
Caloneis bacillum (Grunow) Cleve								0.2			0.3
Caloneis molaris (Grunow) Krammer		0.2			0.7		0.2	1.2		0.2	
Caloneis schumanniana (Grunow) Cleve				0.5	1.6			0.0	0.2	0.0	
Caloneis silicula (Ehrenberg)Cleve								0.4			
Cocconeis pediculus Ehrenberg					0.5				0.2		
Cocconeis placentula Ehrenberg	4.9	14.5	3.4		0.9		0.6	12.4	7.3	8.4	1.2
Craticula accomoda (Hustedt) D.G. Mann in Round, Crawford & Mann								0.2			
Craticula molestiformis (Hustedt) Lange-Bertalot	0.5	0.5	0.2	0.3	0.5		1.8	1.4	0.5	0.8	0.3
Craticula submolesta (Hustedt) Lange-Bertalot	0.3										
Cyclostephanos dubius (Fricke) Round				0.3				0.2	1.4	0.6	0.3
Cyclostephanos invisitatus (M.H.Hohn & Hellerman) Theriot, Stoermer & Håkansson				0.3		0.5		1.7	0.5	1.0	0.6
Cyclotella atomus Hustedt	0.3									0.2	1.5
Cyclotella atomus var. *gracilis* Genkal & Kiss							0.4	0.4			
Cyclotella meduanae Germain								0.2			
Cyclotella meneghiniana Kützing								0.2			
Cyclotella pseudostelligera Hustedt											0.3
Cymatopleura solea (Brébisson) W.Smith				0.3							
Cymbella affinis Kützing		0.2	0.5	2.8	30.5	48.6	0.6	0.2	8.0	1.0	0.9
Cymbella tumida (Brébisson)Van Heurck		2.3	3.6	28.8	0.2	4.8			0.7	4.2	0.9
Cymbella turgidula Grunow *in* A.Schmidt		0.0		2.6					0.5		
Diatoma vulgare Bory		0.2		11.8	0.5	1.9	22.6				
Encyonema gracile Rabenhorst		0.5									
Encyonema minutum (Hilse *in* Rabenhorst) D.G.Mann *in* Round, Crawford & Mann			0.2	2.1		2.9	1.6	4.8	1.1	1.0	
Encyonema silesiacum (Bleisch *in* Rabenhorst) D.G. Mann *in* Round, Crawford & Mann								0.4			
Encyonopsis microcephala (Grunow) Krammer											0.3
Eolimna minima (Grunow) Lange-Bertalot	0.3							5.4		12.2	3.2
Eolimna sp.		0.2	0.5						0.2	1.0	
Eolimna subminuscula (Manguin) Lange-Bertalot & Metzeltin	33.3	7.6	7.9	1.5			7.5	1.4	2.3	9.9	4.4
Epithemia adnata (Kützing) Brébisson											0.3
Epithemia sorex Kützing										0.2	
Fallacia insociabilis (Krasske) D.G. Mann *in* Round, Crawford & Mann										0.2	
Fallacia tenera (Hustedt) D.G.Mann *in* Round, Crawford & Mann					0.2						
Fragilaria capucina var.*vaucheriae* (Kützing) Lange-Bertalot		0.7	8.9	18.8	0.2	20.0	0.2	0.8	2.5	0.2	0.9
Fragilaria crotonensis Kitton										0.8	
Fragilaria nanana Lange-Bertalot						0.7					
Fragilaria ulna "sippen" *angustissima* (Grunow) Lange-Bertalot							0.6				
Frustulia vulgaris (Thwaites) De Toni			0.2								
