

**THE SOUTH AFRICAN DIATOM INDEX
(SADI) –
A PRELIMINARY INDEX FOR INDICATING
WATER QUALITY IN RIVERS AND
STREAMS IN SOUTHERN AFRICA**

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

The core aim of this project was to provide a water quality index, informed by diatom species and abundance, which would encompass South African river and stream conditions and, at the same time, incorporate ecologically relevant characteristics of endemic species – i.e. supporting the derivation of ecological inferences from diatom assemblages. The use of previously-available indices was constrained by their lack of autecological information on southern African diatoms. Creation of the first version of the South African Index has been achieved through the collection, processing, examination and analysis of 768 individual samples – together with their associated water quality information. All of the samples have been curated in the South African Diatom Collection. In addition the extensive notes on the ecology of SA diatoms, compiled by BJ Chohnoky, have been included in this index.

The index has been provided in the form of an upgrade addition to the existing OMNIDIA software, with the intention being that the index will form part of future releases thereof. In this format, the South African Diatom Index (SADI) constitutes the second largest index supported by autecological data currently provided by OMNIDIA. The SADI is based on the same platform as the majority of European diatom indices but, in particular, on the Specific Pollution sensitivity Index (SPI) – see discussion in literature review. The SPI index has the broadest species base of any index currently in use. This species base has been augmented by the addition of endemic SA diatom species, with scores modified for other cosmopolitan and commonly occurring species found in South Africa, thus increasing the accuracy of diatom-based water quality assessments in South Africa.

A secondary aim of the project was to enable skills transfer and training in the use of diatoms as a means to assess lotic environments and to draw ecological inferences therefrom. In this regard the project has been particularly successful, as is evident from the Capacity Building Report. The tools and methods developed during Phase 2 of the Diatom Assessment Protocol have been widely accepted (locally and internationally) and South Africa now has two commercial laboratories offering the procedure. In addition, the DWA is in the process of establishing the protocol across the RHP sites. This phase of the work also saw the second version of the taxonomic key being produced. This version is being hosted on the Internet for both local and international use.

An addition to the original project scope was the inclusion of a Literature Review outlining the use of diatoms as indicators in South Africa, both past and present, as well as the use of diatoms as indicators internationally.

Various sub-aims were completed, these being further assessment of the value of diatoms as assessment tools in the urban river, stream and canal environment, and an evaluation of information of a non-diatom nature contained in the Chohnoky (CSIR) Diatom Collection. During this third phase of development of the Diatom Assessment Protocol, the Chohnoky Collection (now the SA National Diatom Collection) was ceded to SAIAB and moved from the CSIR to North-West University and is under their curatorship. Accordingly, for the first time in many years, this internationally-important collection is once-again under active and professional curatorship.

Advice was sought from Europe during the process of index development. Professor Horst Lange-Bertalot (Frankfurt-am-Main, Germany) has helped with problematic identifications and the description of species new to science, as has Dr Luc Ector from Luxembourg. Dr Michel Coste from France advised on the construction and testing of the diatom index, as well as on the inclusion of species within the OMNIDIA database.

Phase 3 of the Diatom Assessment Protocol has completed the initial aims of providing tools for diatom collection, sample processing and taxonomic identification by adding the interpretive index component. Combining the index with the already available and powerful OMNIDIA software is the most logical manner in which to initially distribute the product of this work. This version (Version 1) of the South African Diatom Index is by no means complete in its present format and further research is required, this being especially true for the Western Cape region. The scope of the necessary work has been proposed to the WRC as Phase 4. Should the research and refinement not continue then application of the Index might not be valid across all South African river and stream environments. Given that so much progress has been achieved in just six years since the inception of Phase 1, it would be illogical to not see the work to completion.

One of the most consistent and common responses, particularly from the DWA, has been that the method is too specialized and difficult to apply. This argument is misplaced in that it does not take into account the level of value and information that can be gleaned from a single diatom sample, a level that outweighs any of the other indicators in terms of assessing water quality and drawing ecological inferences.

Diatom field methods are the simplest of any biomonitoring protocol currently in use. Once the sample has been collected there are two options: Submit it to a commercial laboratory for analysis (several laboratories exist) or perform the analysis yourself.

Diatom species identification can at first seem daunting. The lack of willingness to work with microscopes and taxonomy is an outcome of poor exposure to these skills at the tertiary level, combined with an absence of algological training in South Africa. As with most things, however, proficiency comes with time. This project has shown that with initial intensive training of ~2 weeks, follow up sessions, either in person or via e-mail and after ~6 months of practice a person, with a biological background can become very proficient in diatom analysis.

The second challenge has been why provide such a seemingly complex and intricate method for simple bio-indication?

Diatom indices are not field-based methods and some form of microscopic examination or laboratory-based analysis will ALWAYS need to take place. However, this perceived added effort should not detract from the method. Diatom indices allow for the quantification of a water quality impact in terms of producing an index score, as well as allowing the user to draw specific conclusions regarding the pH, electrical conductivity, nutrient status, organic content and oxygen levels of a stream and is thus a very powerful tool. For a small amount of added effort, a relatively large amount of information regarding the system under question can be gained. This has specific value for the reserve determination process.

This project has continued to demonstrate the versatility of the use of diatoms as an indicator, including its application in environments where all of the other habitat-limited indicators have no use.

This study has identified the following needs for further research:

This study, although fairly comprehensive in terms of covering South Africa, has unfortunately excluded the Western Cape. Samples were collected from different regions in this province but several problems have arisen during their analysis:

- Cholnoky's type material for this region has been lost, thus we cannot cross-check species identifications and his species names are invalid at this time;
- The characteristics of the waters of the Western Cape differ significantly when compared to those in the rest of the country;

- We currently do not have enough samples from this region to be able to meaningfully assign tolerance and indicator values.

Accordingly, a comprehensive regional study is required in order to source type material and expand the SADI V1.0 to incorporate these data.

Ongoing inclusion of results from collections from all over the country are and will remain necessary for some years in order to increase the robustness of the index.

Identification literature

The guide to the common diatoms of South Africa (Version 2) needs to be expanded to include species from the Western Cape and KwaZulu-Natal (sub-tropical). There is also a need to produce floras dealing specifically with each group of diatoms.

ACKNOWLEDGEMENTS

The project team would like to acknowledge the support and contributions of all those who engaged with us during this work, many of whom provided samples and supporting data. Without these contributions we would not have been able to get anywhere near our targets.

Dr Steve Mitchell, formerly of the WRC, is especially thanked for his foresight, support and role in allowing us an opportunity to re-establish the value of diatoms in South Africa after so many years.

STRUCTURE OF THIS REPORT

This project entailed two components, these being

1. A data collection and data analysis component, providing the information necessary to create the South African Diatom Index V1.0 (SADI V1.0). This component formed the bulk of the work undertaken during this project;
2. A reporting component, in which various aspects of the use of diatoms in South Africa, identified during Phase 2 of this work, were addressed further.

With the foregoing in mind, this project report is structured as follows:

Executive Summary

Chapter 1: Literature Review

Chapter 2: Background to the South African Diatom Index V1.0. This chapter is supported by two datasets, these being (a) the results of the individual diatoms sampled during this project, compiled in spreadsheet format; (b) the data associated with the formulation of the SADI V1.0, i.e. the names, codes, S&V values, also compiled in a spreadsheet format. Additionally, also included in electronic format, is the SADI V1.0 upgrade for OMNIDIA. This provides the reader or user of the Diatom Assessment Protocol (DAP V1.0), produced during Phase 2 of this project, to either use OMNIDIA to calculate SADI values (preferred method), alternatively to manually calculate the output using Descy's formula.

Chapter 3: An overview of the second version of the LUCID-based taxonomic key for South African diatoms, V2.0. The key itself is provided for freeware use via the internet.

Chapter 4: An assessment of the value of diatoms as a bioindicator for use in urban canals and stormwater conveyance systems. The supporting data for this component are provided as appendices.

- Chapter 5: An overview of the information, other than for diatoms, contained in the South African National Diatom Collection (SANDC), compiled prior to the Collection being relocated to the Potchefstroom Campus of North West University.
- Chapter 6: A summary of the training and skills transfer effected during this project.
- Chapter 7: A synthesis of future research needs necessary to continue the development of the DAP.

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CHAPTER 1: LITERATURE REVIEW

1. Introduction

1.1 Historical perspectives on biotic index development

Biological communities have been used to monitor water quality for at least the past 150 years. Although sophisticated analytical tools have become available for monitoring water quality, direct measurements of plant, invertebrates, fish and microorganisms are still being widely used as indicators (McKenzie et al., 1992).

The first stream classification systems were based on the responses of aquatic organisms to organic enrichment, this enrichment lead to the formation of conceptual “zones” in streams and rivers, depending on the distance from the source of the pollutant. This ‘saprobien system’, as it became known, eventually gave rise to numerical indices for describing water quality based on the tolerance or sensitivity of certain species.

Robert Lauterborn (1901) was the originator of “the conception of the sapropelic world of life”. He defined the saprobic zone in running waters as the “zone of processes of decomposition”. The following year, Kolwitz and Marsson (1902) presented their seminal account of the new saprobien system based on indicator organisms (plankton) to classify streams. They identified three stages or “zones” of progressive decomposition to classify slow moving waters polluted by sewage. They initially reported on the tolerances of circa 300 species of benthic and planktonic algae and later added another 500 planktonic organisms and benthic animals.

The saprobien system was by no means optimal and there were doubts about assigning a single zone or indicator value to an organism that could also be found outside that zone. Brinkhurst (1969) stated that he “... could see no way in which different saprobity values could be given to each species to account for its reactions to the many different forms of polluting materials”. He found that the system was “... less efficient than the opinion of a qualified biologist expressed in plain language”. Other workers were also critical of using indicator species and rather advocated a community-based approach instead. Fjerdingsstad (1964) was convinced that attached algae (especially diatoms) were the best group to use for this since they are not subject to stream drift.

The saprobien system was however, very important in focusing biologists in their measurement of water resource quality by the presence or absence of a range of indicator organisms. This approach has become more sophisticated and now relies upon specific assemblages or benthic communities and their relative abundance and distribution (Davis, 1995).

The greatest contribution of these saprobien systems was, however, the direct development of numeric biotic indices. Over several decades a variety of methods were tested in order to numerically characterize biological data in a meaningful manner. A number of schemes were used based on trophic function, structural ratios of taxa, feeding requirements, etc. (Washington, 1984). Ruth Patrick, the famous American diatomist, developed a histogram approach (Patrick, 1950) based on seven taxonomic groups. The results were portrayed in a graphical and numerical format but, importantly, demonstrated the effectivity of using community composition rather than population-based analysis. Cairns (1974) felt that Patrick had demonstrated the importance of presenting numerical data rather than long (rather incomprehensible) lists of species to make an estimate of the degree of pollution. Importantly, this allowed for the communication of the information gained to people to whom species lists were meaningless. However, Patrick's approach was too complex for most field biologists, who were generally comfortable assessing only the one assemblage they were most familiar with. Thus the need for a cost effective "rapid" biological assessment method was established. This was not however, easily achieved and it was initially thought that a measure of structural diversity of the community, rather than functional or indicator organisms, may solve this dilemma.

Cairns (1977) saw great potential in the use of diversity indices and felt that the method was "... probably the best single means of assessing biological integrity in freshwater streams and rivers". These indices gained favour with biologists and sanitary engineers. The most popular index was published by C.E. Shannon (Shannon & Weaver, 1949). It is correctly termed the Shannon-Wiener index because Wiener (1948) independently published a similar index at roughly the same time (Washington, 1984). However this index is not without its problems and may fail to provide an accurate reflection of water quality problems for the following reasons:

- an inability to reflect ecological significance,
- a total reliance on structural measures that vary greatly depending on season, sampling equipment and the level of taxonomic resolution,

- the loss of community composition information by the use of a single index value.

Problems also arise as the identity of the species is ignored in the calculation of the indices, thus these measures are not sensitive to compensatory changes in the community. Such changes may alter the taxonomic composition of the community but have little effect on diversity. Hilsenhoff (1977) found little ecological significance when using diversity indices and concluded “the diversity index does not accurately assess the quality of streams, ranking some of the cleanest undisturbed wilderness streams with moderately polluted or enriched polluted streams”. For further discussion see Section 1.4.

In order to produce a more reliable indicator system, Pantle and Buck (1955) produced a “biotic” based directly on the saprobien system. Its simplicity and numeric relationship to the original four zones of stream pollution lead to the development of a widely-used biotic index in the United States (Hilsenhoff, 1982) and could be considered the true predecessor of today’s biotic indices. However, the conceptual modifications to Pantle and Buck’s index, made by Zelinka and Marvan (1961), forever changed the use of the saprobic index (Davis).

Zelinka and Marvan addressed many of the criticisms of the original index, as well as the general use of indicator organisms by adding a ‘saprobic valency’ and ‘indicator weight’ to the original index. The saprobic valency expressed the relative frequency of the species in different degrees of saprobity on a scale that totaled 10. By establishing the saprobic valency, Zelinka and Marvan answered a major criticism of the saprobic index, i.e. its dependence upon arbitrarily assigning saprobic values to a single species that is likely to be present in more than one zone. They also ascribed an indicator weight to organisms from 1 (poor indicator) to 5 (very good indicator). The best indicators were representative of a small saprobic zone, while the lowest indicator weights were given to species found throughout many or most of the saprobic zones. This index has been significantly modified and applied throughout Europe and the rest of the world. More information is presented on the use of the system of Zelinka and Marvan for diatom indices in Section 1.6.

1.2 Biomonitoring in South Africa

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) that 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. Sample collection, handling and preservation introduce a suite of possible variations between the sampled conditions and those ultimately measured in the sample. 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. Diatoms, especially epiphytic forms, are 'glued' in place in the aquatic environment and are not subject to the disturbance and dispersal force effects common to phytoplankton, aquatic insects or fish.

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 – to various degrees – 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 various attributes 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 of years, analysis of benthic algal communities, such as diatom-based indices, have until recently been excluded due to a 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.

1.3 Diatoms and early diatomology studies in SA

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, composed chiefly of hydrated amorphous silica, but which may also 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 Frisch (e.g. Frisch, 1918) and co-worker Rich (e.g. Rich, 1932). During the 1950's and 1960's the acclaimed diatom specialist, Dr. Bela J. Chohnoky, produced over 40 papers dealing with many of the diatom species found in southern Africa (e.g. Chohnoky, 1960). Later, 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.

1.4 Early work on diatoms as indicator organism in South Africa

The potential of diatoms as indicators of water quality was realised in South Africa many years ago. Chohnoky (1968) describes the application of the Thomasson (1925) community analysis, which he adapted to determine water quality using benthic diatom community composition. Use of the 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. Chohnoky (1968) obtained good results using this index, but the user of the Thomasson analysis method needs to have an in-depth knowledge of the aut-ecology of individual diatom species to draw accurate environmental conclusions based on diatom community composition. Chohnoky's application of the Thomasson

analysis method was a forerunner of modern autecological indices (such as those dealt with in this study), which have since 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, with Archibald concluding 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 "saprobial" 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 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.

More recent developments are addressed in Section 1.7.

1.5 Diatoms as indicator organisms

No single group of organisms is 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, rendering in their uneven distribution from headwaters to estuaries. In addition, watercourses that 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 levels (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 phosphorus 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 other water quality problems 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 changes 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.*, 2002). 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 conventional physico-chemical techniques (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.

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 *et al.*, 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 while a

previous phase of the DAP in South Africa saw the development of both electronic and hard-copy diatom keys (e.g. Taylor *et al.*, 2007c).

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).

1.6 Diatom indices

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 this country. 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.* (2004) express the opinion that before an index system can be attempted, there is a need to gather information on dominant South African 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.

There is currently a strong international effort towards the testing and development of diatom indices specific to different countries. Countries that 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 a_j = abundance (proportion) of species j in sample, v_j = indicator value and 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, 1993a).

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' that 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). A summary of the most commonly used indices is provided in Table 1.1.

Table 1.1: Comparison of biotic indices based on diatoms

Biotic Index	Abbreviation	Biotopes sampled¹ (in order of preference)	Sampling equipment	Taxonomic level²	No of taxa used	Identification protocol	Final range	Index	Current usage
Specific Pollution sensitivity Index	SPI	SIC, S, V	Small brush, size not stipulated	G+S+Var	2035	Lab-based	0-20		EU, France, South Africa
Generic Diatom Index	GDI	SIC, S, V	Small brush, size not stipulated	G	174	Lab-based	0-5		EU
Leclercq & Maquet	LMI	SIC, S, V	Small brush, size not stipulated	S	403	Lab-based	0-c.40		France
Commission Economical index	CEC for Community	SIC, S, V	Small brush, size not stipulated	S	208	Lab-based	0-20 based on 2-way table		EU
Artoise-Picardie Index	ADPI	SIC, S, V	Small brush, size not stipulated	S	41G + 91S	Lab-based	0-20		France
Biological diatom Index	CBS	SIC, S, V	Small brush, size not stipulated)	G+S	209	Lab-based	0-20		EU, France
Eutrophication/Pollution Index	CBI	SIC, S, V	Small brush, size not stipulated	G+S	93	Lab-based	0-20		Italy
Sládeček	DSFI	SIC, S, V	Small brush, size not stipulated	S	323	Lab-based	0-20		Czech Republic

Biotic Index	Abbreviation	Biotopes sampled ¹ (in order of preference)	Sampling equipment	Taxonomic level ²	No of taxa used	Identification protocol	Final range	Index	Current usage
Diatom Assemblage Index of Organic Water Pollution	DAIpo	SIC, S, V	Small size brush, not stipulated	S	87	Lab-based	0-20		Japan
Trophic Diatom Index (Germany)	TDI	SIC, S, V	Small size brush, not stipulated	S	105	Lab-based	0-20		Germany
Trophic Diatom Index (UK)	TDI – UK	SIC, S, V	Small size brush, not stipulated	G+S	86	Lab-based	0-100		UK

¹ SIC = stones-in-current (riffles); S = stones (in- & out-of-current); V = vegetation; GSM = gravel, sand and mud

² Var = Variety; S = species; G = genus

In 1979 Descy proposed the first true diatom index using the equation of Zelinka & Marvan (1961) (see Section 1.1) 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 (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).

Leclercq & 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 led 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 BOD₅ (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), this 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).

1.7 Testing diatom indices in SA (recent progress in the use of diatoms)

1.7.1 Initial evaluation of numerical diatom indices in South Africa

The seminal work of Harding *et al.* (2004) and Taylor (2004) could be seen as ushering in a new phase of diatom based assessment in South Africa. Harding *et al.* (2004) and Taylor *et al.* 2005 used information contained in data sheets compiled by B.J. Cholnoky during the 1950s while studying the Jukskei River system (Cholnoky, 1956). The authors used European numerical diatom indices to back-cast water quality for the system. The results showed the technique to be highly successful and to provide a potential tool for unlocking the wealth of information stored in the collection of diatoms made by Cholnoky and other workers. In a similar way, Taylor (2004) and Taylor *et al.* (2007a; 2007b) examined the used of numerical diatom

indices for indicating water quality in some of the most important river systems in South Africa. In general, these studies conclude that European, diatom-based numerical water quality indices could be used with success in South Africa. However, Taylor et al (2007b) highlighted the following potential problems:

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

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

1.7.2 The Diatom Assessment Protocol

Perhaps most important stumbling blocks in the path of the general acceptance of diatom indices into monitoring protocols in South Africa were:

- The lack of any form of illustrated guide to the common diatoms occurring in South Africa.
- The lack of standard methods for field and laboratory procedures.

The Diatom Assessment Protocol (now in its third phase), funded by the Water Research Commission (WRC), was initiated by W.R. Harding (Harding *et al.*, 2004). The initial phase concentrated on the diatom collection housed at the CSIR in Durban, see discussion in section 1.7.1. The second and key phase resulted in the production of a Lucid based multi-access electronic key to the diatoms (Harding *et al.*, 2007) which was accompanied by an illustrated guide in hard copy (Taylor *et al.*, 2007c). This resource was vital for the acceptance of diatom indices. Despite the wealth of literature published on diatom during a period of 40 years by various workers, these works failed to illustrate the commonly occurring diatoms of South Africa. Without such illustration, photographic rather than drawn, it is very difficult to apply diatom indices as the user would then be required to search through European floras. Although such a practice may be instructive and of academic interest, in the day to day practice of routine water quality monitoring, it is impractical and time consuming. The key and guide are also a vital tool for teaching students the process of diatom identification as all key information is summarised within the resource. The key and guide are by no means exhaustive, neither do they pretend to be but instead

have provided a first introduction into the world of diatoms for many students and water utility workers. It still remains for the diatom flora of South Africa to be examined in greater detail and documented in the style of Schoeman and Archibald's abortive "Diatom Flora of Southern Africa" (Schoeman and Archibald, 1976-1980).

A methods manual accompanied by video footage was also produced during the second phase of the DAP. This manual (Taylor *et al.*, 2007d) along with the standard methods of Taylor *et al.* (2005) provide users of diatom indices with the techniques to successfully, and in a scientifically robust manner, collect, preserve, process and analyse diatom samples. Standardisation of techniques is vital for the cross-comparison of data between workers and for the construction of a national index.

1.7.3 Diatom indices and the River Health Programme (RHP)

Diatoms first formed part of a Crocodile West and Marico Water Management Area State of the Rivers Report in 2005 (SoR; RHP, 2005), which in turn paved the way for the preliminary inclusion of diatom indices as part of the RHP methodology (DWAF, 2008c). The SoR was very useful in showing how diatom indices reflect a slightly different picture to that given by other indices. In general the results of the study (Taylor *et al.*, 2007b) showed that the diatoms had a very close relationship with general water quality. However, diatoms cannot be accepted as a part of the RHP until the indices have undergone an accreditation process. For this process to be successful, trained operatives are needed who can be used to test the technique. Not only should these operatives be well trained but they should also have a certain level of experience. Experience is necessary as it is not always possible to successfully identify diatoms from the more common works of literature, operatives should have a good working knowledge of broader diatom literature and sources for identification. This being said, the DWA is currently in the process of training both field and laboratory technicians which will enable the institution to successfully implement diatom indices in the near future.

1.7.4 The role of diatoms in determining the ecological reserve

Setting the ecological Reserve for an aquatic ecosystem includes determining the water quality and quantity, flow patterns and concentrations of water quality constituents (Scherman, 2003). To maintain an ecosystem at a specified state, flow requirements must be set that will maintain the habitat for instream and riparian biota.

Between 1999 and 2007, the 8-step Reserve process has been refined and the assessment tools for setting the Reserve have been improved. The quantity component of the RDM Manual: Volume 3 (DWAF, 1999) has been revised (Louw and Hughes, 2002) and the EcoClassification process refined (Kleynhans and Louw, 2007). The EcoClassification process is an integral part of Ecological Water Resources Monitoring which includes the River Health Programme and Ecological Reserve (Environmental Flow) monitoring for rivers. EcoClassification refers to the determination and categorisation of the Present Ecological State (PES; health or integrity) of various biophysical attributes of rivers relative to the natural or close to the natural reference condition. It is a multi-disciplinary approach that is applied during the Reserve process to gain insights and understanding into the causes and sources of the deviation of the PES of biophysical attributes from the reference condition (Kleynhans and Louw, 2007). This provides the information needed to derive desirable and attainable future ecological objectives for the river.

