THE USE OF CONTEMPORARY AND HISTORIC DIATOM ASSEMBLAGES IN THE DERIVATION OF REFERENCE STATE COMMUNITIES FOR RIVERS IN KWAZULU-NATAL, SOUTH AFRICA

By

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PREFACE

The research described in this thesis was carried out by the author, as a registered student of the University of Cape Town, under the supervision of Professor John J. Bolton. The investigation, however, was undertaken out of Durban, in the Province of KwaZulu-Natal. The study represents original work by the author and the outputs have not been submitted in any form to another University. Where use has been made of other work it has been duly acknowledged in the text and references.

Creation and Providence of Diatoms

All things bright and beautiful
All creatures great and small
All things wise and wonderful
The Lord God made them all

He gave us eyes to see them
And lips that we might tell
How great is God Almighty
Who has made all things well (Cecil Alexander 1818–1895)

'Amphitheatre' at the headwaters of the Thukela River
(KZNCS 2006)

"When I consider thy heavens, the work of thy fingers, the moon and the stars, which thou hast ordained - What is man, that thou art mindful of him."? *(Psalm 8, Verse 4)*

"A reference condition should be a term reserved for describing naturalness at one end of a biological gradient" (Stoddard *et al*. 2006), thus implying the quintessence of biological integrity in the absence of human impact pressures.

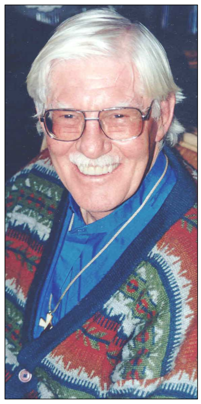
ACKNOWLEDGEMENTS

This research on the freshwater diatoms of the rivers of KwaZulu-Natal is the culmination of a life time interest and commitment to the well-being of the key freshwater resources of this important region of South Africa.

Research posts in KwaZulu-Natal in the late 1960's were rare but I was fortunate to be appointed as a Natal Rivers Research Fellow under the sponsorship of the erstwhile Natal Town and Regional Planning Commission whilst working at the CSIR (KwaZulu-Natal) laboratory in Durban. In this capacity, I was blessed in my early years with the good fortune of being under the guidance of my mentor in biological projects, **Dr B.J. Cholnoky**, a world renowned diatomologist of his time. Furthermore I was part of a Rivers Research team headed by **Dr P.H. Kemp**, the most knowledgeable freshwater chemist of his time in South Africa. I was intimately involved in all Rivers Research programmes involving water quality and freshwater algae in the province. I owe them both a deep gratitude for all I learned while they were alive.

Looking back over forty years, I have to sincerely acknowledge, belatedly perhaps, the unique opportunity of working under the supervision of Dr B.J. Cholnoky. Subsequently my older brother's untimely passing was the inspiration to revive, continue and expand on their valuable and dedicated contributions to research on diatoms of South African Rivers. They were truly leading diatomologists of their time in South Africa.

Dr B.J. Cholnoky
(1899-1972)



Dr R.E.M. Archibald
(1940-1999)

Nonetheless, the full implications of these research projects and specifically the great value of Cholnoky's early research on diatoms of our local river systems in the mid 1950's did not dawn sufficiently on me until my semi-retirement in 2002.

My attention and focus had been diverted from diatoms to the commercial demands and interests of contract research focused mainly on other aspects of water quality of river systems over a period of 25 years. Fortunately, I was later to be challenged by the opportunity and need to rescue and restore the South African Diatom Collection which had been abandoned after my older brother's untimely death in December 1999. I was subsequently encouraged and stimulated, in my semi-retirement, by several personal requests from international and local persons to re-visit the world of diatoms 'hidden' in the South African Diatom Collection. This finally led to the crystallization and formulation of the thesis topic which draws on treasures of 'forgotten herbarium records' of the past and uses these as a platform together with my own recent investigations of KwaZulu-Natal Rivers to benchmark present-day diatom reference state conditions for the future.

I am also sincerely indebted to my supervisor, **Professor John J. Bolton**, of the Botany Department, University of Cape Town, who provided the opportunity to formally register my investigations as a thesis. Much less would have been accomplished without his support, help and advice and this effort would have been somewhat meaningless.