Frustulifera pelliculosa (Brébisson) Lange-Bertalot	0.3		0.2								
Frustulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	15.4	0.9	0.2	0.3			0.2		0.2	1.5	
Gomphonema minutum (Agardh) Agardh	0.0	6.7	11.8	0.5	5.9	0.7	1.6	4.8	28.9	15.7	3.2
Gomphonema parvulum Kützing	4.7	6.5	11.5	3.9	0.0	0.0	14.2	2.9	14.9	3.4	8.8
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	4.9	15.0	0.2						1.8		0.9
Gomphonema truncatum Ehrenberg							0.4				
Gyrosigma scalproides (Rabenhorst) Cleve		0.2	0.5						0.2	0.6	0.9
Gyrosigma spencerii (Quekett) Griffith								0.2			
Hippodonta capitata (Ehrenberg) Lange-Bertalot & Metzeltin *in* Witkowski										0.2	
Luticola mutica (Kützing) D.G. Mann *in* Round, Crawford & Mann	0.3										
Mayamaea agrestis (Hustedt) Lange-Bertalot			0.2								2.9
Mayamaea atomus (Kützing) Lange-Bertalot		3.0	1.0		0.2		0.8			0.4	
Mayamaea atomus var. *permitis* (Hustedt) Lange-Bertalot	7.6		9.6							0.4	1.8
Melosira varians Agardh			0.7	9.8			0.2	1.2	0.9	0.8	0.3
Navicula adamantiformis Archibald	0.3										
Navicula antonii Lange-Bertalot	0.3		0.7			0.2	3.0	8.7	0.9		
Navicula arvensis Hustedt	0.8								0.2		0.9
Navicula capitatoradiata Germain						0.2		2.1	1.4		0.3
Navicula cryptocephala Kützing	0.0	0.5	0.2	0.8	0.0	1.2		2.1			
Navicula cryptotenella Lange-Bertalot	1.3	0.7	0.0	0.3	1.8	1.0	3.5	0.6			0.6
Navicula difficillima Hustedt	1.0	0.2	0.2								
Navicula erifuga Lange-Bertalot			0.7							1.3	2.0
Navicula germainii Wallace		5.1	1.4		0.2		2.4	0.4	4.1	4.4	1.5
Navicula microcari Lange-Bertalot	0.3										
Navicula phyllepta Kützing											0.3
Navicula pseudolanceolata Lange-Bertalot					0.2						
Navicula recens (Lange-Bertalot) Lange-Bertalot			0.7								
Navicula rostellata Kützing		0.5	0.5	0.3			1.0		0.7	2.1	4.1
Navicula schroeteri Meister	0.5										
Navicula symmetrica (Patrick)	1.3	4.8	0.7				0.4		0.9	1.3	
Navicula tenelloides Hustedt		0.5	0.2				0.4				0.6
Navicula tenera Hustedt											0.3
Navicula tripunctata (O.Müller) Bory		0.5									
Navicula trivialis Lange-Bertalot	0.5	0.7									
Navicula vandamii Schoeman & Archibald			0.2	0.3		0.2	0.4	0.2			
Navicula veneta Kützing					0.2		0.4			0.4	0.0
Nitzschia acicularis (Kützing) W.M.Smith				0.3	1.4					0.2	1.2
Nitzschia acula Hantzsch								1.2			
Nitzschia amphibia fo. *rostrata* Hustedt	0.3										
Nitzschia amphibia Grunow		0.2									