According to Kleynhans and Louw (2007) the state of the river is expressed in terms of biophysical components. The 2 main components are:

- Drivers: Water quality or Physico-chemical variables, Geomorphology, and Hydrology). Driver information provides a particular habitat template.
- Biological responses (fish, riparian vegetation and macroinvertebrates), which provide a particular response to a particular habitat template.

An Ecological Category (EC) (A to F; where A represents natural conditions, and F represents critically modified conditions) is assigned to each component. The results of these components are integrated to achieve the EcoStatus of a river. The EcoStatus is defined as the totality of the features and characteristics of the river and its riparian areas that bear upon its ability to support an appropriate natural flora and fauna. This ability relates directly to the capacity of the system to provide a variety of goods and services (Kleynhans and Louw, 2007).

Physico-chemical variables assessed during an Intermediate or Comprehensive Reserve study includes, according to Palmer *et al.*, 2005, and Scherman (2008a, *in press*) inorganic salts, nutrients, physical variables, toxic substances and response variables which include biotic community composition, algal abundance and instream toxicity.

The use of diatoms in South African water quality studies has been very limited and virtually non-existent in ecological Reserve studies. Diatoms were used for the first

time in the Joint Maputo River Basin Water Resource Study during 2007. During this study the Specific Pollution Index (SPI) (Coste in CEMAGREF, 1982) was applied to indicate general water quality. No interpretive results were given and the diatom results were not included in the EcoClassification process (Koekemoer and Taylor, 2007). The diatom results however correlated well with the physico-chemical results. This approach was also followed for the “*Reserve determination studies for selected surface water, groundwater, estuaries and wetlands in the Outeniqua catchment: Technical component – Knysna and Swartvlei*” during 2006 and 2007 (DWAF, 2008b *in press*) and the same approach was followed with results that correlated with physico-chemical results.

In the past, chlorophyll-a was the main algal response variable used in physico-chemical assessments. Based on the success of the inclusion of diatoms as a biological indicator for the State of the Rivers Report on the Crocodile West/Marico catchments (Taylor *et al.*, 2007b) and recent advances in the development of supporting methods and tools, current ecological Reserve studies across South Africa made it possible to apply the approach used in the Crocodile West/Marico RHP study and incorporate water quality diatom indices (specifically the SPI) in the EcoClassification process. The basis for the inclusion of diatoms into the EcoClassification process was to complement the physico-chemical driver component with additional information as a response variable. The Reserve studies included:

- The Comprehensive Reserve Determination study for the Integrated Vaal River System: Upper Vaal Water Management Area (WMA 8).
- The Comprehensive Reserve Determination study for the Inkomati River System (WMA 5).
- The Intermediate Reserve Determination study for the Mokolo River System (WMA 1).

Diatom sampling procedures for reserve studies were followed according to Taylor *et al.* (2007d). The preferred assemblage sampled was epilithon (diatoms that inhabit gravel, stone, rocks and bedrock). Where this substrate was unavailable epiphyton (diatoms attached to macrophytic plants) was sampled. Sampling was conducted concurrently with fish and macroinvertebrate sampling and additional sampling was done during physico-chemical sampling and/or hydraulic data collection where possible. The aim of multiple sampling efforts was to achieve high confidence results

that were representative of different discharges, and to provide a time-integrated indication of possibly unmeasured chemical conditions.

Enumeration of samples was done according to Taylor *et al.* (2007d). The nomenclature followed Krammer and Lange-Bertalot (1986-91). Other sources used to identify diatom species included Prygiel and Coste (2000); Sonneman *et al.* (2000) and Taylor *et al.* (2007c). The biological assessment of water quality for samples was carried out using the OMNIDIA program (version 3.1) (Lecointe *et al.*, 1993).

The scores for twelve water quality diatom indices were calculated using version 3.1 of the OMNIDIA programme (Lecointe *et al.*, 1993). The 12 diatom indices can be divided into four groups according to their objective:

- Saprobic indices: SLA LMI, WAT, DES and ROT.
- Trophic indices: SHE.
- Pollution indices: SPI, GDI, CEC, IDAP, and BDI.
- Indices combining organic pollution, eutrophication, and mineralization: EPI (Zgrundo and Bogaczewicz-Adamczak, 2004).

Although the use (efficacy) of the SPI has been tested in South Africa (De la Rey *et al.*, 2004 and Taylor, 2004), it is foremost a general pollution index, and therefore it was decided that the results of all twelve indices should be studied as the different indices provide information on different physico-chemical components and could identify specific water quality related problems that is not detected by the SPI index. These indices provided additional information with regards to interpretive results while the Ecological Category was based on the SPI index score.

As there is no literature available on the use of diatoms in Environmental flow studies and in particular the EcoClassification process, the approach used in the Reserve studies should be seen as a developmental stage with further refinement needed. The class limit values used in the Crocodile West/Marico RHP study was according to Eloranta and Soininen (2002). Literature reviews showed that the SPI index is the most widely used in Europe and water quality class boundaries has been set by several authors for rivers in Sweden, Finland Belgium and France. The class limits, set by Prygiel and Coste (2000), and applicable to low and mid-altitude areas in Western Europe is generally accepted and therefore was deemed appropriate for the Reserve studies (Table 1.2). Based on the SPI scores from the different sites this index was more representative of the water quality ranges found in the study areas

and the class limits were more appropriate for an EC description. After the first set of samples analysed the class limits set by Eloranta and Soininen (2002) were too high for the different water quality classes. The Prygiel and Coste (2000) class boundaries were adapted for the Reserve studies (Table 1.3) and applied during the interpretation of the results, specifically the water quality class categorization.

TABLE 1.2: Class limit boundaries for the SPI (Prygiel and Coste, 2000)

Index score	Class
$17 \leq \text{SPI} \leq 20$	High quality
$13 \leq \text{SPI} < 17$	Good quality
$9 \leq \text{SPI} < 13$	Moderate quality
$5 \leq \text{SPI} < 9$	Poor quality
$\text{SPI} < 5$	Bad quality

TABLE 1.3: Adjusted class limit boundaries for the SPI index applied in Reserve studies

SPI score	Class	Ecological Category
>17.3	HIGH QUALITY	A
16.8-17.2		A/B
13.3-16.7	GOOD QUALITY	B
12.9-13.2		B/C
9.2-12.8	MODERATE QUALITY	C
8.9-9.1		C/D
5.3-8.8	POOR QUALITY	D
4.8-5.2		D/E
< 4.8	BAD QUALITY	E

The EC derived from the above classification for diatoms at the different sites, showed a good correlation with the physico-chemical ECs for the same sites. Differences between the physico-chemical results and diatom results are mainly due to changes in the flooding regime (i.e. frequent disturbance due to floods and drying events). Physico-chemical results in the Reserve determinations are based on mainly data sets from water quality stations while the diatom results were based on

in situ sampling. As can be expected the water quality data was not continuous and the diatoms provided a time-integrated indication of possibly unmeasured chemical conditions. Diatom index scores were generally higher for sites that were sampled during high flows and resulted in a higher EC than the corresponding physico-chemical category. Higher index scores were generally due to rapid shifts in diatom community composition which is associated with high flows. Generally species that are pioneers and/or have an affinity for well oxygenated waters dominated these samples (e.g. *Achnanthes minutissimum* and *Cocconeis* spp.) and therefore influence the index score. In these cases emphasis was placed on the species that had lower dominance and the occurrence of key indicator species. The composition (diversity and densities) of the less dominant species still gave an indication of the pre-flooding conditions and allowed for the interpretation of the dilution service that the rivers provide and the rate of recovery of the system as diatom samples were also taken during low flows at most of the sites. Inferences regarding water quality changes could therefore be made based on high flow and low flow diatom sample assemblages.

The inclusion of diatoms in the EcoClassification process, and as an additional response variable to compliment the physico-chemical driver, provided valuable additional information, especially at sites where water quality data availability was poor or of low confidence. There are indications that diatoms are very sensitive to water quality related changes and that the community composition indicates a rapid response to these changes. Although index scores were generally higher at sites where flow was elevated (thereby diluting water quality problems), the community composition still indicated which water quality variables were problematic at lower flows. Overall the diatoms provided additional water quality information and in cases where water quality data were limiting, it provided good information on salinity input, toxicants and metal contamination. The quick reaction time of diatoms allows for the biological interpretation of water quality changes (biological water quality). Based on the diatom results in the current Reserve studies, diatom sampling and analysis is recommended for Intermediate and Comprehensive Reserve assessments (DWAF, 2008a *in press*).

1.7.5 The use of diversity indices re-visited

Archibald (1972) tested the usefulness of diversity indices based on diatom communities for reflecting water quality. He found that these type of indices were unsuitable as a very high dominance of a single species occurred in waters both of

very high and very low quality – thus Archibald dismissed these indices for use in water quality assessment in South Africa. However, diversity indices have never been directly compared to autecological indices in terms of their respective accuracy for indicating water quality. De le Rey (2008) revisited these indices (diversity and evenness) and came to the following conclusions:

- Although there is a relationship between diversity indices and water quality, that relationship is not linear.
- Species diversity tends to be higher in moderately impacted water.
- Due to the highly significant relationship of autecological diatom indices with water quality, these indices are deemed more relevant and reliable for use in river monitoring studies.

With this study, questions about the use of diversity indices to evaluate water quality based on diatom community composition have well and truly been laid to rest.

1.8 Diatoms as indicators of pollution in urban waterways

Only a brief summary will be provided here as this work has formed the subject of an in-depth study and has been extensively discussed in another section of this report. Diatoms are uniquely suited as indications of water quality in urban waterways. Canalised environments, in particular, provide little or no habitats or refugia for aquatic fauna and flora. The diatoms, because of their very small size, may colonise habitats unavailable to other organisms. For example the concrete base of a canal makes a perfect substratum for diatom growth; these biofilms can then in turn be removed and processed as a normal riverine diatom sample would be processed. Thus we are able to determine medium term water quality based on an in-stream organism, even when that stream is only a few millimetres deep. Another interesting finding reported on by Kriel (2008) is that diatom indices seem to provide a very good indication of heavy metal contamination in urban streams. In fact the diatom communities showed better relationships with heavy metal concentrations than e.g. nutrient concentrations, one would expect the opposite. In conclusion we may state that the diatoms are a most important water quality indicator in urban streams and that urban stream quality assessment is greatly strengthened by the use of diatom-based indices.

1.9 Diatoms as indicators of land use

Walsh & Wepener (2009) made an in-depth study of the relationship between diatom community composition and land use in urban and agriculturally stressed rivers on the highveld. Their results showed that the community structure for diatoms taken from sites with varying land uses reflected differences attributable to land use. Considering the reference site, which was comprised of diatom species that had preferences for clean, freshwater, diatom community structures were modified and showed specific variation due to identified agricultural and urban water quality impacts. The authors found that agriculture could be split into high- and low-intensity practices, where high-intensity agriculture was indicated by the presence of motile diatom species *Nitzschia*, and low-intensity agriculture was indicated by motile species of the genus *Navicula*. Urban sites contained a combination of species that were tolerant of spikes in water quality. Sites that were impacted by high intensity agriculture were in a 'poor/moderate' class overall, indicating that water quality impacts at these sites were more severe than at urban sites which showed an overall 'moderate' class for diatom community structure. The reference site was classed as 'good' in terms of water quality and diatom assemblage. Walsh & Wepener then went on to state that because diatoms remain in one place for a number of months, they show cumulative effects and an ecological 'recall' of the water quality over this period. This was evident in their study as different pollution types were associated with specific diatom species.

1.10 Community participation in diatom monitoring

Diatom-based techniques should be clearly divided into two stages – sample collection and sample analysis. The first stage, field work and collection of samples, is very rapid and makes use of the simplest equipment available to the householder (e.g. toothbrush, tub, knife, jars, etc.). Thus, it is possible for concerned members of the community to collect samples from rivers or streams of interest, archive and preserve these samples for later submission to a trained analyst. Taylor et al. (2009) clearly demonstrated the value of community participation in diatom monitoring studies. The community was alerted to a section of river which had steadily deteriorated (compared to base-line samples) while the analyst with an interest in diatom taxonomy was provided with unique and interesting diatom samples from streams with no public access. In this way far more territory in South Africa can be surveyed than if only scientists or technicians with an official affiliation collect samples.

1.11 Current trends in the use of diatom indices in Europe

Diatom-based autecological indices still form the backbone of periphyton monitoring in Europe. Indices such as the Specific Pollution sensitivity Index (SPI) and the Biological Diatom Index (BDI) are vital in the implementation of the European Water Framework Directive (e.g. Szilágyi *et al.*, 2008). The basis of the ecological quality estimation according to the WFD is the state of the five biological groups of the aquatic ecosystem (phytoplankton, periphyton (of which diatoms form a part), macrophyte vegetation, macroscopic invertebrates and fish fauna). Achievement of good ecological state or good potential could be assessed by the investigation of these biological groups while other quality elements are considered as providing supporting information (e.g. hydromorphological or physicochemical characteristics) in determining state and potential (WFD, 2000). However, in addition to the autecological indices, several other techniques are being investigated to infer water quality from diatom communities. These techniques are of particular importance in terms of setting reference conditions for rivers and streams and in turn determining the deviation from reference of a given community. The seminal study using these methods was carried out by Gevery *et al.* (2004) who used methods based on artificial neural networks (ANNs) and self organizing maps (SOM). While previous workers have used linear methods such as a correspondence analysis (CA) to determine the environmental affinities of given species ANNs are techniques that have been shown to work well with complex and non-linear ecological datasets (Lek *et al.*, 1995; Scardi, 1996; Recknagel *et al.*, 1997). ANN methods are based on transmission of information through connections similar to those that occur in the animal nervous system. The most common types of ANN used in ecology are supervised multilayer perceptron neural networks with a backpropagation learning algorithm (BPN) (e.g. Brosse *et al.*, 1999; Maier & Dandy, 2000) and unsupervised self-organising maps (SOM) (e.g. Brosse *et al.*, 2001; Michaelides *et al.*, 2001). SOM is usually used for ordination and classification (e.g. Chon *et al.*, 1996; Lek *et al.*, 1999). Gevery *et al.* 2004 concluded that the association of two ANN methods, SOM and BPN, gave satisfactory results for the prediction of diatom assemblages. The map they obtained by the SOM procedure distributed the diatom samples into 12 cells, with each SOM cell representing a specific diatom assemblage according to the environmental conditions of sampling sites assigned in each cell, other ordination techniques such as CA were shown to support this conjecture (Gevery *et al.*, 2004). Despite the initial success of this technique it has yet to be widely adopted by the monitoring community in Europe and other techniques such as the use of

evolutionary algorithms (EAs) to predict exact water quality parameters based on diatom species composition are being investigated.

1.12 Biotic indices in the USA

Although to date water quality monitoring techniques tested in South Africa using diatoms have, almost exclusively, adopted those methods used in Europe we must however mention the very different indices used in the USA. These are known as Indices of Biotic Integrity or IBI and were developed by Karr (1981). IBI, like conventional economic indexes such as the index of leading economic indicators, is a multimetric index that provides a convenient measure of the status of a complex system. Both economic and biological indicators require a baseline state against which future conditions are assessed. For IBI, that baseline – biological integrity – is the condition at a site with a biota that is the product of biogeographic processes in the relative absence of the effects of modern human activity. Multimetric indexes (an average or sum of more than one metric) like IBI integrate multiple biological indicators to measure and communicate biological condition. This robust measure of the biological dimensions of site condition has by now been applied to challenges in basic science, resource management, engineering, public policy, law, and community participation in developing as well as developed nations (Karr, 2006). Initial work with biological indicators concentrated on streams, using fish as focal organisms, but the conceptual underpinnings of IBI have now been applied to diverse environments (streams, large rivers, wetlands, lakes, coastal areas and others) and taxonomic groups (fishes, aquatic and terrestrial invertebrates, algae and diatoms, birds, and vascular plants) (Karr, 2006).

This type of index has been developed, based on diatoms, for streams in the USA. Metzmeier's diatom index of biotic integrity (Kentucky Division of Water, 1994) includes taxa richness, Shannon diversity (Shannon, 1948), the pollution tolerance index (Lange-Bertalot, 1979), and proportion of species sensitive to pollution. These metrics are then translated to a score ranging from 1 to 5, i.e. poor to excellent. The average score for the metrics is Metzmeier's Diatom Bioassessment Index. Hill (1997) developed an index of biotic integrity with ten metrics that successfully distinguished reference and impacted stream sites. Hill's index of periphyton integrity used metrics such as relative genera richness, ratio of diatom cells/all algal cells, live diatoms/dead diatoms, and metrics that decreased as biomass approached nuisance levels. Even though multimetric indices of biotic integrity only summarize the information that diatoms infer about environmental conditions in habitats, they are

valuable management tools. Comparison of indices for different trophic levels and geomorphology provides a spatially, temporally, and ecologically integrated assessment of ecosystem integrity in streams (Plafkin *et al.*, 1989; Hannaford & Resh, 1995). The integrated assessment of composite indices facilitates communication of environmental information to the public and facilitates comparative risk assessment (Stevenson & Pan, 1999).

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CHAPTER 2: THE DEVELOPMENT OF THE SOUTH AFRICAN DIATOM INDEX (SADI) FOR RIVERS AND STREAMS

Introduction

All in the water research community and the vast majority of the general public realize that South Africa is a water-scarce country. This water shortage is most striking when the country experiences droughts in various regions, as it does almost annually. In addition to natural climate variations and a semi-arid climate, population and economic growth has placed water resources under an almost untenable amount of pressure that will continue to increase in the economic hubs of the country.

Very few people would contend that South Africa's water resources are not to some degree polluted. Pollution to some degree is a cost of economic growth and development. South Africa relies to a large extent on mining, primary industry and agriculture to generate revenue. All of these activities have deleterious effects on the environment and particularly on water resources, as does the disposal of inadequately-treated wastewater to our surface waters.

It would be foolish to deny the importance of the above-mentioned activities to the country or to demonize them as despoilers of the environment. Economic growth is a necessity, similarly measuring the impact of mining, industry and agriculture on the environment is a necessity in order that mitigation measures can be appropriately formulated.

Many tools are available for those who wish to measure the impact of man's activities on water resources. The best option of all would be to measure a number of key water quality variables at very regular intervals (e.g. hourly). These data, when interpreted, would give the best indication of all of overall water quality. Why is this not done? Chemical water quality analysis is expensive. So why not reduce the sampling frequency in order to make it more economically viable? The answer lies in two aspects, viz. (1) The rapid changes that can take place in a water resource (minute to minute, hour to hour) and (2) Human activities (extraction, dumping, etc.).

In order to obtain an accurate overview of the condition of a river without the cost associated with high frequency chemical sampling, we need to turn to the biota within the river system. The reason for this is that riverine organisms, especially those that are only locally motile, are subject to the influence of pollutants. Pollutants in a

system with either outright kill an organism or, in most cases, affect a specific organism's viability (growth, reproduction rate, etc.). This will be entirely dependant on the organisms TOLERANCE to pollution. Pollutants, for the most part, will inhibit the reproduction and growth of SENSITIVE organisms, while allowing the growth and reproduction of TOLERANT organisms. Accordingly, inter-species competition and niche occupation dynamics are affected, with the result that community structure and composition will change with respect to dominant and sub-dominant species.

Changes in community composition are what the aquatic ecologist uses to infer pollution and to develop bioindicator protocols. Tolerances of different species, families or groups of organisms are resolved into either descriptive or numeric indices in order to provide an INDICATION of the impact of a pollutant, or group of pollutants, on aquatic ecosystem integrity.

Several organisms are currently in use as bioindicators. It is beyond the scope of this report to detail the merits and downfalls of each particular system; however brief mention will be made of the part diatoms have to play within the current suite of techniques.

What are diatoms?

Diatoms are microorganisms, primary producers that take up nutrients (nitrogen, phosphorous and silica), carbon dioxide and produce oxygen. Mostly less than one millimeter in length and on average around 30 micrometers these organisms are unique in that they have a cell wall composed of biogenic silica rather than cellulose as in other plant cells. The diatoms have many unique life forms, they can be free living as single cells (motile or attached) or often linked together in a colony, forming needle-like colonies or dichotomous branched colonies (Figure 2.1). One could view the biofilm found on most hard substrata in rivers and streams as similar to a forest, with undergrowth and a canopy of trees. Diatoms share the biofilm with other algae and bacteria and are predated upon by microorganisms (e.g. ciliates, amoeba) and by vertebrates (e.g. tadpoles). There are thus several processes happening within the biofilm, the principle ones being:

- Resource utilization (e.g. nutrients – finite resource)
- Interspecific competition (e.g. for light, nutrients, space, etc.)
- Predation

In a natural or undisturbed system these process will in turn be affected by the overall geology of the stream and by climatic conditions. These factors together will shape diatom community structure.

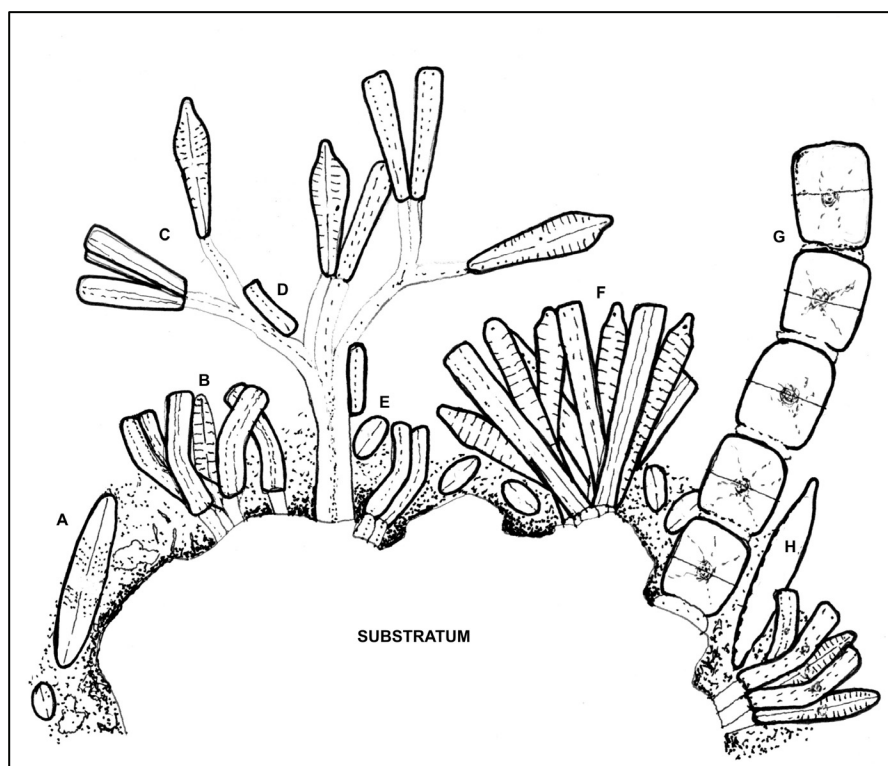


Figure 2.1: Stylized representation of a tiered biofilm on a rocky substratum. A: Free living, motile naviculoid diatom. B: Short stalked colonies of Achanathoid diatoms. C: Long stalked Gomphonemoid taxa. D: Adnate (appressed) Cocconemoid taxa. E: Small free-living, motile Naviculoid taxa. F: Apically attached Fragilarioid diatoms. G: Chain forming centric diatoms (*Melosira* sp.). H: Free living, motile Nitzschoid taxa.