Other professional colleagues and close associates have also played major roles in the challenge to revive diatom research in South Africa. I wish to acknowledge the following persons among this valued scientific community.

Dr Bill Harding jointly and successfully re-launched intense interest in diatom research in South Africa in the 21st century by securing initial funding for key diatom projects, post 2003. This challenge was also driven at the time by the apparent lack of other trained diatomologists in the country. It is gratifying that there are now substantive research efforts and some well trained younger diatomologists in South Africa. **Dr Jonathan Taylor** 'arrived on the scene unexpectedly' and is an inspired and dedicated diatom 'addict' feeding on the opportunity to develop and further the taxonomy and ecology of South African diatoms. Permission for use and access to some additional slide material of samples, particularly those obtained from 'inaccessible high altitude sites,' is acknowledged. These few slides provided an added-value opportunity to examine more material for present day analysis of species assemblages from remote sites within the study area.

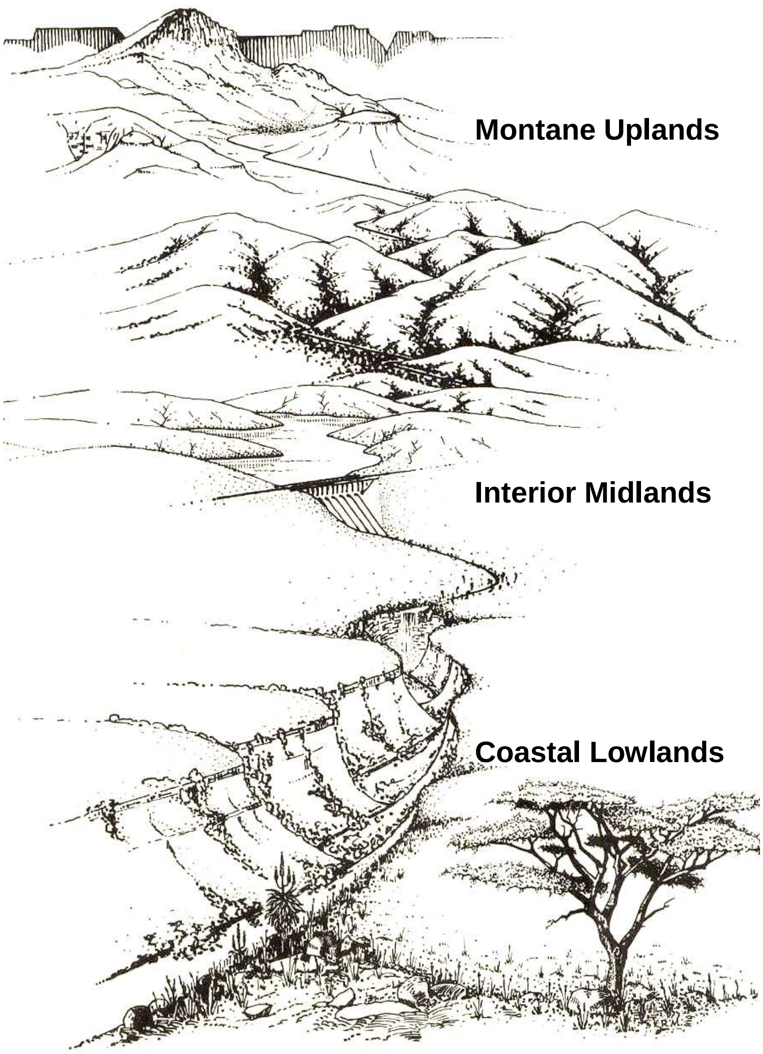
Our collective vision and passion, as a trio, is the restoration of diatoms to a meaningful role as important biological indicators of river 'health' in South Africa. The output from this research, in practical terms, will hopefully make a valid contribution towards turning this vision into a reality. To this end, **Professor Andrzej Witkowski**, University of Szczecin, Poland is also sincerely thanked for being an external catalyst in this revival. Generous assistance and opportunities for training in diatom taxonomy were given to local diatomologists in association with **Professor Horst Lange-Bertalot**.

Craig Morris of the Agricultural Research Council, API-Grassland Science, University of KwaZulu-Natal, provided invaluable expert professional guidance and advice on the practical and theoretical use of multivariate analysis of the diatom community data.