Taxa	Month										
	M	A	M	J	J	A	S	O	N	J	F
Nitzschia bergii Cleve-Euler											2.3
Nitzschia calcicola Aleem & Hustedt										0.2	
Nitzschia capitellata Hustedt *in* A.Schmidt			0.2	0.3					0.2	0.6	
Nitzschia desertorum Hustedt			0.7	0.0						0.2	
Nitzschia dissipata (Kutzing) Grunow			1.0	3.3	18.5	4.6	2.4	2.3	0.2	0.0	
Nitzschia dissipata var.*media* (Hantzsch) Grunow				3.9	1.1	4.3	0.4	2.9	0.2	0.0	
Nitzschia filiformis (W.M.Smith) Van Heurck										0.2	2.3
Nitzschia fonticola Grunow *in* Cleve & Möller	0.5			0.5			2.4		0.2	0.4	0.6
Nitzschia frustulum (Kützing)Grunow	2.3	9.9	1.7		1.4	0.2	0.6	0.6	2.3	5.7	0.3
Nitzschia gracilis Hantzsch					2.0	0.5				0.4	
Nitzschia inconspicua Grunow		0.2							0.2	0.4	
Nitzschia liebetruthii Rabenhorst	0.5	4.8	14.2					0.4	0.5	2.3	0.9
Nitzschia linearis (Agardh) W.M.Smith				1.0	0.9	1.0		0.4			
Nitzschia linearis var. *tenuis* (W.Smith) Grunow	0.3			0.5	1.1	1.0	1.2	0.2			
Nitzschia linearis var.*subtilis* (Grunow) Hustedt											
Nitzschia microcephala Grunow *in* Cleve & Möller		0.2									
Nitzschia palea (Kützing) W.Smith	4.4	4.2	1.0	1.5	2.7	0.5	7.3	2.9	3.7	8.4	41.2
Nitzschia paleacea (Grunow) Grunow *in* Van Heurk			0.5	0.5	0.5		18.5	0.2		1.0	1.2
Nitzschia paleaeformis Hustedt				0.3	0.9	1.0					
Nitzschia pumila Hustedt											0.3
Nitzschia pusilla (Kützing)Grunow			0.7				0.2				
Nitzschia subacicularis Hustedt *in* A.Schmidt											0.9
Planothidium frequentissimum (Lange-Bertalot) Round & Bukhtiyarova	0.8	1.6						2.1		0.2	
Psammodictyon constricta (Gregory) D.G.Mann *in* Round, Crawford & Mann					0.7						
Reimeria sinuata (Gregory) Kociolek & Stoermer	0.0	2.3	1.2	0.3	6.8			0.2	5.5	0.6	1.2
Rhoicosphenia curvata (Kützing) Grunow		0.2			0.2						
Rhopalodia gibberula (Ehrenberg) O.Müller							0.2			0.2	
Sellaphora pupula (Kützing) Mereschowsky		0.2						0.2			
Sellaphora seminulum (Grunow) D.G.Mann								0.2			
Staurosirella pinnata (Ehrenberg) Williams & Round	0.3										
Surirella angusta Kützing				0.3	0.2						0.6
Surirella brebissonii Krammer & Lange-Bertalot				0.8							0.0
Surirella splendida (Ehrenberg) Kützing											0.3
Synedra acus Kützing									0.2	0.4	
Synedra ulna (Nitzsch) Ehrenberg		0.5		0.3			0.6				0.3
Thalassiosira pseudonana Hasle & Heimdal			0.5								
Thalassiosira weissflogii (Grunow) Fryxell & Hasle								0.2			
Tryblionella levidensis W.M.Smith											0.3