If a system becomes impacted by a pollutant, the influence of climate and reigning base geology on diatom community composition will lessen, with the severity of the impact increasing until most, if not all, of the influence of these factors has been overridden by the pollutant(s). As stated before, the pollutant(s) will negatively impact the growth and reproduction of sensitive species, while favouring the growth of tolerant species. In addition life form may also change along with changes in substratum that are often associated with pollution (e.g. sedimentation), which will give motile non-attached diatoms a competitive advantage over stalked attached species (Figure 2.1).

Changes in diatom and other biotic communities have been documented over the last century (see Chapter 1). It is the knowledge of these changes in species composition that have allowed us to develop indicator systems, from the basis descriptive systems used by Cholnoky in the 1950s and 1960s to the autecological indices currently in use.

Diatom indices

Overview

The diatom indices used in this project have been reviewed elsewhere; however a brief description of the foundation and working of these indices will be given here. The type of index chosen for his study is known as an autecological index and incorporates known values for the tolerance and sensitivity of diatoms species weighted by their abundance in the community. This is currently the most common type of index utilized with the European Union. Tolerance and sensitivity have been predetermined and ascribed to diatom species included in the index; abundance is calculated by the analysis and is usually based on cell counts of 400 and above.

Tolerance and sensitivity values were ascribed to species used in European indices based on the following:

- Literature studies
- Empirical methods (statistical analysis such as CCA, PCA)
- Laboratory experiments (very rarely)

Why include these methods in the SA suite of indicators

Diatoms provide an integrated indication of water quality. Simply put, these organisms are directly-dependant on the chemistry of their immediate environment and are reliant on nutrients for their continued growth and reproduction. As attached or locally motile micro-organisms, they cannot simply migrate away from a threat to their well-being but will have their vitality directly influenced by that pollutant. Although they are affected to some degree by the structure of their habitat, habitat requirements for microorganisms are far less rigorous than those of, for example, invertebrates. Figure 2.2 gives a graphic representation of the role that diatoms have to play within a suite of bio-indicator tools.

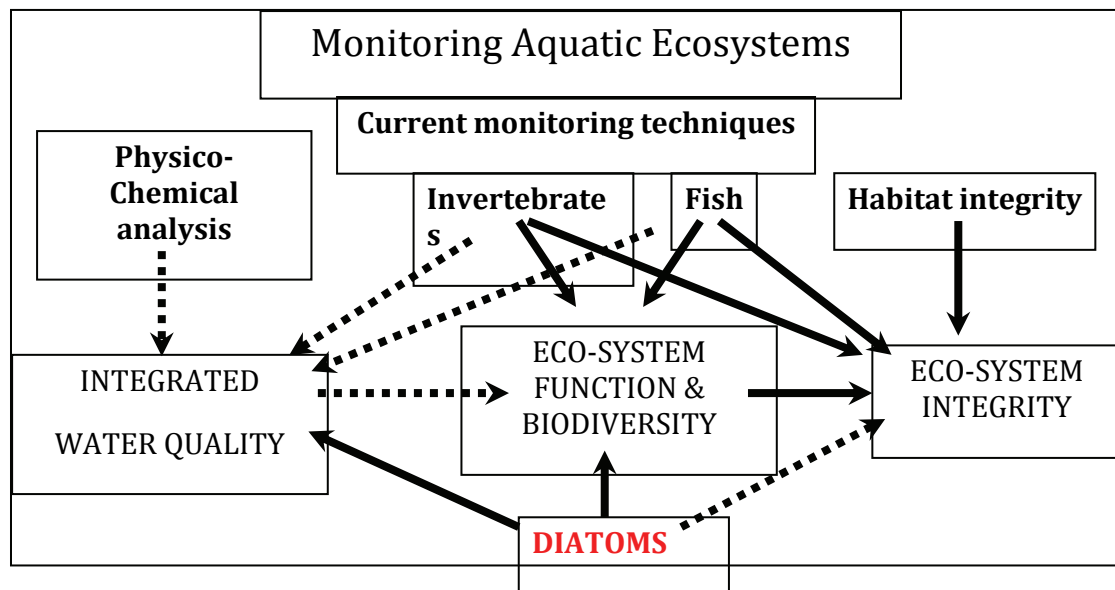


Figure 2.2: Schematic representation of the relationship between bio-indicator organisms/tools used to monitor the environment and what they indicate. Solid arrows represent a strong relationship while dotted arrows represent a weak relationship.

Why a unique SA index

Initial testing of diatom indices in South Africa showed that up to 98% of the species present in the sample were cosmopolitan taxa, this accounting for the success of European indices for monitoring SA waters. These initial studies were undertaken in river systems with, at best, moderate water quality impacts. Although tolerant endemic taxa were present, they were not abundant enough to influence index scores. However, in later catchment-wide studies, it was found that some sites, especially those near-natural mountain streams, were dominated by the SA endemic *Achnantheidium standeri* (Cholnoky) JC Taylor, E morales & Ector (Appendix 2), for which no tolerance of indicator values were included in the index. This resulted in index scores, based on the composition of these diatoms, being inherently flawed and pointed to the need for a modified index unique to SA.

SADI

The South African Diatom Index (SADI) is a modified version of the Specific Pollution sensitivity Index (SPI) developed in France. This index currently has the highest resolution of any diatom index, as it includes the highest number of taxa of any index. Indicator and tolerance values for SOUTH AFRICAN ENDEMIC species were added to the dataset already present in the SPI, yielding the SADI (Appendix1). It was

deemed more appropriate to modify an index than create a totally new one as many of the species occurring in South Africa occur in similar conditions in Europe (i.e. pollution tolerant cosmopolitan species).

The SPI is often perceived as a “complex” index as it contains more species than other indices. This is not the case as the index uses the same calculation as other indices and just has a larger species base. An index with 2000 species is no more complex than an index with 200 species. Hence, at this time there is no good reason for reducing the species base of the SPI/SADI as this will not reduce complexity but very well could reduce accuracy.

Compilation of the South African Diatom Index

The index has been formulated using data from 768 samples collected and analysed during this project. All of the data utilized are appended to this report in electronic format. The output, created as an upgrade or add-on to the OMNIDIA software package, is also provided electronically.

The manner in which this has been done is illustrated for a Case Study using *Achnantheidium standeri*, as described below:

Determining tolerance and indicator scores for SA endemics

Case study – *Achnantheidium standeri* (Cholnoky) JC Taylor, E Morlaes, Ector

Cholnoky, when describing this species, recorded a pH of 5.8 at the type locality and also noted that the rather species-poor diatom community in which *A. standeri* was found, was dominated by taxa requiring oxygen rich, fast flowing waters.

Canonical Correspondence Analysis (CCA) was used to create a bi-plot of species distribution vs. water quality variables. Figure 2.3 clearly corroborates Cholnoky's finding in that this species (ringed) is found in conditions with high oxygen and low nutrients and salinity. This has proved the case when analysis several data sets during this project and thus it becomes possible to assign both a high indicator and a high sensitivity value to this particular taxon. CCA was the most commonly used method during the project used to ascribe indicator values.

Alternative methods of assigning indicator and tolerance values

Unfortunately some of the diatom data sets were not accompanied by chemical data, either as such data was not available, not reliable or simply not submitted to the project team. Although chemical data is important for assigning these values, it is not vital. Instead we made use of Principal Component Analysis (PCA) to determine inter-specific relationships within diatom communities. In this way it has become possible to assign indicator values to species. For example if species X consistently ordinated next to *Nitzschia palea*, we will then know that species X occurs under similar conditions to *N. palea* and we are then able to assign appropriate indicator and tolerance values to this species.

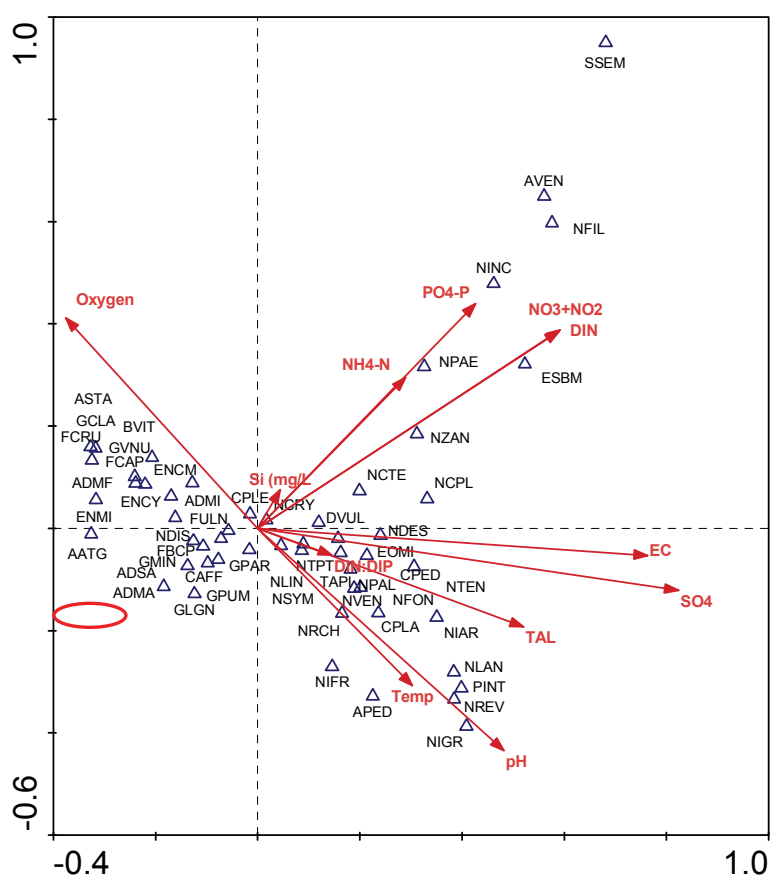


Figure 2.3: CCA biplot showing the relationship between dominant diatom species in the Crocodile and West Marico WMA. Circled code denotes *Achnanthes standeri*.

CHAPTER 3: THE LUCID-BASED TAXONOMIC KEY (VERSION 2.0)

The key produced during Phase 3 of the development of the South African Diatom Assessment Protocol (DAP), is the second version of this guide to be released. The first version was released on CD-ROM, whereas this version is released to a wider, local and international audience, via the Internet, hosted on the LucidCentral website at the University of Queensland, Australia. Stand-alone versions, having very modest system requirements, are available on request from the WRC.

Version 2 of the key contains 300 species, together with illustrative information for each in the form of photomicrographs captured using light or scanning electron microscopy.

The key has again been produced using the LUCID software tool that is not platform-specific (variants for Windows, Mac OSX and Linux are available free as downloads). The key is in a multi-access format, presenting hierarchical queries that do not require to be answered in a specific order. As more features are entered into the key, the number of possible solutions reduces to a final solution. This process also provides a powerful learning tool as the user becomes more familiar with the features associated with different species.

Use of the key is fundamentally self-explanatory. Features identified under the microscope are ticked in order to arrive a species solution. The entry of insufficient information will not yield a unique solution but will produce a choice of options which can then be matched against the unknown. The software contains a suite of options, for example the pruning of redundants, which can be used to reduce the list of possible options. Diagnostic options are also available to highlight features unique to certain species, i.e. highlighting unique diagnostic features.

Further details on how LUCID works may be obtained from the website <http://www.lucidcentral.org/Home/tabid/56/language/en-US/Default.aspx>

CHAPTER 4: THE USE OF DIATOMS FOR MONITORING WATER QUALITY IN URBAN STREAMS

Introduction

Urban canals and stream are often highly impacted and carry water from both point and non-point sources. The major contributor to the pollution of waterways in most developed and developing countries is the domestic component of sewage. The quality of stormwater is often compared with the quality of raw or treated sewage, and stormwater generally equals or exceeds sewage in terms of suspended solids, metals and/or biological oxygen demand. Stormwater may contain between 5 and 40 % of the levels of nutrients in secondary treated sewage. Leaking pipes or groundwater infiltration in sewerage systems causes stormwater pollution problems. Roads contribute mainly metals and sediments, and the higher the density of traffic, the greater the concentrations of metals such as cadmium, zinc, copper and lead (John, 2002).

Urban canals present unique challenges for the implementation of bio-monitoring. There is a limited range of habitats, in addition the water is usually very shallow and may become very warm in the summer months when solar radiation is absorbed by substances such as concrete which may be used to line waterways. Urban streams may also be subject to periodic flooding and scouring both from natural as well as unnatural drainage and may also be subject to cleaning by municipalities.

Benthic biota such as macroinvertebrates and algae can integrate the effects of multiple environmental stressors, such as those mentioned above, over time (Walker and Pan, 2006). The biggest stumbling block in the way of such monitoring in urban canals and streams is the lack of suitable habitats for aquatic organisms. However, when you turn to micro rather than macro organisms, the restrictions of habitat become less significant. Diatoms are small algae usually with an average size of ~30 μm and comprise about 40% of the total algal community (Round *et al.*, 1990). The diatoms are found in almost every aquatic and semi-aquatic habitat. Diatoms are distinguished by having a cell wall composed almost completely of silica.

This unique cell wall provides several advantages when using diatoms for bio-monitoring:

- The organisms are robust and not easily damaged during sampling.
- They are easy to preserve (20% Ethanol)

- Organic material is easily removed from inside the silica wall
- The distinctive patterning of the perforations of the wall makes species identification possible under the light microscope.

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

- they occur in all types of aquatic ecosystems,
- they collectively show a broad range of tolerance along a gradient of aquatic productivity, individual species have specific water chemistry requirements (Werner, 1977; Round, 1991),
- they have one of the shortest generation times of all biological indicators (Rott, 1991). They reproduce and respond rapidly to environmental change and provide early warnings of both pollution increases and habitat restoration success. It takes two to three weeks before changes are reflected to a measurable extent in the assemblage composition (Round, 1991; Kelly et al., 1998).
- they are sensitive to change in nutrient concentrations, (Pan et al., 1996). Each taxon has a specific optimum and tolerance for nutrients such as phosphate (Hall & Smoll, 1992; Reavie et al., 1995; Fritz et al., 1993; Bennion, 1994, Bennion et al., 1996) and nitrogen (Christie & Smol, 1993), which can usually be quantified to a high degree of certainty,
- assemblages are usually diverse and therefore contain considerable ecological information. For this reason, and because it is easy to obtain large numbers of individuals, robust statistical and multivariate procedures can be used to analyze assemblage data. (Dixit et al., 1992),
- they respond rapidly to eutrophication and recovery (e.g. Zeeb et al., 1994). Because diatoms are primarily photoautotrophic organisms, they are directly affected by changes in nutrient and light availability (Tilman et al., 1982),
- rapid immigration rates and the lack of physical dispersal barriers ensure there is little lag-time between perturbation and response (Vinebrooke, 1996),
- the taxonomy of diatoms is generally well documented (e.g. Krammer & Lange-Bertalot, 1986-91). Species identifications are largely based frustule morphology,
- diatoms can be found on substrata in streambeds even when dry, so they can be sampled at most times of the year (Stevenson & Pan, 1999),

Why add diatoms to a monitoring programme?

In addition to the advantages listed above these organisms also have few habitat restrictions (to diatoms a “river” could be a few mm deep and wide – Round, 2001) and provide a slightly different picture to that reflected by other organisms already in use in national monitoring programmes.

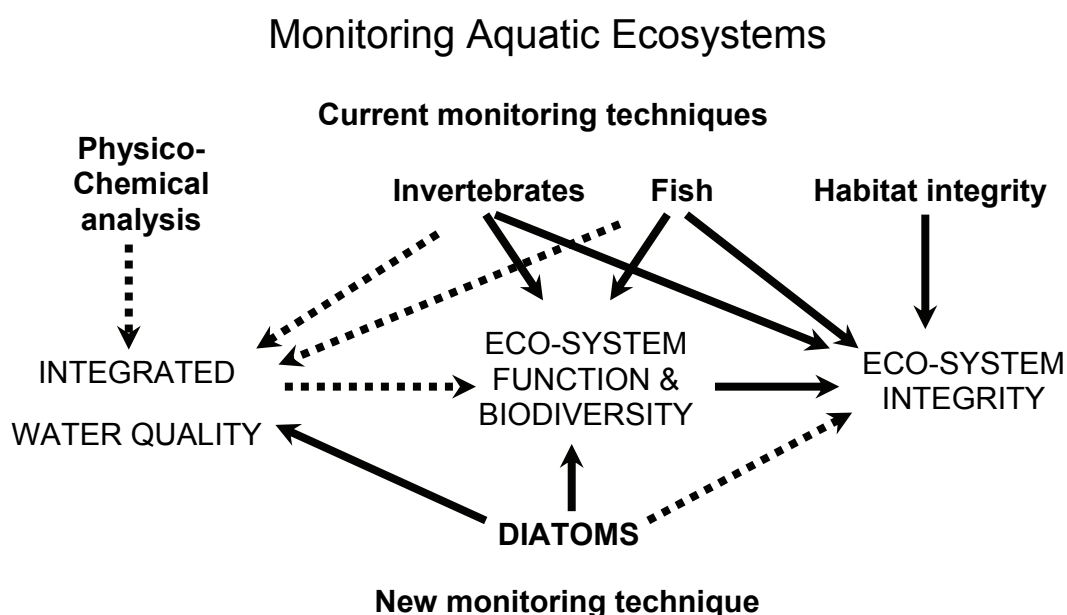


Figure 4.1: Schematic representation of the relationship between parameters used to monitor the environment and what they indicate. Solid arrows represent a strong relationship while dotted arrows represent a weak relationship.

Figure 4.1 provides a conceptualisation of the relationship between biological indicators and what they in fact may tell us about the environment. Larger organisms such as macro-invertebrates, because of habitat affinity, food requirements, reproductive cycles, etc. have a stronger relationship to the functioning and the ecological integrity of their direct environment and thus may be used as indicators of these parameters. On the other hand the diatoms (as micro-organisms and primary producers) are directly influenced in the main by chemical water quality. This is because diatoms need nutrients for growth and reproduction and their osmotic balance is influenced by salinity and pH. In addition, the deposition of the cell wall may be disturbed or interfered with when heavy metals are present in the water

resulting in the cells being deformed (see Appendix 1, Plate 3, Figures 8-24; Morin et al., 2008; Cattaneo et al., 2004).

Recently several studies have found diatoms to be useful for indicating general water quality in South African rivers and streams (de la Rey et al., 2004, Harding et al., 2005, Taylor et al., 2007a; 2007b) and guides to the methodology for collecting, studying and identifying these organisms have been produced (Taylor et al., 2005; Taylor et al 2007c;2007d). The present study is however, is one of the first to propose and test the use of diatoms for monitoring urban waterways in South Africa.

Diatom community structure and the relationships between these organisms and urban water quality have been the subject of various studies world-wide. Duong *et al.* (2006) studies the impacts of urban pollution on receiving rivers in Vietnam as well as testing the use of various diatom indices in these environments. They found close correlations between the Specific Pollution sensitivity Index and physico-chemical analysis of water quality variables and concluded that these indices were useful in studies of these types of hydrosystems. Interestingly, Newall and Walsh (2005) in a study of the response of diatom assemblages to urban influences in Australia found that European derived diatom indices were useful indicators of general water quality and they suggested that these indices may be transportable beyond their continent of derivation. Walker and Pan (2006) found that diatom distribution in urban streams was highly correlated with conductivity and dissolved nutrients and found that diatom assemblages were consistently different between urban and rural sites. They concluded that diatom assemblages can be used as a biotic indicator of urban stream conditions.

It will be shown in the results below, as in the above mentioned studies, that diatoms could be collected from most sites visited and that the index results accurately reflected impacts as well as degrees of impacts.

Methods summary

Sampling equipment is simple and is illustrated in Figure 4.2.

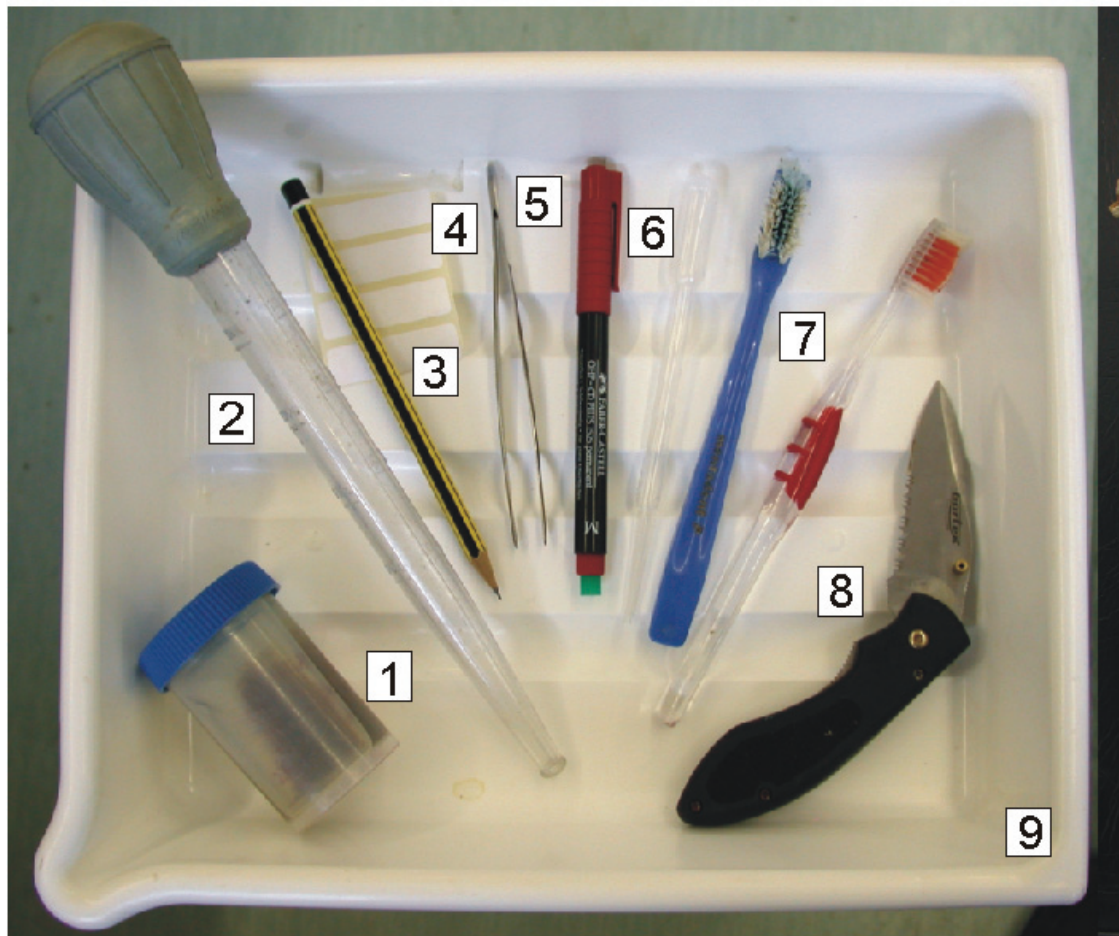


Figure 4.2: Field sampling equipment. 1. Sampling bottle 2. Large pipette 3. Pencil 4. Labels 5. Forceps 6. Waterproof marking pen 7. Old tooth brushes 8. Knife 9. Plastic dish.