APPENDIX 4 – DIATOM INDEX SCORES

	Month	DIATOM INDEX SCORES - MARCH 2002 TO FEBRUARY 2003													
		SPI	SLA	DES	LMI	SHE	WAT	EPI	ROT	GDI	CEC	BDI	APDI	TDI	%PTV
SITE V1	M	10.1	11.2	13.2	12.4	10.5	10.3	6.9	12.4	10.7	9	12	11.1	64	22.2
	A	14.5	13.7	15	14.6	14.3	13.5	11.5	14.2	12.8	13.7	14.1	13.8	53.1	7
	M	13.3	13.1	14.1	13.8	13.4	15.3	11	14.2	13.1	13	13.2	12.7	54.1	9
	J	13.1	13.5	12.9	12.5	16.2	16.3	12.9	16.6	13.9	12	14	11	19.5	7
	J	12.8	11.8	15.9	12.3	14	19.3	12.2	12.8	14.3	12.8	15.1	11.2	53.8	3.6
	A	15.9	13.7	17.2	14.3	14	17.9	11.2	13.9	13.7	14.7	14.8	14.9	63.3	5.2
	S	9.7	11	13	10	8.6	12.6	7.5	11	8.5	10.3	12.6	8.6	88.5	16.8
	O	9.8	9.2	12.8	9.4	8	11.1	6.1	10.1	12.4	9.2	12.2	9.2	82.3	14.1
	N	11.2	9.4	15	11.7	9.9	9.6	4.7	10.6	12.4	10.1	11.5	10.6	67.8	5.4
	D	14.8	12.9	14.4	14	13.7	14.1	11.4	13.7	13.3	13.4	13.3	13.5	63.5	3.6
	J	15	13.5	14.4	14.6	14	13.8	11.6	13.7	13.9	13.4	14.4	14	51.1	5.1
	F	12.1	12	12.9	14	14	14.5	10.9	13	13	11.8	14.9	11.1	63.3	13.4
SITE V2	A	14	14.4	16.3	14.2	9.6	9.2	12.8	8.5	17.1	17.3	11	13.8	28.2	0.6
	M	14.2	12.6	17	13	12.1	13.6	11.4	12.4	14.8	16	11.5	13.7	55.3	2.5
	J	14	11.4	18	12.1	12.7	15.1	11	12.2	14.4	15.4	11.2	13.3	71.7	1.4
	J	13.8	11.8	18.5	11.5	13	15.3	10.5	12.4	14.3	15.8	10.6	12.6	68.2	2.1
	A	15.4	11.6	19	12.3	12.7	16.4	11.5	11.9	15.5	16.2	12.9	14.7	73	0.8
	S	14.7	12.8	17.2	12.9	12.1	14.7	10.6	11.4	14.9	15.6	12.2	14	52.4	4
	O	13.6	12.2	18.2	12.6	10.5	11.9	10.4	9.9	15.1	15.8	10.6	13	53.2	1.3
	N	14.1	12.5	17	13	11.8	13.3	10.6	11.5	14.6	15.1	11.6	13.4	57.2	3.1
	D	14	12.1	17.7	12.6	11.8	14.4	10.6	11.6	14	14.7	12.3	13.8	67.4	4.8
	J	13.9	13.3	17	13.3	10.8	11.2	10.7	10.6	15.1	15.3	10.8	13.4	47.6	3.6
	F	12.6	12.9	17.4	13.1	10.8	11.1	10.2	11.2	13.9	14.1	10.5	12.3	53.2	6.8
SITE V3	M	6.4	10.4	8.3	8.8	8	10.1	7	9.7	9.1	5.6	8.9	8.8	83.7	33
	A	14.3	13.7	15.1	14.4	13.7	11.8	10.2	13.8	12.6	12.2	12.8	14.2	52.7	5.7
	M	8.7	10.8	11.5	11.5	10.5	11.5	9	11.9	8.9	7.5	7.6	9	74.8	20.5
	J	11.4	11.2	13.2	10.7	12.1	12.5	8.4	12.1	9.3	10.7	10.9	9.6	71.7	22.6
	J	11.2	11.4	13.3	11.8	13	12.1	9.8	13.5	10.5	10.9	10.1	9.9	51.4	20.4
	A	8.5	11.6	10.6	11.3	10.2	11.7	9.8	11.5	8.6	8.6	10	8.7	72.8	21.8
	S	12	12.8	14.1	12.2	12.7	13.3	10.7	13.3	10.9	11.3	11.1	10.5	53.2	19.3
	O	14.7	13.4	17.4	13.5	9.6	10.3	10.4	12.3	15.3	14.5	10.8	13.2	38.9	3.1
	N	13.3	13.1	14.8	12.9	11.1	11.4	10.6	12.9	12.7	11.6	11.4	10.5	41.4	15.7
	D	11.1	12.7	14.2	12.3	11.5	12.8	10.2	13	10.3	10.3	11.1	8.2	64	26.3
	F	7.5	12.4	8.7	10.5	9.2	12	9.6	13.3	8.5	7.3	9.4	8.8	84.1	46.4
SITE V4	M	10.1	11.6	14.1	11.8	9.9	11.5	9.6	12.3	9.6	10.3	10.1	11.2	68.6	30.4
	A	9.1	10.7	7.3	9.3	8	12.9	9.5	14	10.7	7.7	7.9	10.9	56	36.4
	M	11.8	12.5	15	12.9	10.8	14.1	10.4	13	9.8	13.5	9	12.4	55.4	44.6
	J	11.3	12.4	10.8	9.7	8	14.6	10.2	11	11.6	11.8	9.7	10.3	66.4	39.6
	J	10.6	10.9	14.6	11.9	9.6	14.6	9	13.3	8.9	10.3	11.4	10.9	82.4	20.4
	A	12.5	11.2	16.2	12.2	11.5	17.2	9.2	12.7	12.4	12	12.7	11.3	68.2	12.9
	S	11.7	11.7	16.4	12.7	8	9.5	9.3	8.4	14.8	12.6	9.7	11.2	40.7	8.1
	O	11.4	11.2	15.4	12.1	9.6	13.7	9.6	9.2	12.7	11.6	11.2	10.7	51.4	21.6
	N	11.7	11.4	16.4	12.6	11.8	16.8	9	11.8	11.6	12	13.2	11.2	59.9	19.1
	D	14.4	13.3	15.9	14.5	13	15.2	10.4	13.1	13	14.1	12.3	14	55.4	3.2
	J	14.4	13.9	15.4	15	13	11.9	10	14	12.4	13.5	12.6	14.2	53.3	7.3
	F	10.8	12.4	13	13.1	12.7	14	10.1	13.3	9.5	11.8	12.7	12.2	69.2	28.6

	Month	DIATOM INDEX SCORES													
		SPI	SLA	DES	LMI	SHE	WAT	EPI	ROT	GDI	CEC	BDI	APDI	TDI	%PT
SITE V5	M	8.4	10.7	14.9	10.5	9.9	10.6	5.8	10.6	6.8	7.7	7.9	8.3	73.9	44
	A	10.9	12.4	15	13.2	13.4	12.8	10	13	8.4	11.5	9.7	14.1	59.8	30.3
	M	12.1	11.7	15.4	11.8	13.7	16	11.4	12.6	10.9	11.5	8.5	13.9	62	11.7
	J	9.5	9.6	16.1	10.2	10.2	12.2	5.3	11	10.9	9.2	8.1	10.2	87.5	10.9
	J	11.5	9.7	17.5	9.9	10.8	13.5	6.8	12.6	12.1	12.4	10.7	11.4	90.1	29.1
	A	12.1	8.6	16.9	8.5	8.6	12	6.2	11.3	12.3	12.2	13.5	10.6	97.1	61.7
	S	8.6	8.6	14.7	8.3	8.6	12.2	5.4	10.2	10.9	9.4	10.4	9.2	95.4	47
	O	8.8	8.5	13.9	8.4	7.3	10.3	4.8	9.6	10.2	7.8	9.7	8.8	90.2	42.3
	N	5.7	9.5	7.1	8.9	8.3	9.6	5.9	10.6	8.3	4.8	7.5	9.3	81.4	41.7
	D	13.3	13.5	15.2	14.1	13.4	11.3	6.9	14.5	12.6	7.3	11.5	14.3	53.5	2.6
	J	3.5	9.3	3.3	7.7	6.1	7.1	4.6	11	6.6	2.1	5.9	8.9	75.4	47
	F	5.7	10.1	10.9	6.8	8.9	10.9	5.7	10.1	8.1	4.8	7.1	7.2	82.7	34.6
SITE V6	M	12.7	11.3	15.2	13.6	11.5	9.1	1.8	12.3	12.2	8.6	11.8	13	56.7	6.3
	A	9.3	11.6	13.9	11.8	12.4	12.1	6	12.3	11.2	7.5	8.4	12.3	60	12
	M	9.8	11.4	12.9	10.7	15.3	14.1	5.9	11.6	11.8	0	4.4	10.6	63.5	2.3
	J	8.8	9.1	13.2	10.3	9.2	8.8	3.6	9.6	11.2	7.7	8.7	9.7	74.3	4.1
	J	8.2	8.9	11.3	9.2	12.1	10.3	6.3	10.2	10.9	6.7	5.5	6.8	72.9	1.6
	A	8.6	8.8	13.3	9.9	9.6	9.9	4.4	8.9	10.9	6.7	5.6	8.1	83.1	2.2
	S	10.4	9.9	15.8	10.9	9.9	10.5	5.3	14.2	10.5	0	7.7	10.9	82.1	1.2
	O	9.9	10.2	13.9	10.7	11.5	10.6	4.7	10.4	10.7	7.7	6.5	10.6	73.8	1.7
	N	7.6	9	12	9.7	10.8	7.5	6.4	11.3	11.3	4.8	7.7	9	76	7.8
	D	8.5	10.2	14	10.9	12.1	10.4	5.2	11.4	12	7.1	7.3	9.8	71.9	3.5
	J	9.3	9.9	14.6	10.3	10.2	6.3	2.5	8.7	12.6	7.8	8.4	8.3	74	12