There are in essence 3 basic sampling strategies

1. Scrubbing the diatoms from a portable substratum such as a rock or a reed stem.
2. Scraping the diatoms from the concrete bottom of the canal with a knife or spoon (the most used technique in this study).
3. Sucking up sediment dwelling diatoms with a large pipette or syringe.

Field work is rapid and simple, an advantage when the operator's safety may come into question. At least five sub-samples make up a single sample from a site (e.g.

five rocks are scrubbed, 5 discrete portions of canal bottom are scraped, etc.), the sample is collected into a plastic dish and then well mixed. The resulting suspension is stored in a sample bottle and preserved with ethanol.

On return to the laboratory the sample is left to stand in potassium permanganate for 24 hours to remove the organic material, it is then boiled in dilute hydrochloric acid to remove the permanganate and any calcium which may be present. After rinsing the acid from the sample with water, by centrifugation, slides may be made. Strewn mounts are made by drying diluted diatom suspension onto a glass cover-slip. Finally the cover-slip is mounted on a slide and is then ready to be examined under the microscope. For a detailed explanation of the methods please refer to Taylor et al. (2005) and Taylor et al. (2007c).

The index tested in the present study is known as the Specific Pollution sensitivity Index or SPI (Cemagref, 1982), calculated using the formula of Zelinka and Marvan (1961) using OMNIDIA 4.1. The maximum value of 5 (converted to 20 by the software package OMNIDIA; Lecointe et al., 1993) indicates pristine water.

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

Canonical Correspondence Analysis (CCA), using CANOCO version 4.5 (Ter Braak & Šmilauer, 1998), was used to determine the relationship between diatom species and measured environmental variables. Abbreviations used in the CCA diagram may be found in Appendix 2.

Results and Discussion – Urban streams in Gauteng

TABLE 4.1					
Index Score	Locality		Sampling date	Co-ordinates	Substratum
6.8	Lynnwood, Spruit	Waterkloof	23/05/2006	S 25,77750° E 28,28273°	Canal btm.
8					Vegetation
7.7					Rocks
6.9					Vegetation
6.7	Koedoespoort Spruit		23/05/2006	S 25,71531° E 28,27196°	Canal btm. below inflow
3.5					Canal btm. above inflow
2.9					Canal btm. at inflow
6.9	Walker Spruit		23/05/2006	S 25,76469° E 28,22156°	Sediment
11.2	Canal // to Walker Spruit				Canal. btm
7	Apies Wonderboompoort		23/05/2006	S 25,69348° E 28,18618°	Canal btm.
9.8	Mootspruit				Sediment/rocks
7.6	Mabopaan Highway		23/05/2006	S 25,70490° E 28,13989°	Reeds/vegetation
1.6	Drainage canal and Skinner Spruit		23/05/2006	S 25,74363° E 28,15695°	Canal btm.
5.9					Sand
4.2					Sediment, right bank
3.6					Rocks, right bank
11.1	Canal through Len Rutter Park, Florida		23/05/2006	S 26,15959° E 27,90628°	Canal btm.
1.2	Boksburg Lake, canal upstream of lake		24/05/2007	S 26,21775° E 28,25403°	Canal btm. and sediment
1.5					Canal btm. at gauging weir
1.4	Jukskei River, Bez. Park		24/05/2007	S 26,18341° E 28,09877°	Canal btm.
9.5	Observatory Spruit u/s Jukskei confluence		24/05/2007	S 26,18359° E 28,05794°	Rocks
8.3	Sand Spruit at Melrose Arch, next to M1		24/05/2007	S 26,13676° E 28,06779°	<i>Cladophora</i> sp.
8.2	Braamfontein Spruit		24/05/2007	S 26,17323° E 28,01673°	Seep at side of canal
8					Canal btm.

The results of the first part of the study are presented in Table 4.1. The index scores can be interpreted with the aid of Table 4.2. Some well known indicator species, encountered in this study, are illustrated in Appendix 1. The index used is known as the Specific Pollution sensitivity Index or SPI. This index has been tested at several other locations in South Africa and has been found to provide a good indication of water quality (Taylor et al 2007a, Taylor et al 2007b, de la Rey et al., 2004).

TABLE 4.2	
Interpretation of index scores	
Class	Index score SPI
high quality	>17
good quality	15-17
moderate quality	12-15
poor quality	9-12
bad quality	<9

All of the sites were either classified as having bad water quality (red) or as having poor water quality (orange). Some of the lowest diatom index scores ever recorded from South African rivers and streams were recorded in this study. These very bad quality sites (index score below 2 out of 20!) include a drainage canal into the Skinner Spruit, the Jukskei River at Bezuidenhout's Park, and the canal just upstream of the Boksburg Lake. Water with such a low quality (considering the volume of water carried by the Jukskei) will without doubt have an impact on any receiving water body and the biota that are found there.

A rather interesting site was the Walker Spruit in Pretoria. The Walker Spruit itself had a very low score (6.9 out of 20) and could thus be classified as having bad quality water. Running parallel to the Walker Spruit, and separated by only a few meters is another drainage canal with considerably better water quality (11.2 out of 20) and a completely different species composition. These two canals were thought to have carried water of a similar quality.

The only locality from where no meaningful diatom sample could be collected was the Spartan Drainage Canal. The canal bottom was covered with fine grey sediment. On examination of live material in the laboratory, the only cells visible were bacteria. After cleaning the material a few diatom cells (*Nitzschia palea*) were found, but not

enough for a meaningful count to be made. The absence of diatoms is an indicator that the water in this stream is highly toxic, as, even in the most concentrated industrial effluents, diatoms are often abundant. Apart from the presence of bacteria and cyanobacteria this stream can be considered as “sterile” and a great threat to any receiving water body.

Diatom index scores do not simply just give a water quality classification but they may also be used to indicate different degrees of impact within, for example, the class “bad water quality”. The water quality of the Sand Spruit and the Braamfontein Spruit is of bad quality, but bordering on poor quality while the quality of Jukskei River is of *extremely* bad quality.

Diatoms may also give an indication of heavy metal pollution. Heavy metals interfere with the deposition of the diatom’s silica cell wall and lead to the deformation and distortion of the cell shape (Morin et al., 2008; Cattaneo et al., 2004). Such distorted cells (*Fragilaria capucina*) were found in a side seep of the Braamfontein Spruit (for illustrations see Appendix 1).

Results and Discussion – Urban streams in the Cape Town metropolitan area

The following section is included to demonstrate the statistical relationship between both diatom index scores as well as the relationship between diatom community composition and environmental variables.

Diatom index scores generated for 15 sites in the Cape Town metropolitan area are presented in Table 4.3. The SPI scores may be interpreted with the aid of Table 4.2. In addition to the SPI the %Pollution Tolerant Valves (%PTV) is presented. This is a measure complimenting the Trophic Diatom Index of Kelly & Whitton, 1995, but is also a useful measure even when used alone. When the PTV rises above 20% it indicates that there is a significant influence of organic material at the site.

Table 4.4 shows some correlations between the SPI, the PTV and various environmental variables. Interestingly, the number of species found at each site also correlates to some degree with environmental variables. However, far stronger correlations are found between the SPI and environmental variables. The SPI shows strong negative correlations (as the variables increase, index score decreases) with all of the variables except COD. COD is a measure of the organic material in a sample, the decomposition of which will require oxygen. COD is positively correlated

to %PTV showing that as the COD increases so the number of pollution tolerant diatoms also increases.

TABLE 4.3				
Sample number	Population	No species	Specific Pollution sensitivity Index (SPI)	%Pollution Tolerant Valves (%PTV)
07-223	416	17	2.4	23.1
07-224	418	28	4.4	23.7
07-225	418	35	8.3	21.3
07-226	415	33	9.5	10.6
07-227	402	47	11.9	22.1
07-228	403	49	11.1	21.6
07-229	411	38	11.2	28
07-230	406	41	7.8	29.8
07-231	413	20	13.2	7
07-232	411	30	9.4	2.9
07-233	437	32	9.3	6.4
07-234	401	46	8.7	13
07-235	402	24	11.1	6
07-236	403	39	8.3	19.1
07-237	416	37	10.3	9.4

TABLE 4.4			
Pearson correlation matrix. Marked correlations are significant at $p < 0.10$. $n = 15$ (casewise deletion of missing data).			
Variable	No species	Specific Pollution sensitivity Index (SPI)	%Pollution Tolerant (%PTV) Valves
pH	-0.5024 p=.056	-0.6597 p=.007	-0.4052 p=.134
Conductivity	-0.4946 p=.061	-0.7909 p=.000	-0.0628 p=.824
Chemical oxygen demand	-0.2162 p=.439	-0.185 p=.509	0.486 p=.066
Total Oxidisable Nitrogen	-0.6286 p=.012	-0.6265 p=.012	0.036 p=.899
Soluble Ammonia	-0.3913 p=.149	-0.7818 p=.001	0.3561 p=.193
Soluble Nitrite + Nitrate	-0.3185 p=.247	-0.5665 p=.028	-0.1618 p=.565
Total Phosphorus	-0.4093 p=.130	-0.8144 p=.000	0.2765 p=.318
Soluble Reactive Phosphorus	-0.4557 p=.088	-0.8425 p=.000	0.2561 p=.357

The relationship between the diatom species encountered in the study and the measured environmental variables is shown in Figure 4.3. It is of the utmost importance, in a study of this nature, to demonstrate that, in addition to a high correlation to index scores, the diatom species themselves respond in the expected manner to changes in environmental variables. If species did not react in the predicted manner than the transfer of diatom indices across continents would not be valid.

Conclusions and Recommendations

Diatom samples could be collected from all of the randomly selected urban canals and streams with the exception of a single site. Diatom species encountered were similar to those found in the rivers and streams of South Africa and not species specific to canals were encountered. Diatom index scores are useful for classifying the water quality of urban streams and are also useful and accurate for classifying degrees of water quality impact in urban streams. Diatoms may also indicate heavy metal pollution when the cell walls become deformed. Diatom species reacted to changes in water quality variables in the expected manner, showing that indices can be used outside their continent of derivation. It is recommended that the diatoms, as indicators of water quality, be further investigated and studied with the aim of producing a robust bio-monitoring tool for urban canals and streams.

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CHAPTER 5: ADDITIONAL DATA RESOURCES/INFORMATION CONTAINED IN THE CHOLNOKY COLLECTION

During Phase I of this project (WRC TT242/04), there was an indication that valuable information and data of a non-diatom nature were included in the Collection. This inference was drawn from a brief initial examination of certain files and the fact that Dr BJ Cholnoky had a tendency to draw and record any species that he had not previously encountered. Additionally, primary records held in the form of original field notebooks and annotated maps similarly indicated that these sources might provide the basis for supporting the linkage of historical data to sites or river reaches, thus underpinning the use of historical diatom-derived ecological inferences to determine antecedent reference conditions.

At the time of this deliverable being included in this phase (Phase III) of this project, there was no indication that any attention would be paid, in the short to medium term, to the curation of the Collection. An offer by the North West University to curate and actively manage the Collection had been declined. At that time the only data extraction from the Collection, with the exception of the summary prepared during Phase I, was the preparation of a bibliography of South African published papers (the latter undertaken as part of Phase I).

Subject to the inception of this project, however, there have been some significant changes to the aforementioned scenario. A database project was funded by SANBI and which has supported the generation of a catalogue of the Collection's content (reference to website). Additionally, the Collection is now being transferred into the care of SAIAB and will, in all likelihood, be physically housed and curated at North West University, as per the original proposal made by this project during Phase I.

This deliverable (Deliverable 16 of Phase III) has undertaken a manual audit of the Analysis Sheets, Notebooks and Maps stored with the Collection (for full details of the contents of the Collection please refer to WRC TT242/04). It should be noted that other maps and sample records have been identified by the SANBI project from other resources within the Collection.

Analysis Sheets

The analysis sheets, covering the various data 'Series' (see WRC TT242/04) contain the original counts of diatoms made by Dr Cholnoky). These records contain numerous scaled sketches of diatom frustules and, in some instances, equally

detailed sketches of specimens from other algal Divisions (see **Appendix A**). The raw data analysis sheets are for the following systems:

- Pretoria Sewage Works
- Lake Chrissie
- Harmony Gold Mine
- Hennops River
- De Hoek
- Rayton Vlei
- Rustenburg area
- Tugela River
- Jukskei-Crocodile
- Waterburg / Nylstroom / Ellisras area
- Olifantsvlei
- Swartkops River
- Cape Province

Two factors were immediately apparent from this audit: Firstly, the amount of information other than for diatoms is relatively sparse; secondly, information pertaining to other algal groups is largely confined principally to two specific studies conducted by Dr Cholnoky – these being an analysis of the Pretoria Sewage Works and an examination of the limnology of Rayton Vlei (now Van Wyks Vlei) (see **Appendix A**). Other than this there are occasional notes and references entered with the analysis sheets.

On a regional basis the most (geographically) comprehensive set of Analysis Sheets exists for the Cape Province. This spans some 420 samples between Lamberts Bay and the Grahamstown / Peddie area. Most of the sample sites should be readily locatable via their descriptions annotated with or on the Analysis Sheets.

Within-region detailed sets exist for the Waterberg area, the Jukskei- Crocodile system and for the Swartkops River. Sample site maps are available for the Jukskei-Crocodile, Rayton Vlei and the Berg River.

Field notebooks

Original field notebooks (see examples in **Appendix B**), containing abbreviated information, regarding where samples were taken plus, in certain cases, primary field data such as weather conditions, water temperature and pH, exist for the following systems:

- Rietvlei Dam (these data for were a limnocorral study)
- Orange Free State / Lesotho (this work comprehensively published by Archibald)
- Jukskei – Crocodile
- Diatom series E, G, H and K
- Cholnoky notebooks (in German / Hungarian)

The use of maps or diagrams in the notebooks is almost non-existent (see following). In some cases the descriptions are sufficient to reasonably locate the original sampling location. While Cholnoky provided notes on almost every site, the use of this information will require deciphering his handwriting and translating into English.

Maps

The map series rendered very little information (see **Appendix C**). There are some annotations (markings) on some map sheets but these were only found to relate to sample sites for the Berg River system in the Western Cape and for the Berg River in the Western Cape and the Swartkops River near Port Elizabeth.

Other resources

The Collection is supported by a large and impressive library of texts pertaining to diatomology. The true value of this material, mostly all in German or other languages, is of value only for purist taxonomists. However, there are some materials, such as the rare and hugely illustrative set of Helmcke-Krieger SEM photographs (see **Appendix D**) describing diatom ultrastructure and features, which constitute invaluable teaching aids. Once the Collection is ultimately housed at the North West University, resources such as this may be deployed for the training of aspirant diatomologists.

CHOLNOKY'S "ECOLOGY OF THE DIATOMS"

During 1969 Dr Cholnoky published (in German) a book entitled "The Ecology of the Diatoms". This work is based exclusively on his many South African and southern African field expeditions. From this audit it was apparent that the bulk of the data and information contained within the paper records in the Collection are summarized in this publication. As such, The Ecology of the Diatoms constitutes the single most important resource of South African diatom-based data and information that exists today. The book not only contains the diatom information but also the descriptions of the systems examined and the ecological inferences that Cholnoky drew from his observations. Attempts to have this book translated into English and re-published have failed to attract funding from a sponsor. While the original publishers have agreed to republish and remarket the work, they are not prepared to fund the costs of translating and the necessary taxonomic updating of species that is required.

VALUE OF THE DATA FOR COMPARATIVE STUDIES

The information contained in the Collection provides the basis for comparative historical vs. present day assessments. Reworking of the historical data, using current methods and interpretive tools such as OMNIDIA, would support the determination of Reference Conditions for the systems and regions covered by the Collection. They also provide a valuable basis for new students to conduct then and now analyses of any of the systems (e.g. the Swartkops River, Lake Chrissie or Rayton Vlei). Sight should not be lost of the value of such comparative analyses for providing general indications of the degree of change that has occurred as a result of anthropogenic development during the 50-60 years since the first studies were undertaken by Dr Cholnoky.

SUMMARY

Contrary to initial expectations, the paper records housed in the Cholnoky Diatom Collection contained very little information pertaining to algal types other than diatoms. Similarly, details of sampling sites, either annotated on maps or in field notebooks, were minimal and are confined to specific studies conducted by Dr Cholnoky. Primary field data were also extremely limited.

Detailed data, supported by maps and notes, were found to be extremely sparse and limited to a few specific investigative studies. In this regard the results were disappointing.

The K-series of diatom records, collected from the Cape Province, is the most comprehensive and supported by reasonably clear descriptions of where the samples were collected. This series encompasses more than 400 sample locations.

Most of the paper record information attributed to Cholnoky appears to be summarized in his 1968 text entitled 'The Ecology of the Diatoms' (in German). Most of the work post 1970, undertaken by Archibald and Schoeman, has similarly been summarized and reported in various detailed publications. The Cholnoky work, currently out of print, is considered to constitute the single most valuable summary of the bulk of the Diatom Collection (pre- 1970). Translation and re-publication of this work would provide an extremely valuable resource of information describing many South African aquatic ecosystems between the mid-1950s and 1970.

APPENDIX A: ALGAL INFORMATION

Rayton vlei - 4.6.1952		III.	Klein in den Finstappen Viel spärlich (Finstappen) abtötende Trümpel bleibend, sonnig, die Kugelung abgebrannt. 8.5 (1 1/2 - 1) ^{nicht 1 1/2 mm! erst jetzt!} hervor aus dem Quell!
D3			
1. <u>Lyngbya</u> ✓	a <u>Microcystis</u> <u>Microcystis</u> <u>Kongressus</u> beide <u>Kongressus</u> <u>et al.</u> (jetzt nicht!)	—	<u>Seraplova</u>
2. <u>Coelastrum</u> <u>cyathia</u> <u>De Not.</u> ✓	a 70 p 10 Pistonside vado, da a <u>diagnostisch</u> nicht abge. — <u>Seraplova</u> !		
3. <u>Coelastrum</u> <u>pyramidalium</u> <u>Boib.</u> ✓ u. var. <u>gigantium</u>	a 129 p 72.5 : 25 <u>Seraplova</u>		
4. <u>Peridinium</u> <u>libellula</u> (<u>Foote</u>) <u>Ward</u> ✓ var. <u>intermedium</u> <u>W. & G. Ward</u>	a 172.5 p 33		
5. <u>Dermidium</u> <u>schwanzi</u> <u>Dy.</u> ✓	8 26.5 x 20 x 10		
6. <u>Peridinium</u> <u>crispum</u> (<u>De Not.</u>) <u>Witt</u> ✓	a 26.5 p 14 x 11 a <u>piläum</u> a <u>montel</u> <u>filii</u> <u>latarini</u> ammod! — <u>Seraplova</u>		
7. <u>Peridinium</u> <u>cucurbitarium</u> , <u>Boib.</u> ✓	a 75 p 32.5		
8. <u>Desiderata</u> <u>dentoides</u> <u>Boib.</u> var. <u>angulosa</u> (<u>Kautsky</u>) <u>W. & G. Ward</u> u. f. <u>angusta</u> ✓	a 228 p 150 x 47 <u>Lodras</u> <u>Kautsky</u> , nicht <u>Ward</u> <u>diagnostisch</u> , <u>epithel</u> <u>atralis</u> a <u>keim</u> <u>algar</u> <u>mag</u> <u>feld</u> .		
9. <u>Peridinium</u> <u>navicula</u> <u>Boib.</u> var. <u>crispum</u>	a 38 p 15		

Figure A1: Example of an analysis sheet with non-diatom algal records (Rayton Vlei)

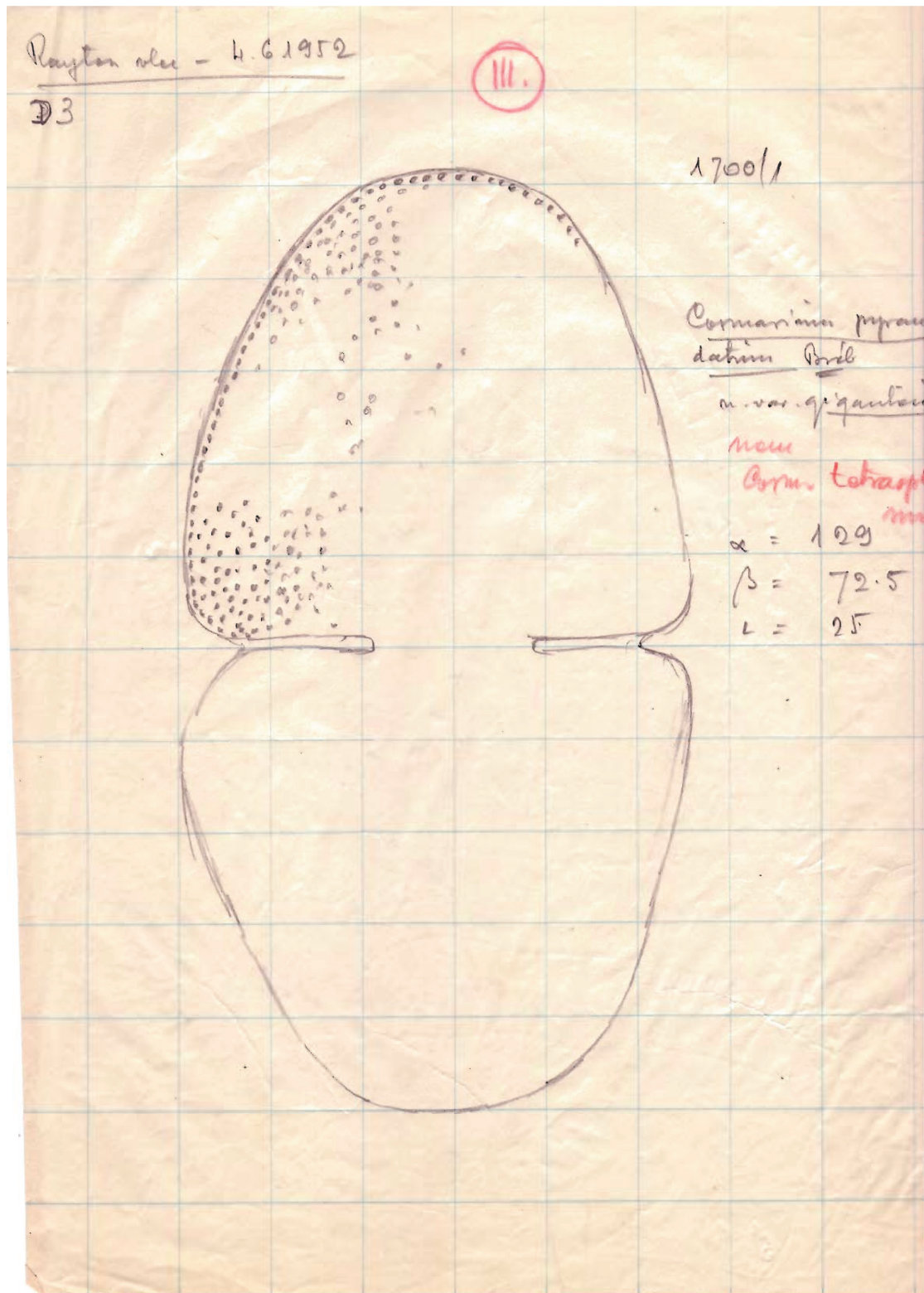


Figure A2: Sketch and dimensions of *Cosmarium* sp. collected from Rayton Vlei.

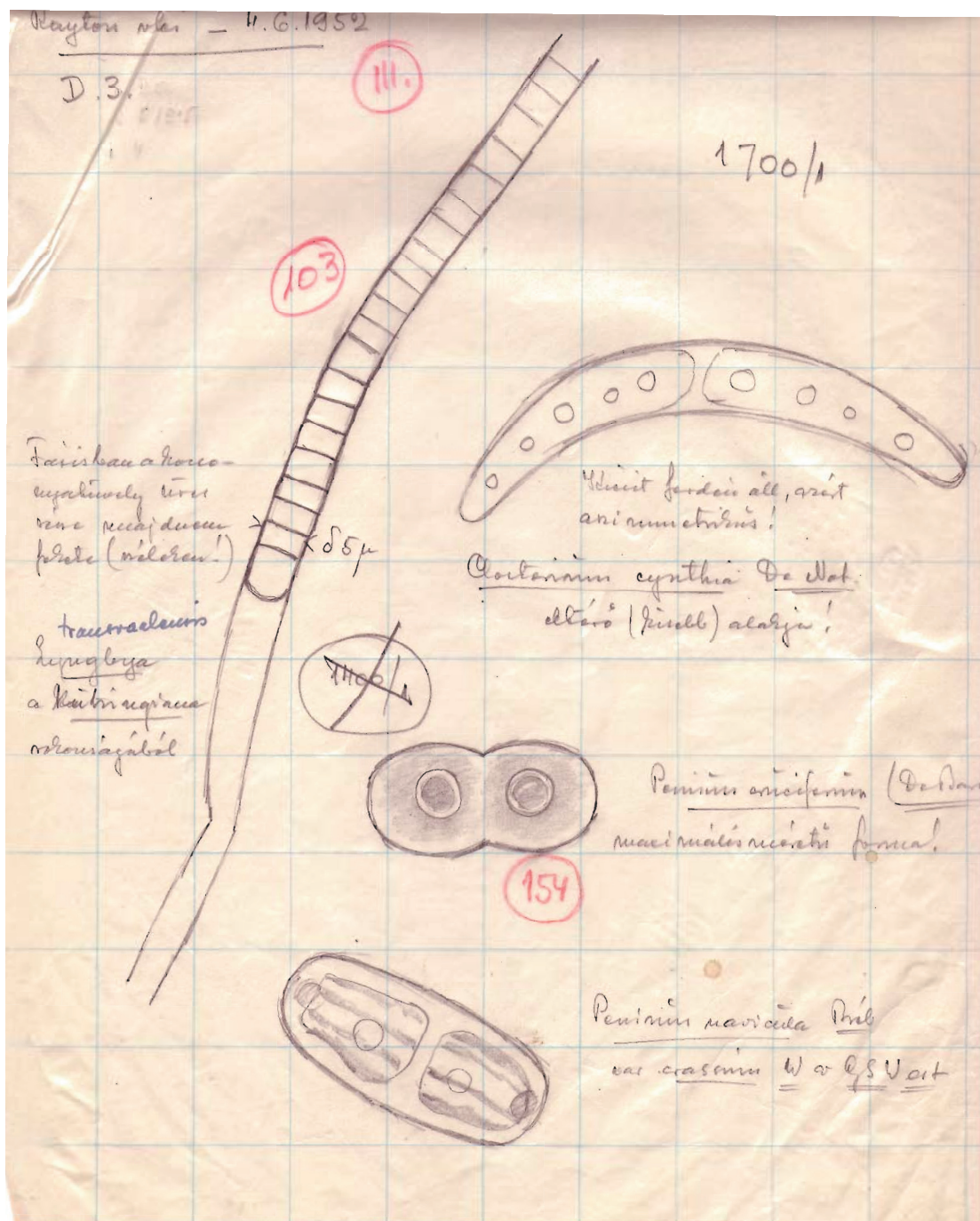


Figure A3: Sketch of *Lyngbya*, *Closterium* and *Perinium* sp. from Rayton Vlei.

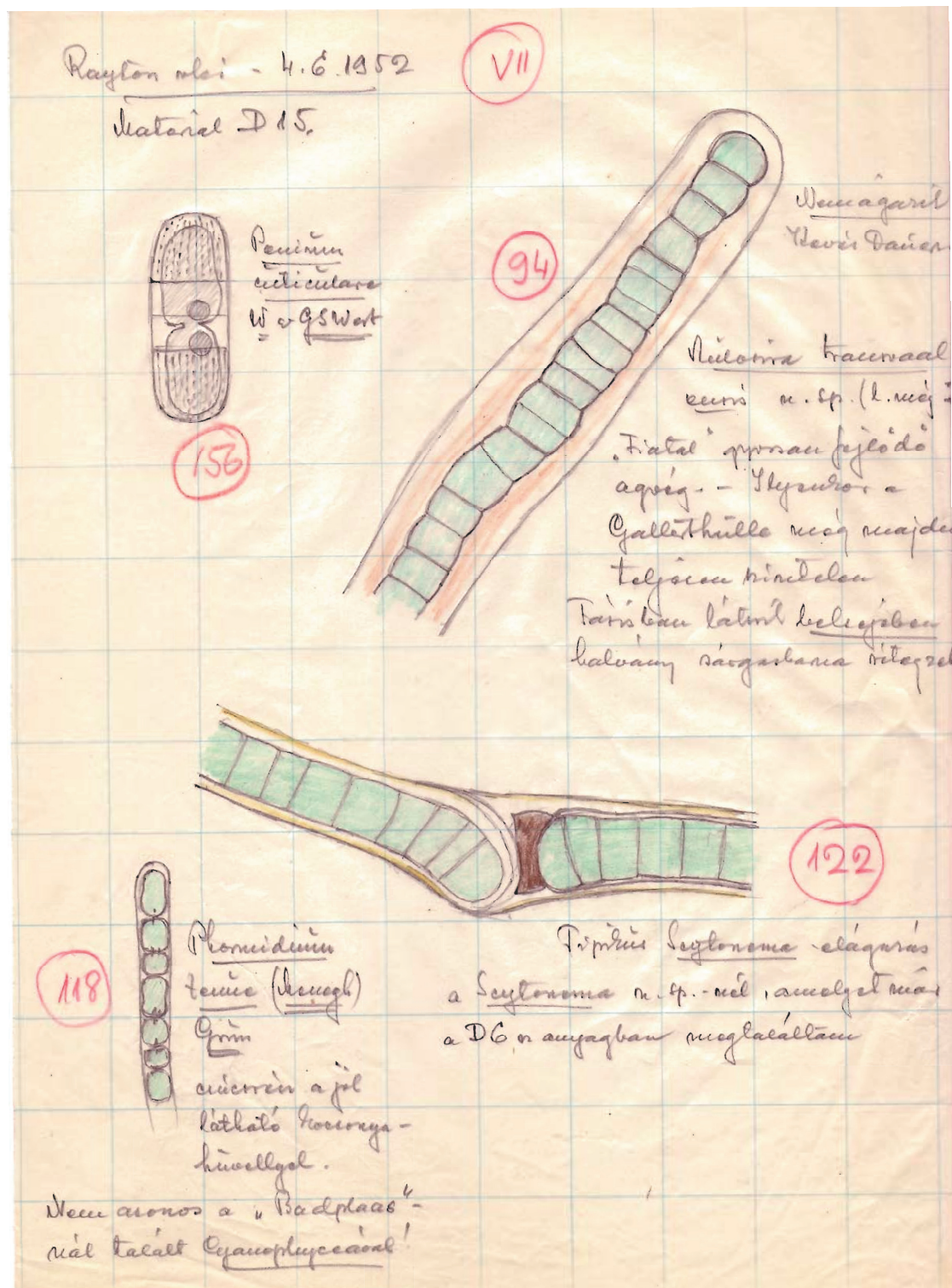


Figure A4: Sketch of Scytonema and Phormidium observed in Rayton Vlei.

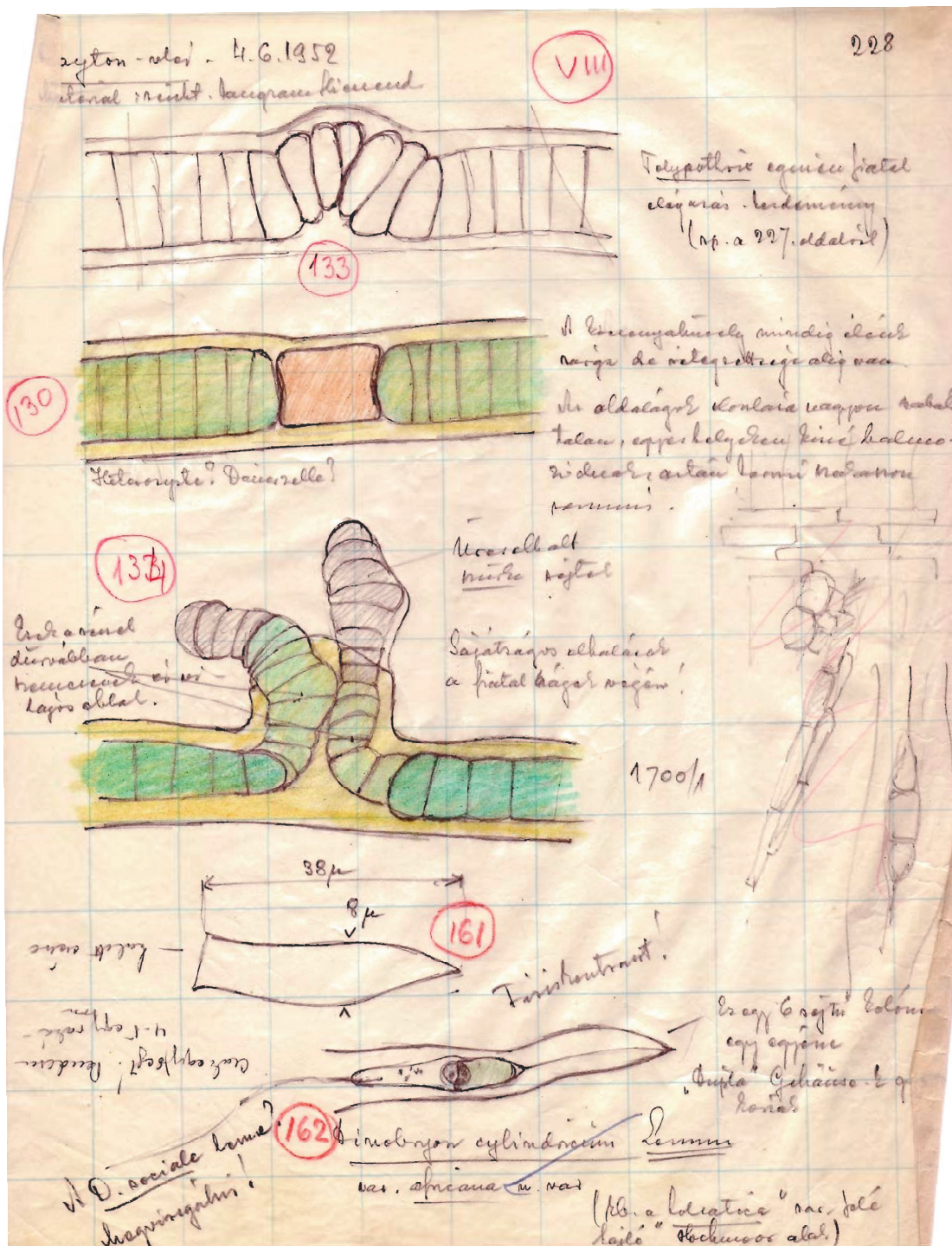


Figure A5: Detailed sketches of *Tolypothrix* and *Dinobryon* from Rayton Vlei.

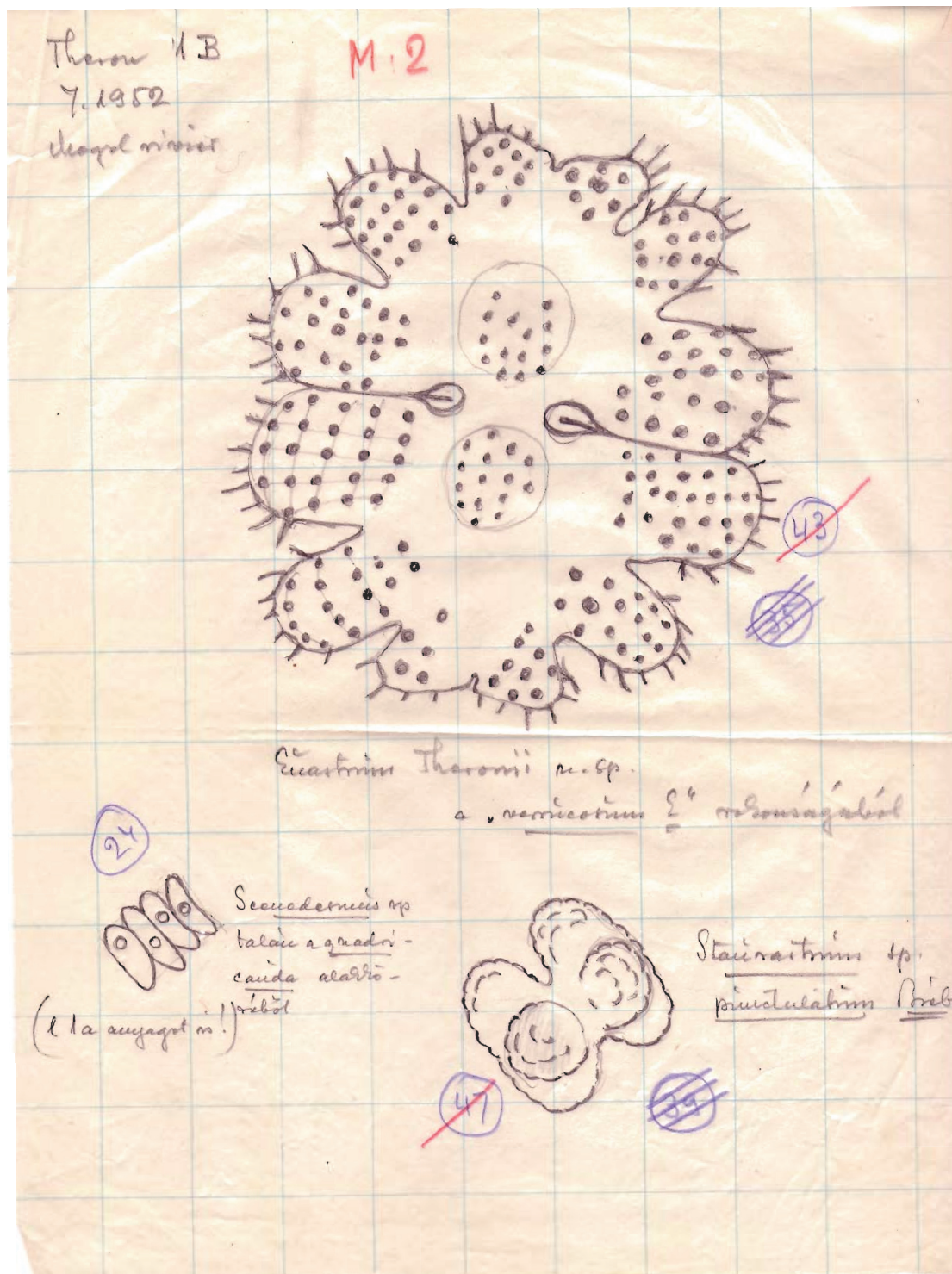


Figure A6: Sketch of *Euastrum theronii* collected from river near Ellisras.

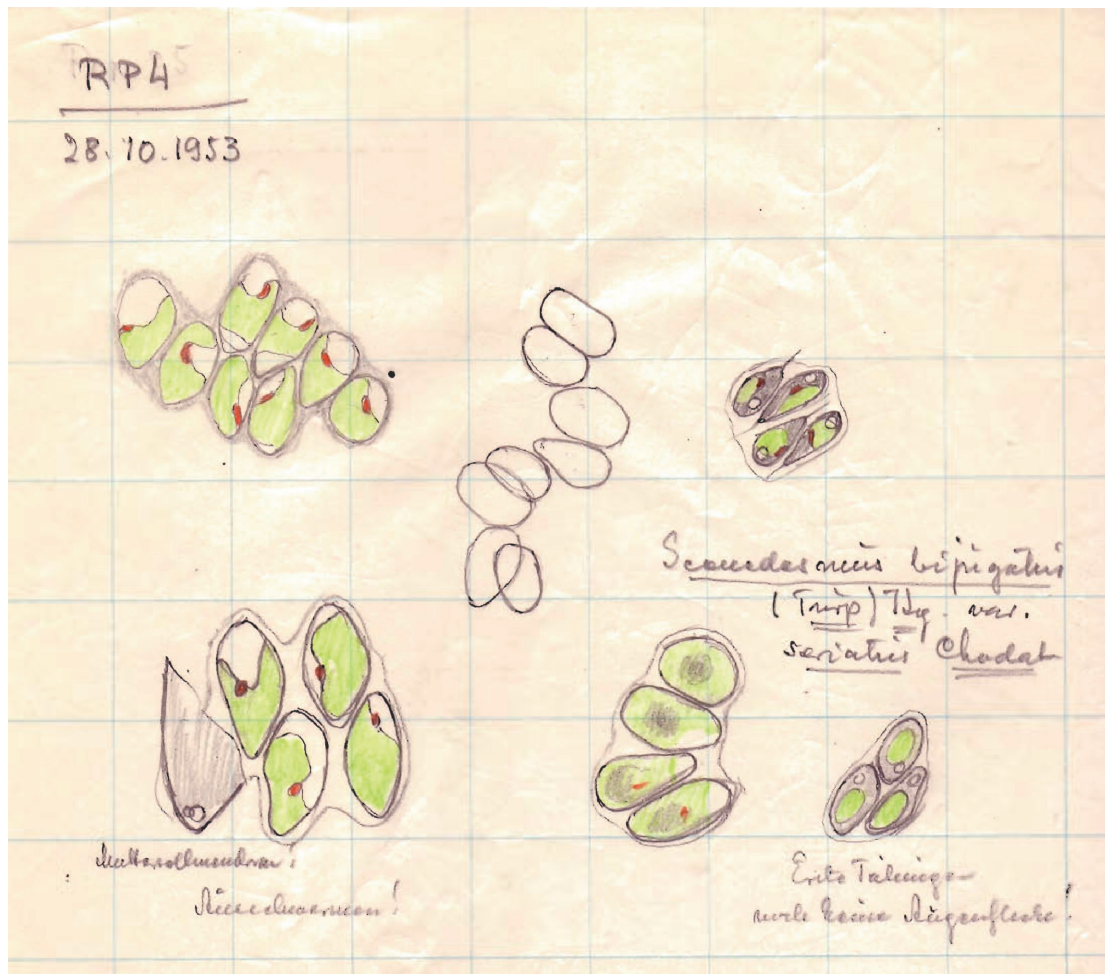


Figure A7: Sketch of *Scenedesmus bijugatus* collected from Pretoria Sewage Works.