	F	3.1	8.8	2.1	6.7	4.2	7.4	6.1	10.2	2.8	2.1	6.4	8.1	76.3	78.7
SITE V7	M	10.8	10.9	14.4	13.4	10.8	8.6	2	11.9	11.3	8	11.2	12.5	58.6	10.2
	A	8.4	11.7	11.5	12.5	10.5	10.1	6.3	12.3	10.6	7.5	10.6	12.7	64.1	6.7
	M	7.6	9.6	10	11.3	8	9.6	7	12.3	10.1	4.4	7.2	11.4	67	5.9
	J	8.4	11.2	9.2	10.3	9.2	10.4	8.9	12.6	10	0	7.8	10.4	72.9	3.9
	J	7	8.7	10.6	8.5	8.9	9.8	7.6	11.6	10.2	6.3	6.1	5.4	78	3.7
	A	7.1	7.9	13.7	7.9	8.9	9	8	17.6	10.9	5.9	5.1	4.7	91.2	1.5
	S	5	7.4	8.8	6.7	5.4	9.1	6.9	10.9	10	3.1	4.3	3.4	88.9	5.3
	O	5.9	7.9	11.4	9.5	6.7	9.1	4.6	9.6	9.5	5.6	7.3	7.2	78.7	23.5
	N	6.2	8.4	14.6	9.2	8	9	3.7	9.8	10.6	5	6.5	6.9	84	25.3
	D	7.1	7.8	13.9	9.1	7.3	6.6	2.1	8.5	10.3	6.5	8.6	7.7	78.1	28.4
	J	5.2	9.5	8.4	9.3	8.9	9.3	3.6	10.6	9.2	3.3	6.2	8.6	73.8	29.9
	F	5.4	10.6	8.7	8.5	8.6	10.5	6.1	10.9	7.7	4.8	7.7	8.6	76.3	29.4
SITE V8	M	4.6	7.4	5.8	7	4.5	8.3	3.6	7.4	10.2	4	7.2	5.7	86.5	26.8
	A	8.7	9.6	8.9	9.1	7.7	10	5.9	10.2	9.3	0	7.3	6.3	79.2	7.3
	M	8.7	8.7	13.8	8.3	9.9	10.7	7.8	10	10.2	5.8	6.6	6	81.6	6.7
	J	7.1	8.5	12.6	8.7	8.3	9.6	6.9	9.7	9.4	5.6	6.2	5.9	85.1	16.1
	J	6.5	7.8	11.9	8.9	8	8.8	6.8	8.7	10.2	4.8	5.6	4.8	83.3	10.9
	A	5.7	8.1	8.4	7.9	7.7	9	7.5	9.6	8.4	4.8	5.9	4.1	83	18.9
	S	7.2	10	10.6	8.9	9.6	9.7	7.8	11	9.2	6.3	8.1	6.2	72.2	5.1
	O	6.7	11.5	15.5	8.8	10.2	11	5.1	11.1	5.1	6.3	6.9	6.6	84.4	60.6
	N	6.6	8.9	12.3	7.5	7.3	9.2	4.4	9	9.6	4.8	7.6	6.6	88.7	31.2
	D	6.8	10.6	14.9	9.1	11.8	11.8	7.7	11	8.5	4.8	6.4	6.7	79.9	31.1
	J	7.4	9.2	10.9	6.4	8.6	11.2	7	8.9	2.6	5.9	6.4	9.4	77.6	84
	F	6.5	11.7	14.7	10.4	10.2	10.7	4.7	11.3	5.3	6.3	5.8	6.4	78.6	64.3