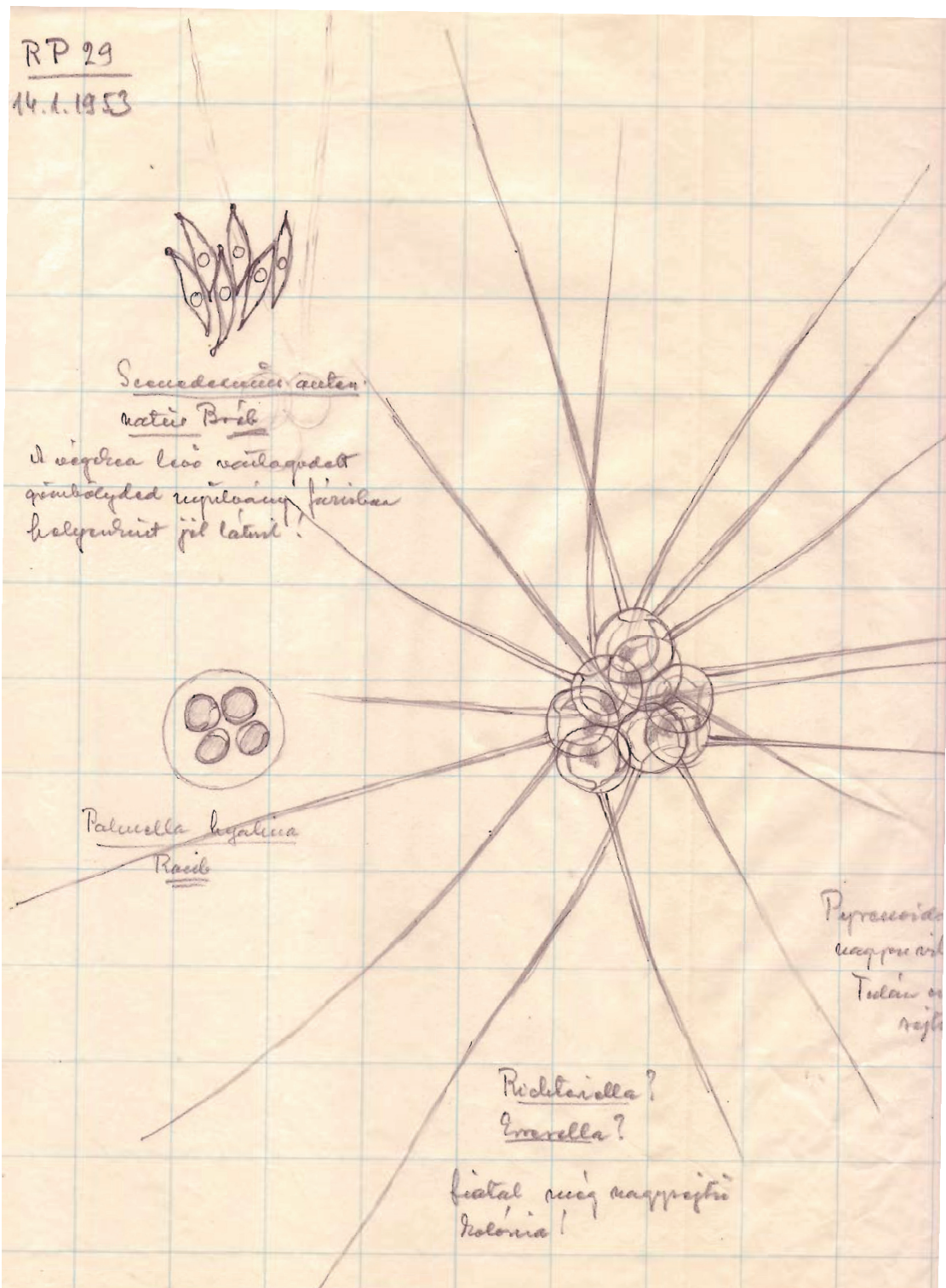
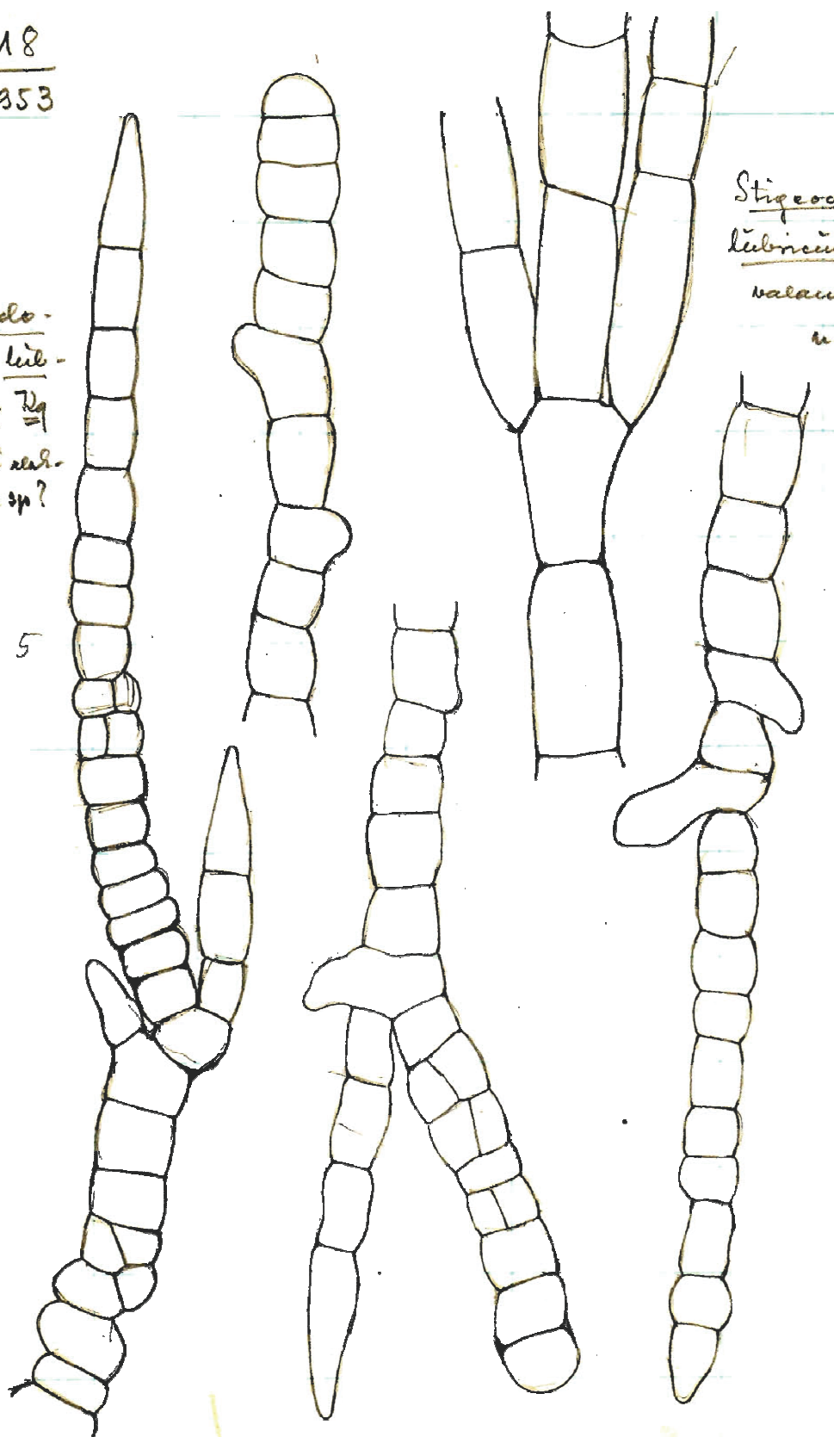


Figure A8: Sketch of unconfirmed sp. of *Richterella* collected from Pretoria Sewage Works.

RP 18

2.12.1953

Stigeodo-
nium leib-
nium Dg
valan val-
pa? n sp?



Stigeoc
leibnizii
valan
n

Figure A9: Detail of *Stigeoclonium* filaments observed at the Pretoria Sewage Works.

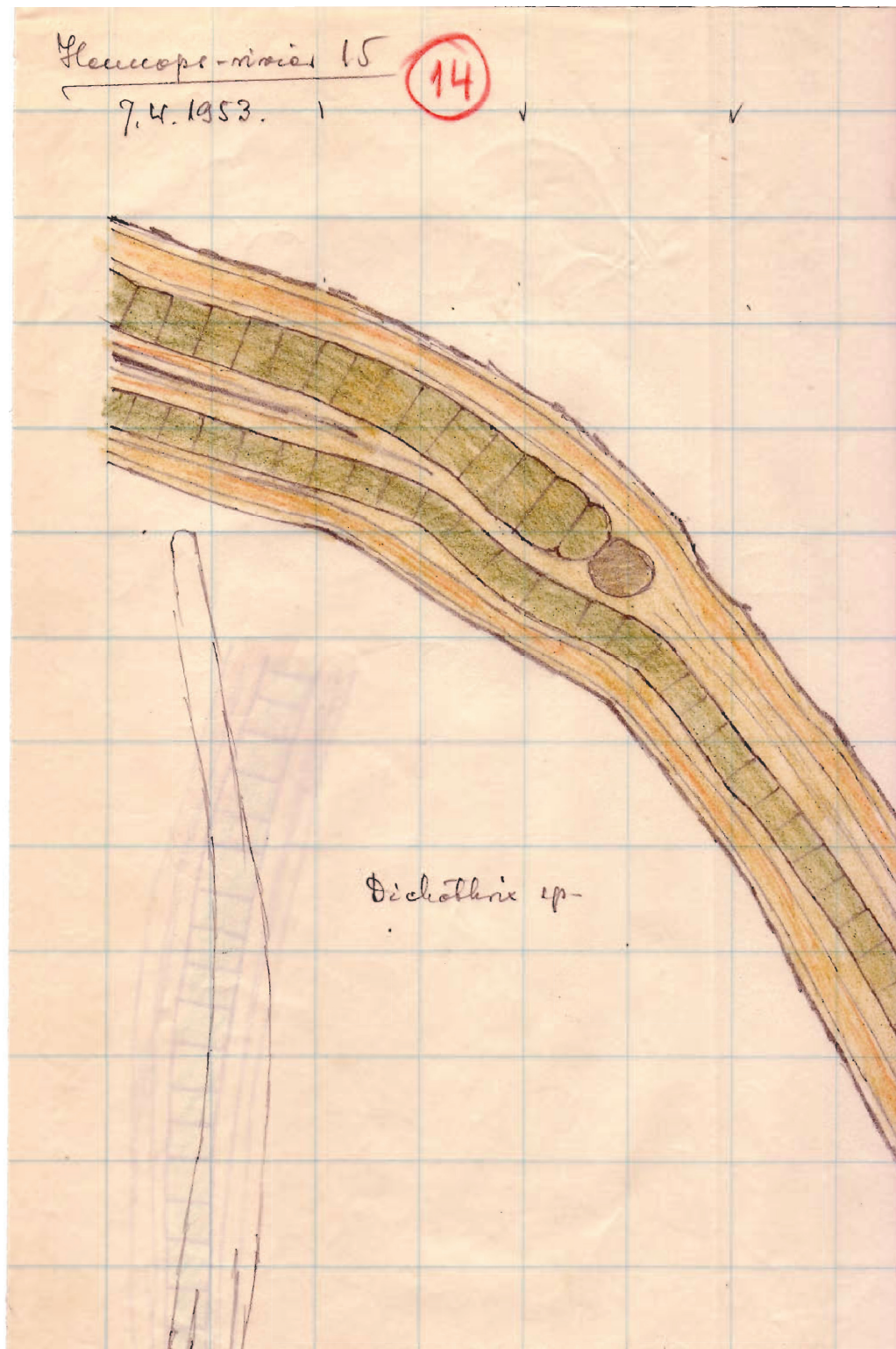


Figure A10: Sketch of *Dichlothrix* filament collected from the Hennops River.

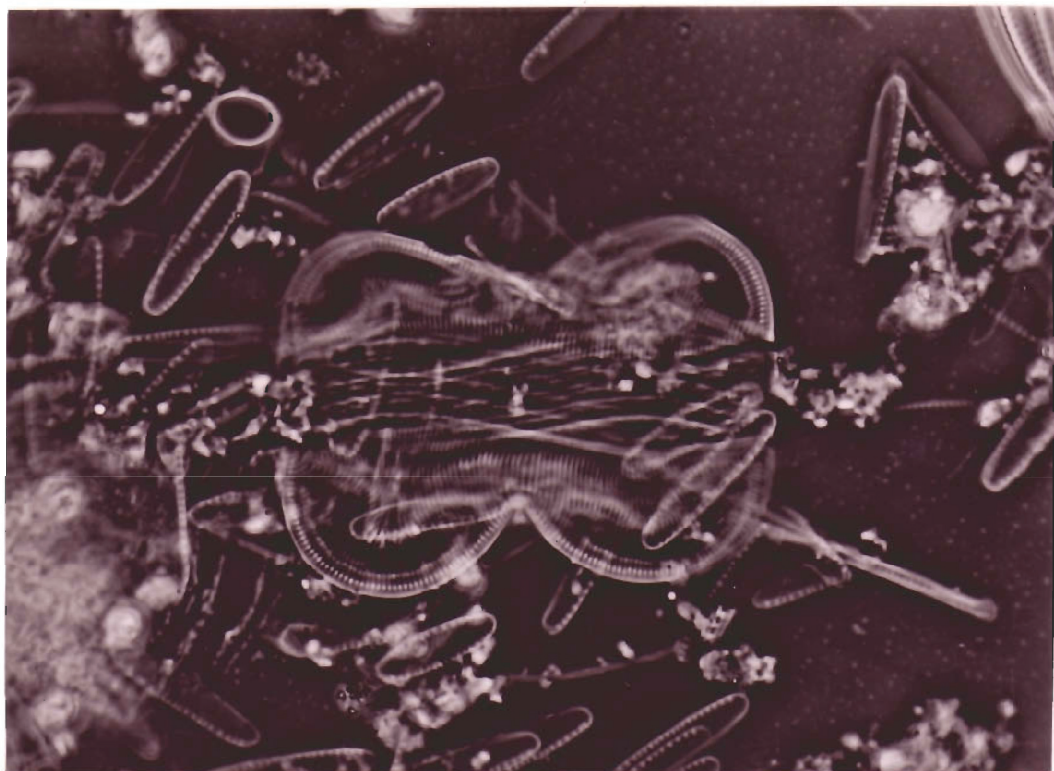
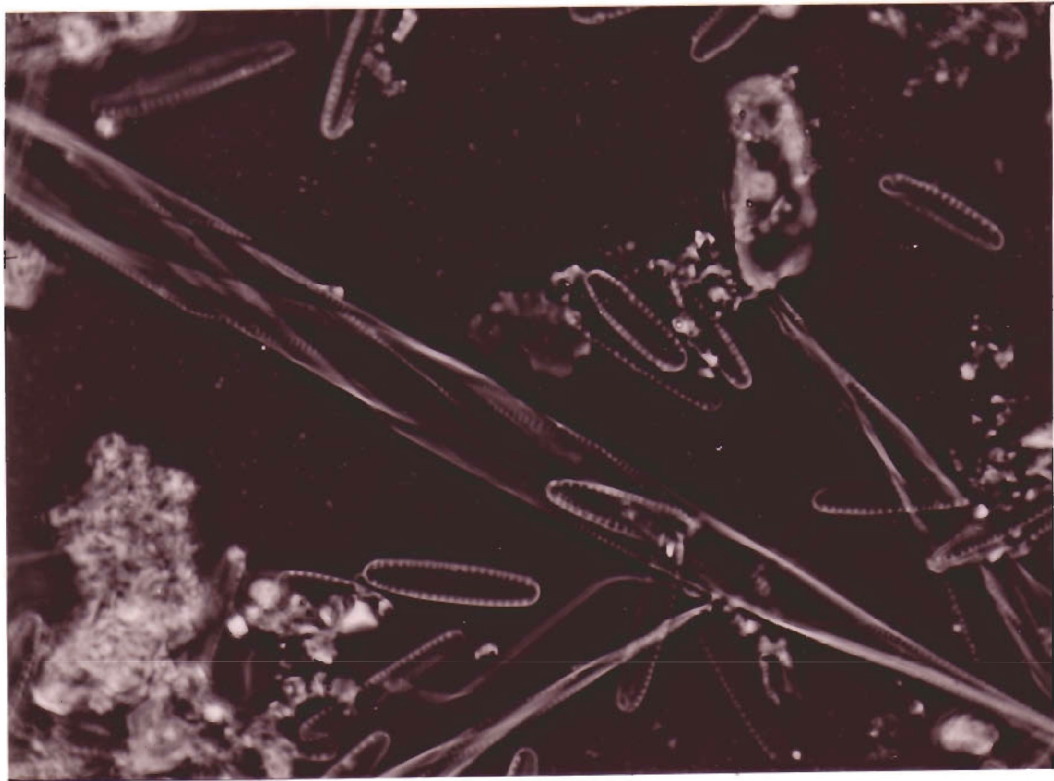


Figure A11: Photomicrographs of *Cylindrothecae gracilis* (top) and *Amphiprora paludea* (bottom) collected from Harmony Gold Mine.

APPENDIX B: NOTEBOOKS

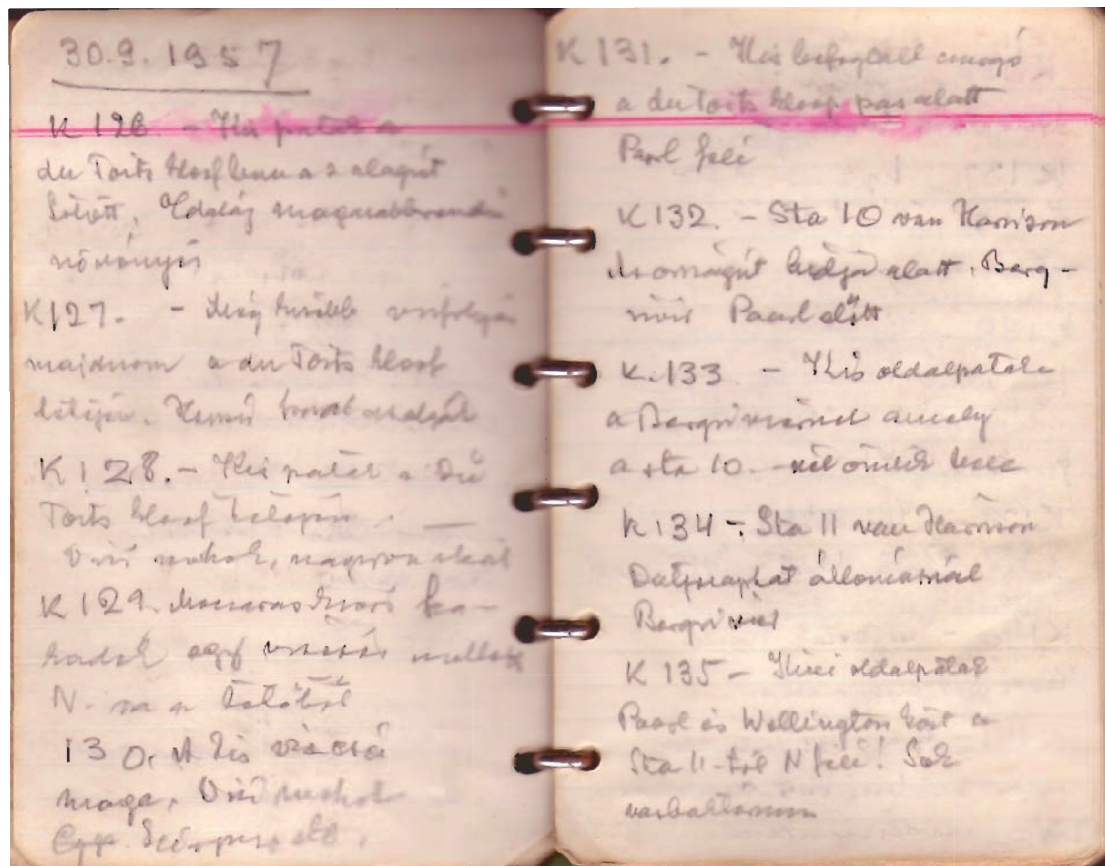


Figure B1: Example of Cholnoky field notes for some Western Cape sites.

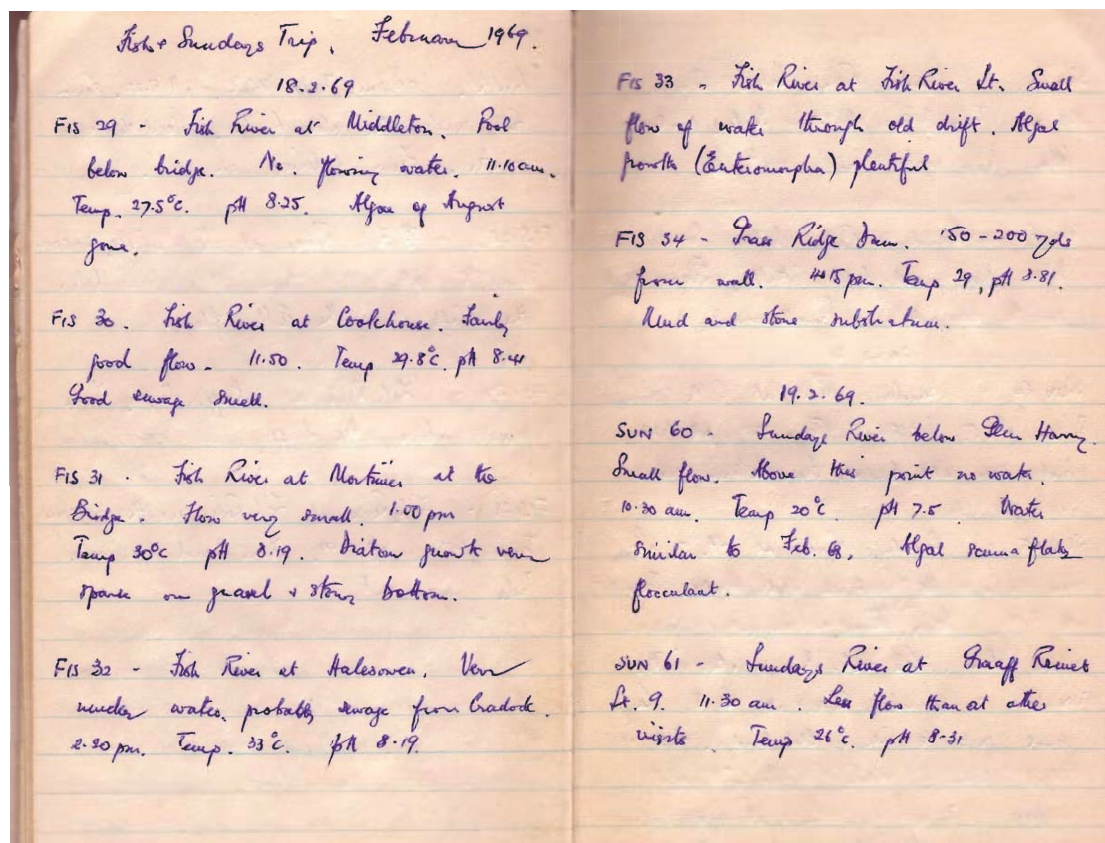


Figure B2: Example of Archibald field notes for the Sundays and Fish Rivers.

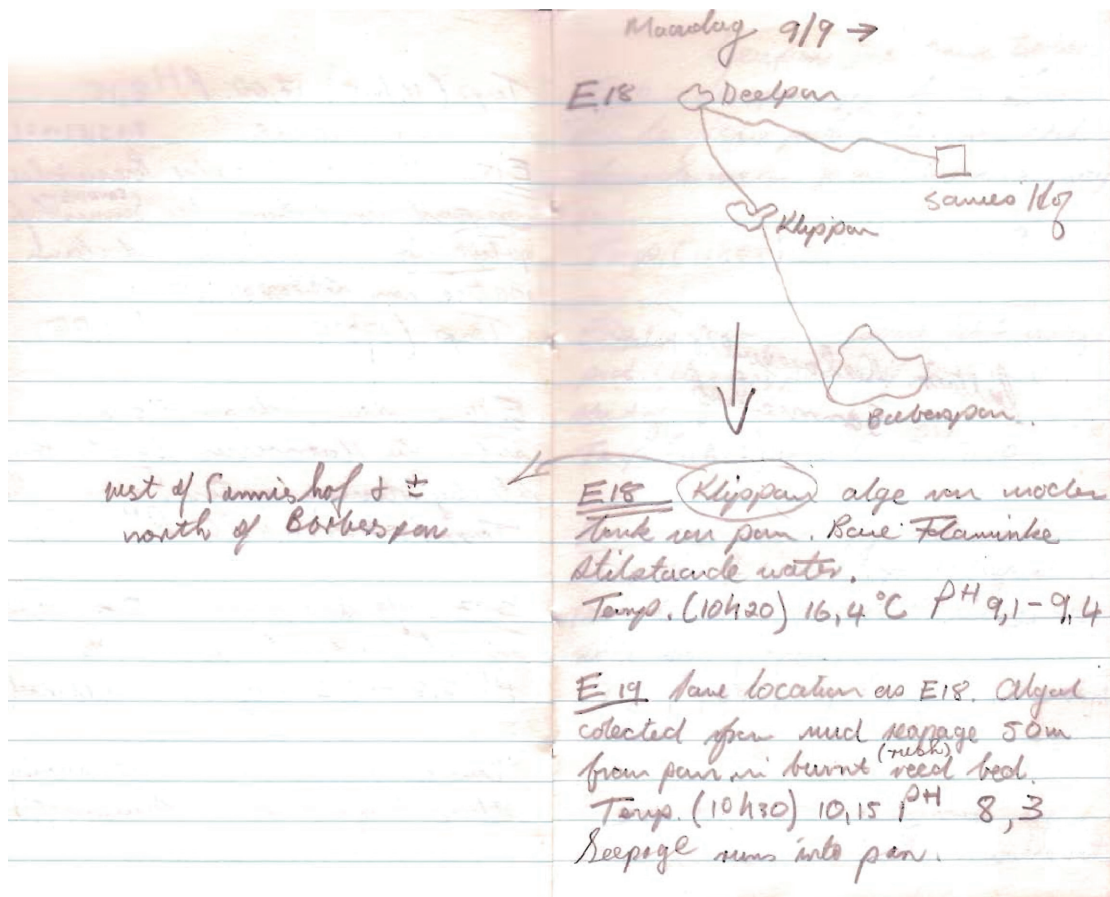


Figure B3: Example of field notes detailing samples collected near Barberspan.

old
H42 Montagu Pass at
Stinkhoutdree (Railway
arches). Algae from
rocks & moss along
roadside. Trickling water
(temp 10h30) 16°C
pH 6.3
Water sample H42.
25/2/85
H43 George Van Riebeeck
Park towards mountain
algae from grass & rushes
in marshy seepage area.