	Month	DIATOM INDEX SCORES													
		SPI	SLA	DES	LMI	SHE	WAT	EPI	ROT	GDI	CEC	BDI	APDI	TDI	%PT
SITE V9	M	4.6	9.4	4.2	7.7	4.8	7.5	7.7	11.2	6.4	5.6	9.2	7.9	80.6	67.5
	A	6.2	9.9	13.5	9.5	11.1	10.3	5.3	10.5	3.8	5.9	6.4	6.2	78.8	49
	M	6.4	10.7	11.3	11.1	10.5	10.7	4.2	10.6	8.2	6.3	6	7.8	77.5	31.7
	J	11.6	12.4	18.1	12.6	13	15.8	6.4	12.4	9	13	9.3	13.1	90.9	14.4
	J	12.6	11.8	18	12.7	13.4	16.3	8.2	12.7	10.9	13.7	11.1	14.2	92.4	8
	A	13.9	12.6	17.8	13.8	13	15	6.7	13.3	10	14.7	11	13.9	82	6.2
	S	11.7	10.8	17	9.1	14.3	12.3	10.1	12.8	6.6	8.6	12.5	6.9	88.8	34.1
	O	8.6	9.4	16.1	10.6	9.9	11.2	7.4	14.1	9	7.8	7.4	9	88.5	17.1
	N	10.1	10.6	16.5	12.4	9.6	11.1	7.7	16.3	9.7	7.5	9.6	12.5	85.8	13.7
	D	11.8	10.4	18.9	13.1	9.9	11.5	8.1	17.2	11	9.7	9.5	12.7	92.2	10.7
	J	7.8	12.2	14.2	10.8	10.5	11.4	10.4	13.6	5.2	6.5	7.5	7.8	80.2	52.3
	F	8.6	13	15.4	13.1	11.5	11.1	5.2	12.8	10.2	7.3	8.7	11.4	70.3	22.9
SITE W1	M	7	11.3	11.7	11.6	10.2	10.6	5.6	11.6	9.6	5	7.5	10.7	72.4	27
	A	7.1	8.2	12	8.3	5.8	8.1	3.7	6.6	10.2	7.7	8.4	6.7	92.7	13.5
	M	10.1	12.1	13.9	11.7	9.2	13	6.4	12.7	10.4	10.9	11.4	8.8	74.5	25.9
	J	9.2	9.1	12.8	9.1	9.2	13.6	9.4	11	10.2	9.4	9.5	8	81.5	39.9
	J	12.1	10.6	15.2	11.1	13	18.3	10.2	13.9	15.2	12.8	13.4	12.2	65.4	6.7
	A	15.2	14	16.5	13.6	15.6	16.8	13.3	15.5	14.7	15.1	14.9	14.8	34.2	9.5
	S	14.5	13.7	16	12.8	17.2	18.7	14.1	16.6	16.3	13.2	15.1	12.9	22.7	3.1
	O	9.6	10.8	13.4	10.1	8.6	13.6	7.6	11.6	9.5	10.5	12	8.7	94.9	23.2
	N	13.1	12.2	15.1	12.5	10.2	11.5	9	13.3	13.6	12.4	12.1	13.3	59.3	13.7
	J	11.9	12.2	12	11.3	11.5	16.7	10.6	13.8	13.1	11.5	12.6	10.2	62.4	21.8
	F	8.3	10.3	11.5	9.8	8.9	10.4	7.3	12.1	10.4	8.6	10.8	8.9	75.1	35.6

APPENDIX 5 – THE IMPORTANCE OF SCANNING ELECTRON MICROSCOPY IN DIATOM ECOLOGICAL STUDIES

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THE IMPORTANCE OF SCANNING ELECTRON MICROSCOPY IN DIATOM ECOLOGICAL STUDIES

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The diatoms (Bacillariophyceae) comprise a ubiquitous, highly successful and distinctive group of essentially unicellular algae, whose most obvious distinguishing characteristic is the possession of siliceous cell walls (frustules).

Existing diatom systematics relies exclusively on their morphology, especially on the diagnostic features of the siliceous frustules as revealed by SEM. Hence even in studies in which DNA is isolated from diatom cells, it is always necessary to study the ultrastructure of any given assemblage to make sure that all the diatom cells belong to the same species[1].

Diatoms are readily available for collection and are becoming increasingly important as ecological indicators of water quality, particularly of pH, conductivity, salinity and trophic status[2]. Two morphologically similar species may have different ecological specificities to various factors determining water quality. The correct identification of diatoms occurring in South Africa is important when determining the relationship between diatom species and the chemical state of a particular water body.

Light microscopy (LM) is used for the routine identification of diatoms in ecological monitoring studies[3]. However, the introduction of the Scanning Electron Microscope (SEM) led to the revelation of taxonomically important structures previously virtually invisible under LM. A study was undertaken to determine the relationship between diatom communities of the Vaal River and water quality. SEM was used in this study to confirm and correct doubtful species level identifications made using LM.

Preparation of these cells for SEM examination is simple due to the robust nature of the silica wall. Either fresh or acid cleaned material is filtered through a 1.5µm Millipore® membrane. The membrane is rinsed with ethanol and allowed to dry in a desiccator, coated with gold-palladium and examined under high vacuum at 15kV, with a spot size ranging from 2.3 to 3.5 depending on magnification.

Two groups of diatoms the centrales and the pennales are encountered in freshwater habitats. The centric diatoms are the group in which one relies most heavily on the use of SEM for correct identification of species. Structures such as rimoportulae and fultoportulae must be interpreted three dimensionally. The occurrence and pattern of these structures (Figure 1) are critical diagnostic criteria.

Although differences between species of pennate diatoms may be more easily observed using LM, confusion may arise in some of the pennate genera e.g. *Cymbella*. The number, grouping and internal structure of isolated pores or stigmata are the bases for distinguishing between morphologically similar species within this genus (Figure 2 & 3).

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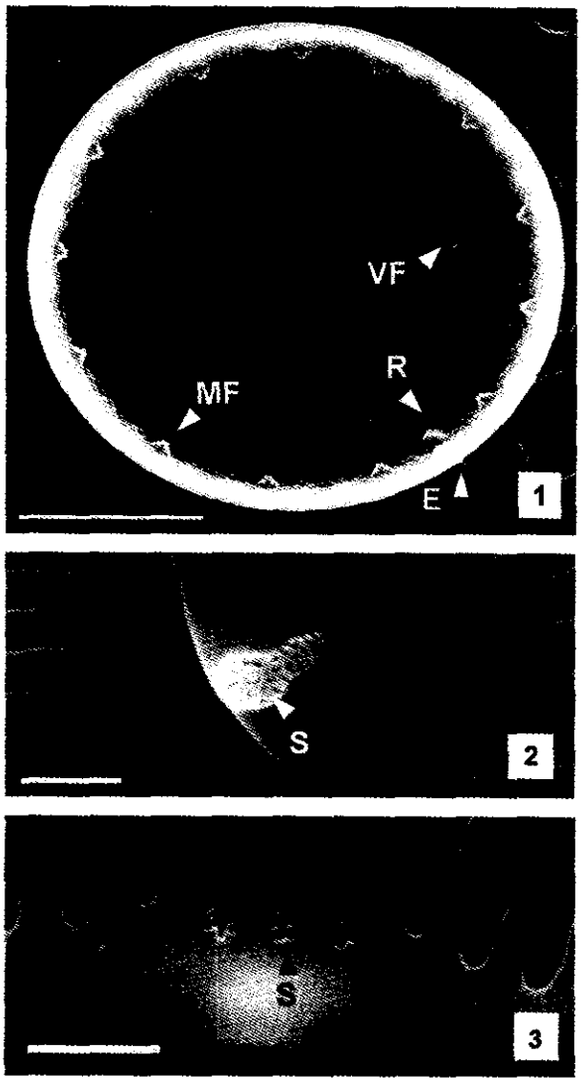

Fig. 1. The position of several processes found in the centric diatom *Stephanodiscus agassizensis*. R - rimportula; MF - mantle fultoportula VF – valve face fultoportula and E - external opening of rimoportula. Scale bar = 5µm.
Fig. 2. Structure of the stigma (S) on the inner valve face of the frustule of *Cymbella tumida*. Scale bar = 2µm.
Fig. 3. Structure of the 3 stigmata (S) on the inner valve face of the frustule of *Cymbella turgidula*. Scale bar = 2µm.