Figure B4: Field notes for H-series samples collected in the vicinity of George.

APPENDIX C: MAPS

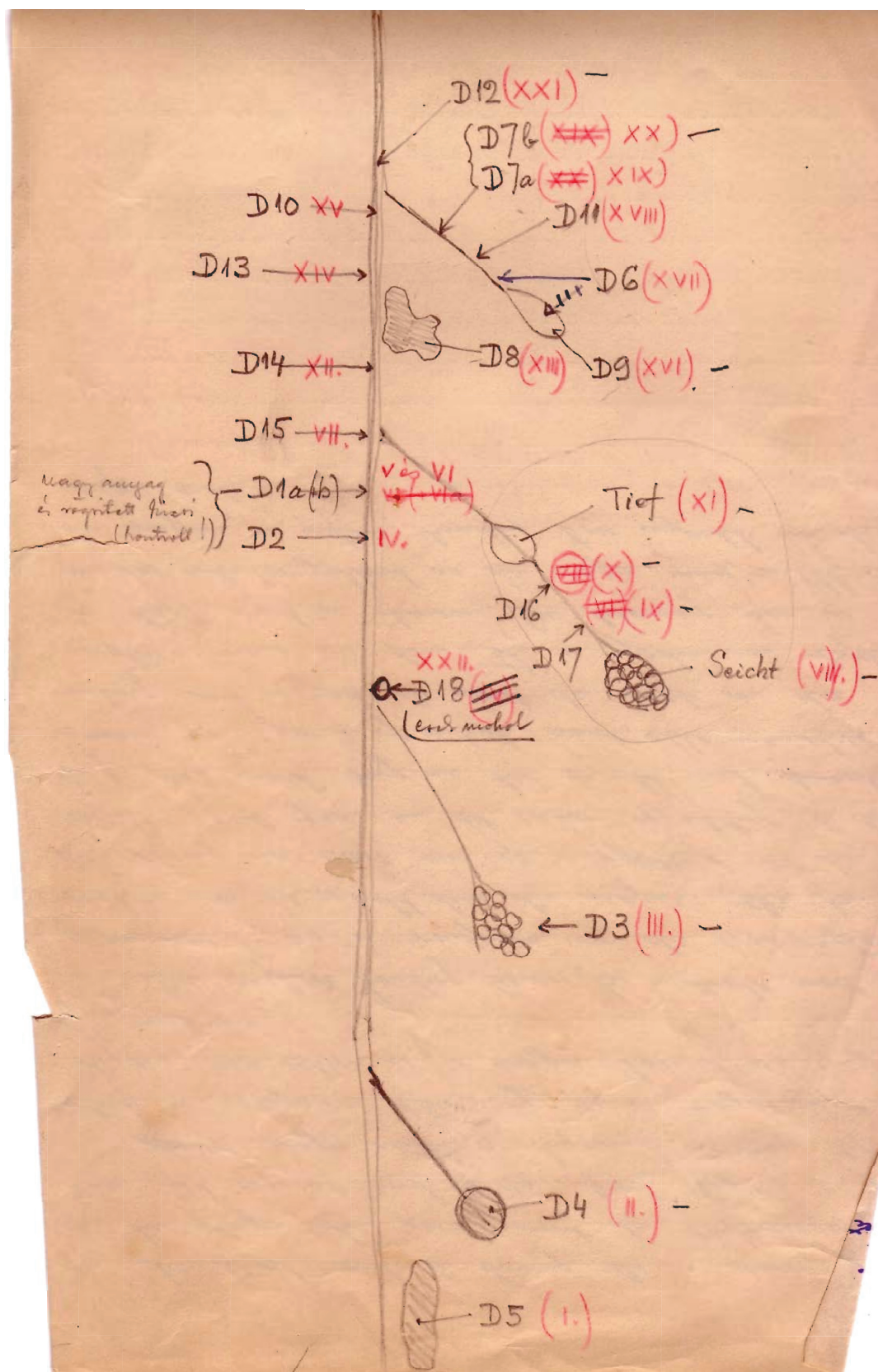


Figure C1: Cholnoky's sketch of his map for samples collected at Rayton Vlei.

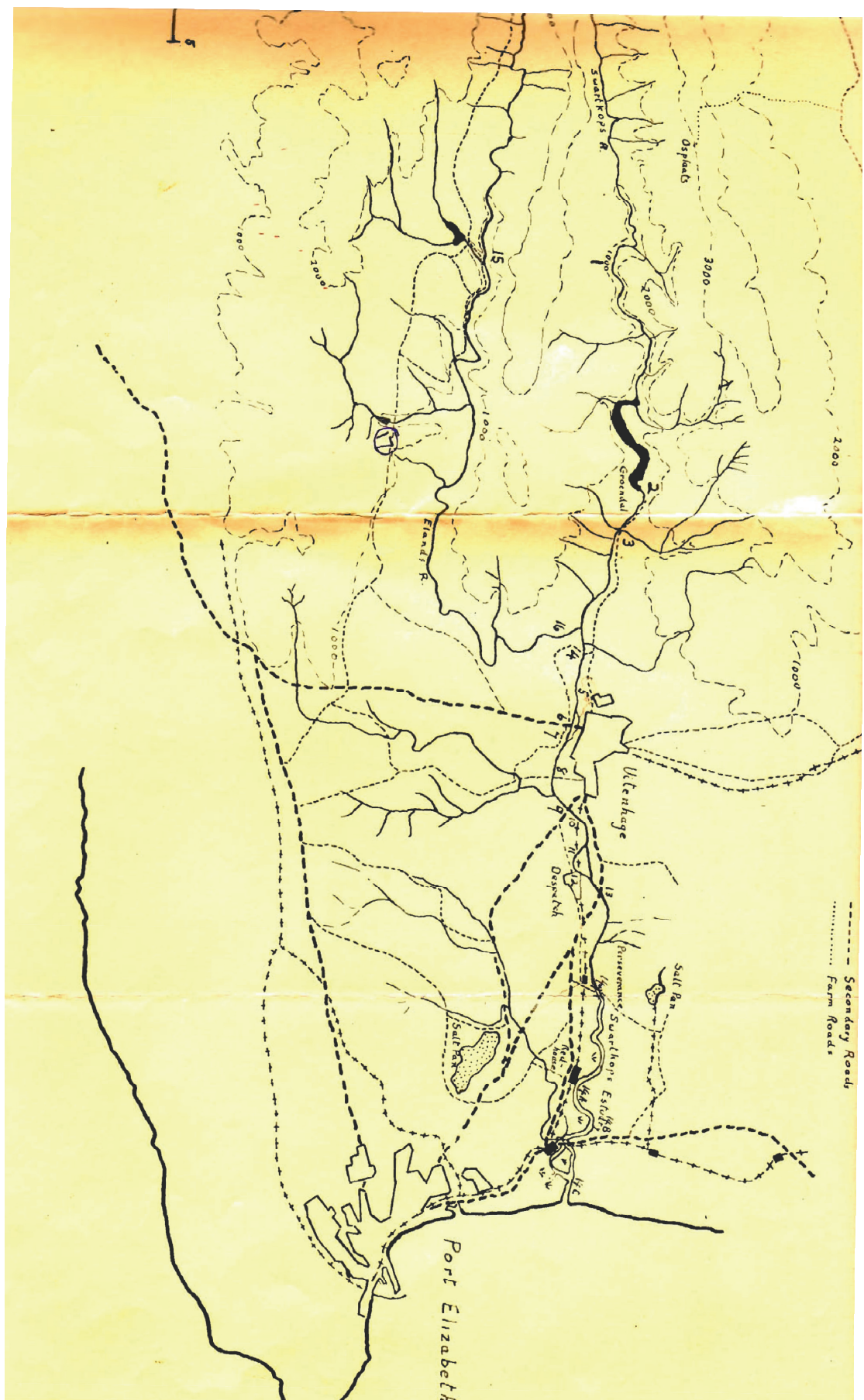


Figure C2: Map of the Swartkops River showing sampling sites (see Figure C3).

Diatom Samples sent to Dr. Cholnoky, April 1959.

ZWK 54.	Stn. 2	Swartkops River.	From vegetation.	19a
ZWK 68.	" 12,	" "	Mitenhage end of Despatch	28
ZWK 69.	" 13,	" "	Nationaal Road Bridge	29
ZWK 70.	" 9, a	" "	From causeway. de Mist Bridge	25a
ZWK 71.	" 9, b	" "	From stones in stream.	25b
ZWK 72.	" 11,	" "	Venter's Pore	27
ZWK 73.	" 3,	" "	From vegetation and causeway. ^{Rovikraus}	20
ZWK 74.	" 6, a	" "	From trickle from location.	22a
ZWK 75.	" 7,	" "	Below outflow from wool washery.	23
ZWK 76.	" 6, b	" "	From "main" stream. Niven's Bridge	22b
ZWK 77.	" 10,	" "	Below sewage outfall.	26
ZWK 78.	" 8,	" "	Below tannery.	24
ZWK 79.	" 14, a	" "	Below causeway (i.e. tidal).	30a
ZWK 80.	" 14, a	" "	Above causeway (i.e. not tidal).	30b
ZWK 81.	" 2, b	" "	Stream below dam.	19b
ZWK 82.	" 4,	" "	Below confluence with Elands River.	21

Figure C3: List of sample site descriptions for Swartkops River samples.

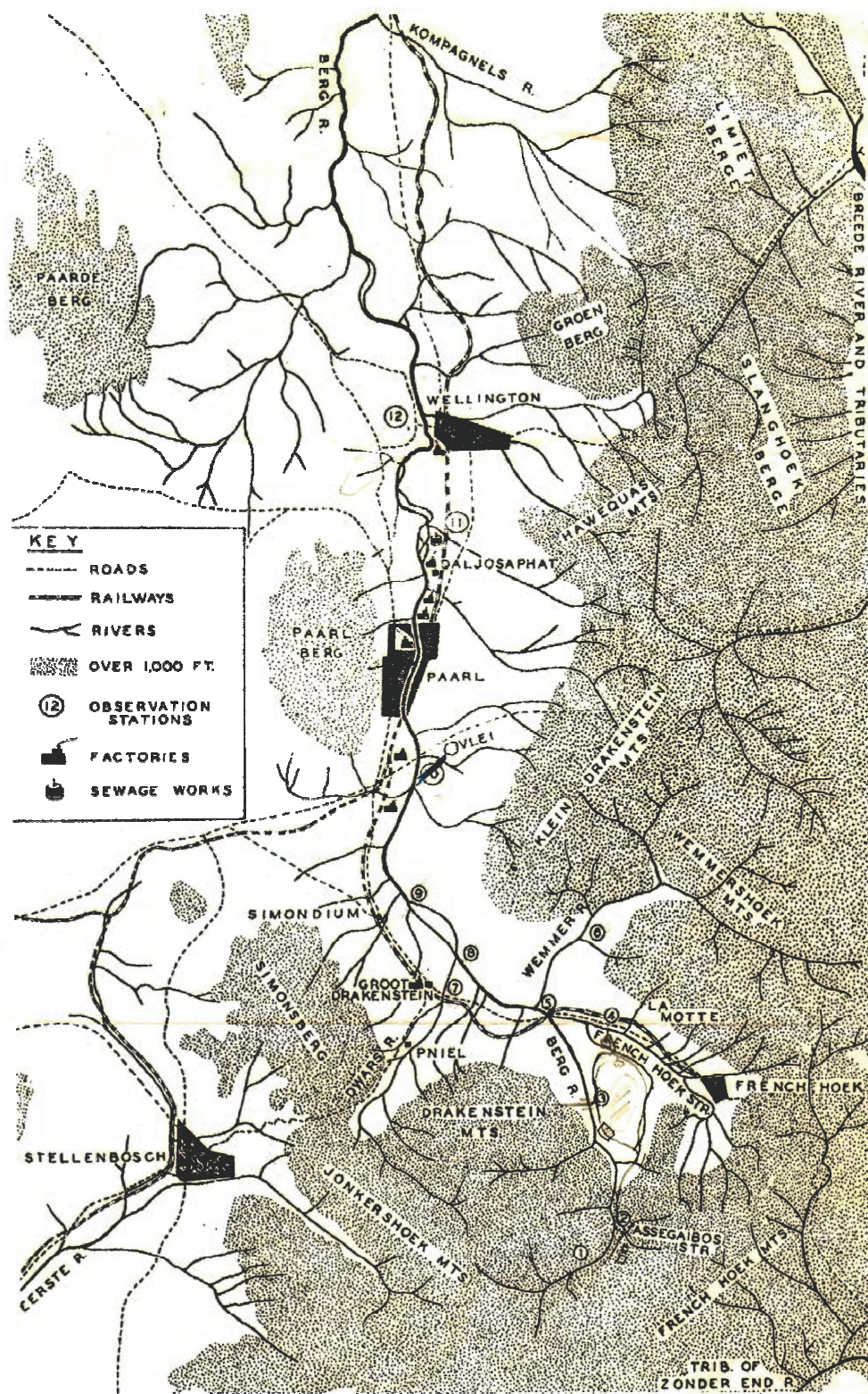


Figure C4: Upper Berg River (Cholnoky sample sites).

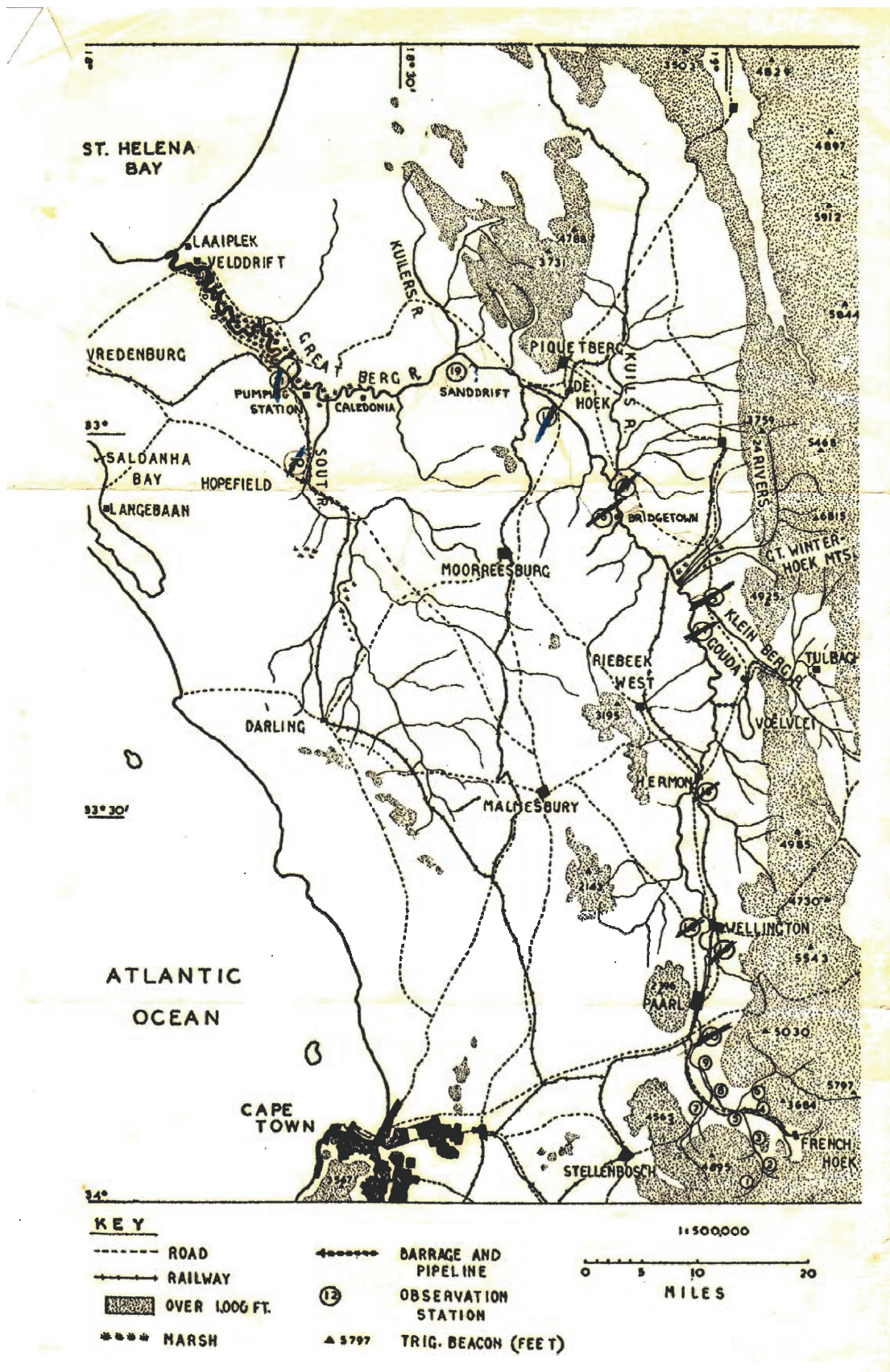


Figure C5: Mid and lower Berg River (Cholnoky sample sites).

- Weg nach Hauptstadt
- 1.10.1956
- ~~K.1.~~ - Die Toits Klaf zwischen Worcester und Paarl. ~~Das~~ Das dortige Ziegung der Schicht
Ein Bach im Felsschliff neben dem Wege. Kleiner Wasserfall. Schieferige Kalksteine
des Felsen
- ~~K.2.~~ - Die Toits Klaf. Ebenda. Ein kleiner Entwässerungsgraben neben der Landstrasse
unterhalb des vorigen Wasserfalls. Viel Lycopodium.
- ~~K.3.~~ - Die Toits Klaf. In der Schicht. Ein kleiner Bach des Hauptbaches ~~des~~ der Schicht.
Grüne, grüne Algenmatte.
- ~~K.4.~~ - Die Toits Klaf. Ein grüner Seitenbach ebenda. Bodenüberzüge auf abgerundeten,
~~gerundeten~~ Felsen.
- ~~K.5.~~ - Die Toits Klaf. Der Hauptbach der Schicht. Bodenüberzüge ~~von~~ der Nähe der Mündung
des nahe genannten Seitenbaches.
- ~~K.6.~~ - Die Toits Klaf. Braune, schieferige Kalksteine auf dem Felsen im Felsenwasser
neben dem unteren Ende der Toits Klaf-Tunnel.
- ~~K.7.~~ - Die Toits Klaf. Ebenda. Der Bachlein neben ~~dem~~ der Landstrasse, das aus
dem oben off. Becken ebener Felsenwasser entsteht.
- ~~K.8.~~ - Ein kleiner Bachlein etwa 10 m über Meeresspiegel nördlich Camps Bay auf der
Kapschen Halbinsel
- ~~K.9.~~ - Zwischen Camps Bay und Landudus. Auf dem Felsen hervorragendes Wasser
neben der Landstrasse, etwa 20-25 m ü. Meeresspiegel. Kapschen Halbinsel.
- ~~K.10.~~ - Kapschen Halbinsel. Auf sehr steilen Felsen hervorragendes Wasser nördlich Kaps Bay.
- ~~K.11.~~ - Kapschen Halbinsel. Eine kleine Quelle am Wege mit einem Trichterstein neben
dem
Nammatje. Die aus Felsen hervorstehende Wasser
- ~~K.12.~~ - Kapschen Halbinsel. Ebenda. Abfluss neben der Quelle.
- ~~K.13.~~ - Kapschen Halbinsel. Weg von Meeresspiegel neben Smith's Point Bay. Abfluss einer
kleinen Quelle mit sehr vielen Algen.
- 2.10.1956
- ~~K.14.~~ - Sir Lowry's Pan. Somerset-West und Grabouw. Kleine Quelle am Felsende des
Pans ^{unter} auf dem östlichen Kaps. Abfluss der Quelle.
- ~~K.15.~~ - Sir Lowry's Pan. ~~Die~~ Kapschen Halbinsel. Ein Graben mit mehreren kleinen Wasser
unterhalb derselben Quelle.

Figure C6: Portion of Cholnoky's descriptions of his Western Cape Sites (K-Series).

Proben aus East London und Buffalo-River.

22.1.1961.

EL 1 - Die Mündung des Gulu-Flusses, die von dem Meere durch eine Nehrung getrennt ist. Bodenbelag in einem seichten Ufertümpel.

EL 2 - Ebenda. Eine sehr seichte Bucht des nördlichen Ufers. Dicke Bodenüberzüge.

EL 3 - Epiphyten auf submersen Zweigen in den oberen Teilen der Mündung

EL 4 - Ebenda. Überzüge auf Steinen auf der südlichen Seite des Causeways. Das TDS ist hier angeblich 2600.

EL 5 - Der Gulu-Fluss oberhalb des Causeways. Etwa 10 m von dem vorigen Fundort, aber 2 m höher.

23.1.1961.

EL 6 - Maiden Dam (Stausee) nördlich King Williams Town. Epiphyten höherer Pflanzen und Bodenüberzüge am unteren Ende des Staudammes.

EL 7 - Maiden Dam. Ein sehr kleiner Seitenbach in dichtem natürlichem Walde auf Felsplatten.

EL 8 - Buffalo Fluss in dem Pirie Urwald oberhalb des Stausees Maiden Dam.

EL 9 - Unter dem Staudamm des Stausees Maiden Dam. Felsplatten zwischen kleinen, aus Natursteinen gebauten Wasserbrechern.

EL 10 - Buffalo-Fluss unterhalb des Staudammes Rooikrantz-Dam. Kleinere und grössere Wasserfälle auf Felsplatten. Überzüge und Cladophora-Watten.

EL 11 - Ein Seitenbach des Buffalo-Flusses unterhalb des Rooikrantz-Stausees in die Richtung King Williams Town. Der Bach heisst angeblich "XENXE" Schnell fließendes Wasser unterhalb eines Durchlasses.

EL 12 - Buffalo-Fluss oberhalb King Williams Town oberhalb der alten und neuen Brücke der Landstrasse (Sili-Brücke?) oder (Izili-Brücke?). Überzüge der Felsen.

24.1.1961.

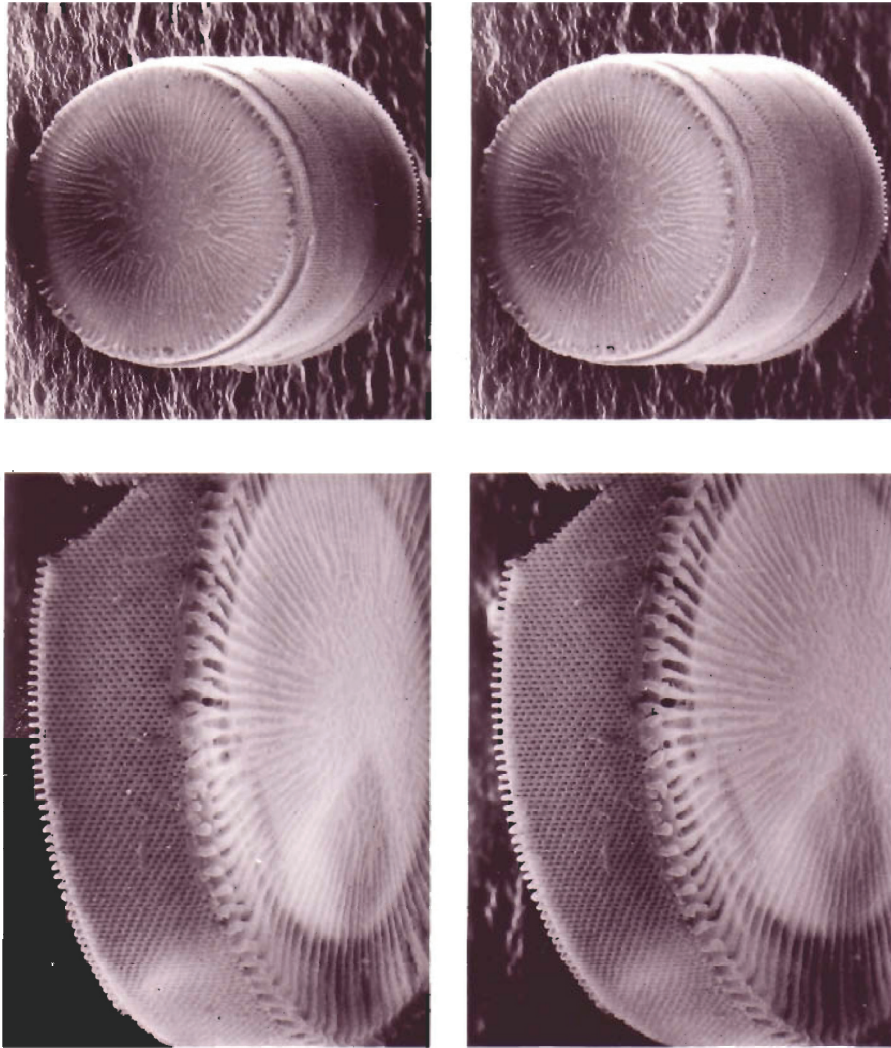
EL 13 - Buffalo-Fluss. Everett's Weir unterhalb King Williams Town und der Gerberei. Zähe Überzüge der Felsplatten mit sehr vielen Cyanophyten.

EL 14 - Dicke, schleimige Überzüge auf den Betonplatten des Everett's Weir-s in dem Buffalo-Fluss unterhalb der Gerberei.

EL 15 - Location Drift unterhalb King Williams Town, wo dieser Zufluss schon das ganze Abwasser der Lokation führt. Seichtes Wasser auf den Zementplatten der Furt.

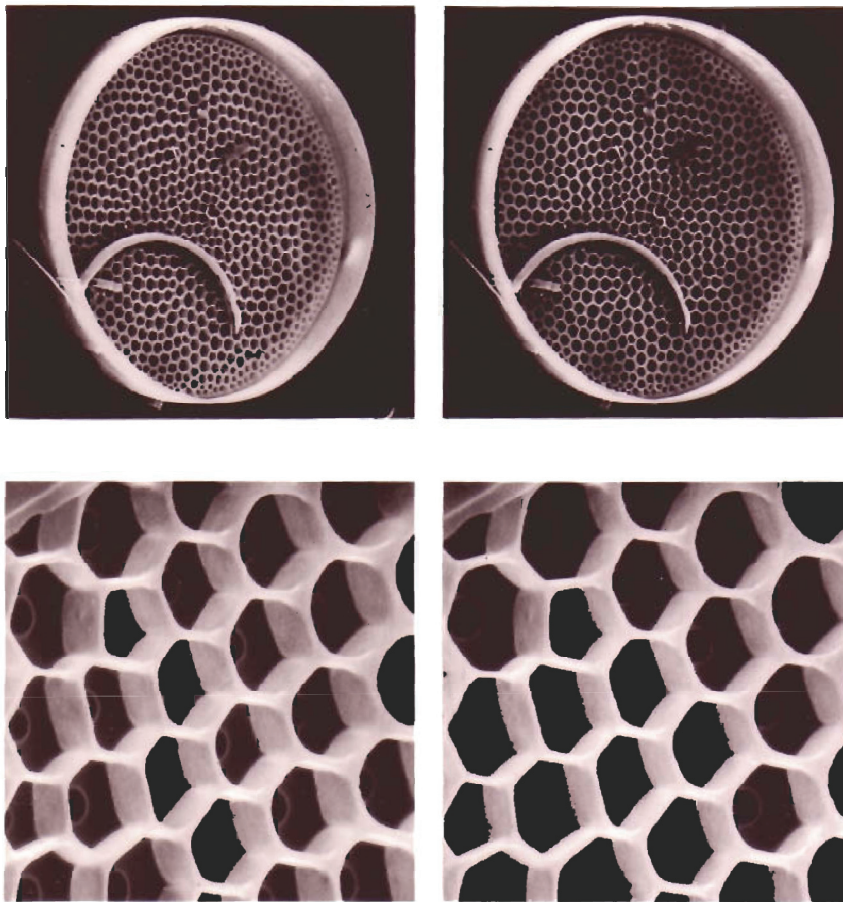
Figure C7: Site descriptions for Cholnoky sites in the East London area.

APPENDIX D: OTHER RESOURCES



Tafel 719 *Melosira arenaria* Moore
 oben: SEM, 1135:1
 unten: SEM, 1920:1

Figure D1: Helmcke-Krieger photomicrographs. *Melosira arenaria* detail.



Tafel 773 *Coscinodiscus* spec.
 oben: Aussenansicht, SEM, 1400:1
 unten: Aussenansicht der Schale mit Kammern, SEM, 5150:1

Figure D2: Helmcke-Krieger photomicrographs. Fine detail for *Coscinodiscus* sp.

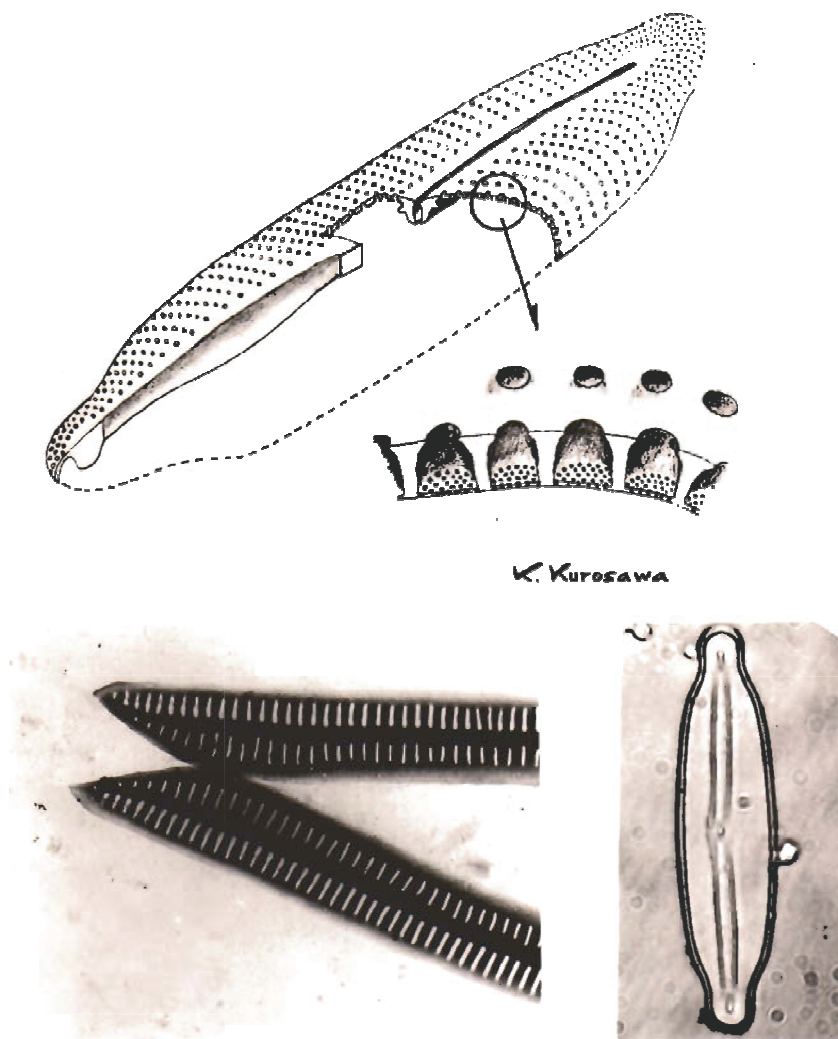


Plate 862 *Frustulia vulgaris* (Thwaites) De Toni

Bottom left: Intercalary band, TEM, 7500:1

Bottom right: Light micrograph 1500:1

Reconstructed diagram, showing fine structure of raphe and loculi (K. Kurosawa)

Figure D3: Helmcke-Krieger photomicrographs. *Frustulia vulgaris* detail.

CHAPTER 6: SKILLS TRANSFER

The following people were trained during this project.

Ms. Malebo Matlala

Ms Matlala is a full-time Masters student in Environmental Sciences at the North-West University (NWU) and is receiving continuous training in both diatom sample preparation and identification as part of her masters project. Ms Matlala's studies were funded from this project.

Ms Matlala received one-on-one training at the North-West University and has now completed the laboratory work and identification of her samples for her M.Sc. Her dissertation has been submitted and awarded.

Ms. Kathy Milford

Ms Milford is a full-time employee of Umgeni Water and a participant in project K5/1707.

Ms. Milford was trained in all aspects of diatom sampling and taxonomy (as detailed in the capacity building report). She has completed the collection of 40 samples in the Umgeni catchment. Safety rules at Umgeni Water prevented her from processing the samples after hours as per our agreement. All samples were thus dispatched to NWU for processing, this is now complete and finished slides have been dispatched to Kathy for analysis.

As Kathy lives at quite a distance from the NWU questions on identification and methodology have been dealt with telephonically and via e-mail (images sent for identification). Kathy continues with her sample analysis for the project.

Mr. Justice Madonsela

Justice is a full-time employee of the University of Venda and is registered for a part-time honors degree in Environmental Science at the NWU.

Justice has now completed the analysis of all of his samples. He attended a one day refresher course in Potchefstroom in June 2008, during this session outstanding species identifications were dealt with as well as data formatting and analysis and the use of Omnidia software. Justice returned to NWU later in 2008 for assistance with data processing and has now completed the write-up of his honors degree dissertation which will be submitted for marking at the end of October 2009.

Ms. Wilma van Staden

Ms. Van Staden is the most recent student to have joined the project. She is a full-time Masters student at the NWU working on the algae and diatoms of Marion Island. In addition she is analyzing samples for K5/1707 in order to familiarize herself with the diatom flora of the South African mainland; all in all she will analyze 150 samples by the end of her project. Wilma shows a great aptitude for the diatoms and has proved to be skilful both in laboratory preparation techniques as well as in diatom analysis. She is the most likely student to proceed with Ph.D. studies.

She has day-to-day interaction with members of the project team and has received formal training in the following aspects of diatomology:

1. Introduction to the biology of the diatoms
 - a. Life cycle, reproduction, etc.
 - b. Screening of the film "Diatoms: Life in glass houses"
 - c. Collection and investigation of living diatom material
 - d. Introduction to field and laboratory techniques
2. Training in methodology
 - a. Practical exercises in collecting in the field from a variety of substrata, pebbles, rocks reeds, sediments
 - b. Sample preservation
 - c. Sample preparation and laboratory techniques
 - d. Cleaning samples
 - e. Slide preparation
 - f. Archiving
3. Analysis techniques
 - a. Brief overview of the history of microscopy and introduction to optics and associated terminology.
 - b. Techniques for achieving optimum resolution with alight microscope.
 - c. Brightfield, phase contrast and DIC microscopy techniques.
4. Structure and function of diatom cells
 - a. Outline of cell morphogenesis
 - b. Gross morphology of the diatom valve
 - c. Fine structures of the diatom valve
 - d. Function of fine structures

5. Identification of diatoms
 - a. Introduction to the morphological characteristics of diatoms important in identification.
 - b. Identification and delineation of genera
 - c. Chief characteristics of each of the main fresh water genera.
 - d. Identification of diatom species
 - e. Focus on differential characters between closely aligned species.
6. Diatom counting, analysis, microscopy and photography
 - a. Software for counting.
 - b. Rules for counting diatoms.
 - c. Introduction to uploading species data and calculating diatom indices.
 - d. Introduction to high resolution microscopy.
 - e. Overview of digital photomicroscopy.

Ms. Van Staden is one of the first students to benefit from the donation of a microscope to the NWU by the WRC, allowing for prolonged and uninterrupted periods of work.

Ms. Gina Walsh (University of Johannesburg)

Gina used diatoms as part of her Masters degree project in the Department of Zoology at the University of Johannesburg. She attended an introductory course in diatom identification on the 13th of June 2007 where the WRC report TT282/07 was used to provide a complete overview of the common South African diatom genera and species.

Gina has been schooled in the collection of diatom samples through the medium of the video material forming part of WRC report TT281/07 as well as attending the knowledge dissemination workshops held by the project team members of K5/1588.

Gina completed 3 days training on diatom identification and enumeration from the 23rd to the 25th of July 2007. She used this time to count and identify samples which will be used in her M.Sc. thesis.

Gina has now graduated and has also submitted the results of her study for publication in water SA

Ms. Linette Ferreira (University of Johannesburg)

Linette is examining the impact of various water quality variables on diatom communities as part of Masters project at the University of Johannesburg. She attended an introductory course in diatom identification on the 13th of June 2007 where the WRC report TT282/07 was used to provide a complete overview of the common South African diatom genera and species.

Linette will further received training on the identification and enumeration of diatoms at the North-West University from the 26th till the 30th of May 2008. In addition there has been active correspondence with Ms. Ferreira via e-mail on the use and application of diatom indices. All results from Linette's project were processed by the staff of NWU and submitted to her.

Linette has now graduated.

Eastern Cape DWAF

Ms. Thulani Guzana and two other members of the Eastern Cape DWAF biomonitoring team attended an introductory course on the use of diatoms in river monitoring studies held at the North-West University, Potchefstroom Campus from 5th November 2007 to 8th November 2007.

The team received a full day of field training on sampling and collection techniques and then over the next two days were presented with an introduction to diatom biology, laboratory techniques for cleaning and preparation as well as a basic introduction to diatom identification.

Ms. Jessica Brislin (University of KwaZulu-Natal)

Jessica is examining diatom species found in the high mountain wetlands of the Drakensburg as part of her honors degree at UKZN. She will received field training at her sites from the 16th till the 18th of June 2008 and then received a week of training at the North-West University from 23rd to the 27th of June on all aspects of diatom sample preparation as well as basic identification skills. Although not currently part of the project team capacity building was considered important as she is a potential candidate for a masters degree project using diatoms as indicators of water quality. She has now completed all analysis for her project and was remotely assisted in identification via e-mail.

Jessica graduated with a B.Sc. (Hons). Although she is now working the project team members are actively encouraging her to publish her dissertation as well as to continue in her study of the diatoms.

Ms. Pashni Pillay (Associated with UCT FRU)

Pashi is an ex-employee of Umgeni Water where her expertise lay in the identification of freshwater planktonic algae. She was trained at NWU in the identification of diatom and has since been able to apply this knowledge to assist with another WRC project at UCT FRU.

Pashni attended at a course on all aspects of diatom preparation and identification at NWU from the 25th of June till the 6th of July 2007. She continues to receive aid with identification and methodology via e-mail.

Ms. Luisa Riato (Wetlands Consulting Services, Pretoria)

Ms. Riato received a day of training at the NWU on basic diatom collection and cleaning methodology at NWU on the 19th of August 2008. She collected diatom samples that she then brought to NWU for processing during October 2008. Ms. Riato is planning to establish in-house expertise at wetlands consulting services to use diatoms as indicators of wetland water quality, especially desiccated wetlands.

Luisa has now established her own laboratory at Wetlands Consulting in Pretoria. She received a morning of follow-up training on the 20th of August 2009. The training included the use of counting software as well as an introduction to the Omnidia software. She will again visit NWU to be trained on the detailed aspects of cell identification.

Ms. Ratidzo Dhlembeu (University of Cape Town)

Ms. Dhlembeu has included diatom analysis in her project. However, UCT does not have a license for the Omnidia software so her data was sent through for processing to NWU.

Mr. Elijah Mogakabe DWAF RQS

The Department of Water Affairs, Resource Quality Services plans to develop in-house capacity for diatom analysis. Elijah Mogakabe attended a week long training course from 12-18 October 2008 at NWU.

Topics covered by Elijah during his visit to NWU include:

1. Introduction to the biology of the diatoms
 - a. Life cycle, reproduction, etc.
 - b. Screening of the film "Diatoms: Life in glass houses"
 - c. Collection and investigation of living diatom material
 - d. Introduction to field and laboratory techniques
2. Training in methodology
 - a. Sample preparation and laboratory techniques
 - b. Cleaning samples
 - c. Slide preparation
 - d. Archiving
3. Analysis techniques
 - a. Brief overview of the history of microscopy and introduction to optics and associated terminology.
 - b. Techniques for achieving optimum resolution with a light microscope.
4. Structure and function of diatom cells
 - a. Gross morphology of the diatom valve
 - b. Fine structures of the diatom valve
 - c. Function of fine structures
5. Identification of diatoms
 - a. Introduction to the morphological characteristics of diatoms important in identification.
 - b. Identification and delineation of genera
 - c. Chief characteristics of each of the main fresh water genera.
 - d. Identification of diatom species

Elijah has now been promoted to a management position within DWAF and with thus no longer be heading up the diatom assessment program. Paul Botes (Senior Freshwater Specialist Scientist) at RQS is now heading the initiative and will identify suitable candidates for further training.

Ms. Nina Woods – Mogalakwena Research Centre

In early 2009, NWU was approached by Paul Foché of the University of Venda to train one of his graduate students – Nina Woods. Nina works as the research manager at Mogalakwena Research Centre in Limpopo and will be doing her masters degree research on the quality of the Mogalakwena River, especially that section near to the research centre. She will be evaluating water quality using both diatoms and macroinvertebrates.

She attended a training course at the NWU from the 27th to the 29th of May 2009 and the following topics were covered:

1. Introduction to the biology of the diatoms
 - a. Life cycle, reproduction, etc.
 - b. Screening of the film “Diatoms: Life in glass houses”
 - c. Collection and investigation of living diatom material
 - d. Introduction to field and laboratory techniques
2. Training in methodology
 - a. Sample preparation and laboratory techniques
 - b. Cleaning samples
 - c. Slide preparation
 - d. Archiving
3. Structure and function of diatom cells
 - a. Gross morphology of the diatom valve
 - b. Fine structures of the diatom valve
 - c. Function of fine structures

Once she is finished with sample collection Ms. Woods will return to the NWU for training in diatom identification.

Mrs Lynda Muller

Mrs Muller of Muller Environmental has received training in the collection and preservation of diatom samples from a range of aquatic habitats. Mrs Muller is assisting DH Environmental with the collection of samples from the Western Cape.

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

One of the most consistent and common responses, particularly from the DWA, has been that the method is too specialized and difficult to apply. This argument is misplaced in that it does not take into account the level of value and information that can be gleaned from a single diatom sample, a level that outweighs any of the other indicators in terms of assessing water quality and drawing ecological inferences.

Diatom field methods are the simplest of any biomonitoring protocol currently in use. Once the sample has been collected there are two options: Submit it to a commercial laboratory for analysis (several laboratories exist) or perform the analysis yourself.

Diatom species identification can at first seem daunting. The lack of willingness to work with microscopes and taxonomy is an outcome of poor exposure to these skills at the tertiary level, combined with an absence of algological training in South Africa. As with most things, however, proficiency comes with time. This project has shown that with initial intensive training of ~2 weeks, follow up sessions, either in person or via e-mail and after ~6 months of practice a person, with a biological background can become very proficient in diatom analysis.

The second challenge has been why provide such a seemingly complex and intricate method for simple bio-indication?

Diatom indices are not field-based methods and some form of microscopic examination or laboratory-based analysis will ALWAYS need to take place. However, this perceived added effort should not detract from the method. Diatom indices allow for the quantification of a water quality impact in terms of producing an index score, as well as allowing the user to draw specific conclusions regarding the pH, electrical conductivity, nutrient status, organic content and oxygen levels of a stream and is thus a very powerful tool. For a small amount of added effort, a relatively large amount of information regarding the system under question can be gained. This has specific value for the reserve determination process.

This project has continued to demonstrate the versatility of the use of diatoms as an indicator, including its application in environments where all of the other habitat-limited indicators have no use.

This study has identified the following needs for further research:

This study, although fairly comprehensive in terms of covering South Africa, has unfortunately excluded the Western Cape. Samples were collected from different regions in this province but several problems have arisen during their analysis:

- Cholnoky's type material for this region has been lost, thus we cannot cross-check species identifications and his species names are invalid at this time;
- The characteristics of the waters of the Western Cape differ significantly when compared to those in the rest of the country;
- We currently do not have enough samples from this region to be able to meaningfully assign tolerance and indicator values.

Accordingly, a comprehensive regional study is required in order to source type material and expand the SADI V1.0 to incorporate these data.

Ongoing inclusion of results from collections from all over the country are and will remain necessary for some years in order to increase the robustness of the index.

Identification literature

The guide to the common diatoms of South Africa (Version 2) needs to be expanded to include species from the Western Cape and KwaZulu-Natal (sub-tropical). There is also a need to produce floras dealing specifically with each group of diatoms.