Successive Directors of the Division of Environmentek (CSIR) have been generous in allowing me full access to historic material contained in the South African Diatom Collection. Ex-colleagues in the CSIR (KwaZulu-Natal) Regional Laboratory in Durban provided analytical services and facilities for the safe preparation of diatom material and analysis of some water samples collected in more recent times. The final acknowledgement, but no less important, is traditionally reserved for close family.

To my late parents, **Brian and Irene**, who spared nothing to provide the best available school and university education in my formative years.

To my wife, **Barbara**, and children **Murray** and **Vanessa** -'*sincere thanks for the enduring love, support, and encouragement and to* **Margaret Adam**, who also provided continuous support.



Sketch by H. Few (NTRPC 1973)

"Time, to an atom locked in a rock, does not pass"!

"*An atom at large in the biota is too free to know freedom; an atom back in the sea has forgotten it. For every atom lost to the sea, the prairie pulls another out of the decaying rocks".* [Leopold (1949): An extract from the epilogue (Likens *et al.* 1977)].

"*Comparisons of biogeochemical data from natural ecosystems with those that have been manipulated by man provide important information about the functional efficiency or 'health' of an ecosystem"* (Likens *et al.* 1977).

ABSTRACT

The Use of Contemporary and Historic Diatom Assemblages in the Derivation of Reference State Communities for Rivers in KwaZulu-Natal, South Africa

Colin George Mostert Archibald

Reference state conditions of minimally disturbed headwaters were identified from present-day and historic diatom data of key rivers within the selected study area, with the main purpose of establishing diatom reference state communities. No meaningful research on the diatom communities of KwaZulu-Natal Rivers has been undertaken since 1970 and none has followed a dual '*a priori' and* '*a posteriori'* approach for benchmarking diatom reference communities in the rivers of South Africa. An '*a priori'* classification of the local rivers resulted in the delineation of three headwater spatial zones, namely the Montane Uplands, the Interior Midlands, and the Coastal Lowlands based on natural geological characteristics and geomorphological features peculiar to the region. A small spatial scale improved the correspondence between diatom responses and near natural river conditions by reducing the influence of the natural heterogeneity of river styles and the high variability of water quality parameters.

Suppositions were made that headwaters of rivers, free of human disturbance pressures, would be expected to engender diatom assemblages of high ecological status, conforming to the degree of naturalness associated with a reference state condition. The notion of an objective, chemistry-free diatom-based classification of reference state sites makes no theoretical predetermined assumptions as to the environmental factors influencing the distribution and condition of the associated reference state diatom communities. The correspondence between diatom responses and river reference state conditions was determined using relative abundance data, at the species level, by the initial grouping of reference state sites using Agglomerative Cluster Analysis. An 'a *posteriori'* interpretation of the site ordinations and diatom responses to unmeasured environmental gradients was achieved with Non-metric Multidimensional Scaling and Principal Components Analysis.

The main findings from this study determined that (i) an '*a priori*' physical classification of headwaters and an '*a posteriori* chemistry-free diatom-based approach was a practicable, relevant and tractable scientific protocol for identifying diatom reference state communities. (ii) Key scientific information of the diatom species composition was retrieved from a refined data set extracted from 570 historic and 200 present-day 'near natural' type specific sites in the headwaters of KwaZulu-Natal Rivers. *Tabellaria flocculosa, Achnanthidium minutissimum, and Cocconeis placentula* were the dominant diatom species representative of historic reference state taxa associated with the undisturbed, chemically dilute headwaters of rivers originating in the Montane Uplands. (iii) There was a significant change to a co-dominance of *Achnanthidium crassum* in the present-day samples of these same headwaters. A further difference was recorded in the headwaters of the rivers originating in the Coastal Lowlands where *Psammothidium oblongellum* was the main co-dominant species (iv) The historic diatom data, held dormant in the South African Diatom Collection for more than 50 years, is of great scientific value and relevance in benchmarking near-natural historic conditions, as evidenced by the world-wide paucity of such information and by the absence of appropriate extant diatom data for the selected rivers. Reference water quality conditions of these rivers was defined by retrospective analysis of the unique historic environmental information retained by the original diatom communities (v) The responses of these dominant species were comparable with other findings from headwaters of rivers elsewhere in the world thus evoking the real possibility that diatom water quality indices, developed outside of the study area, are sufficiently valid for application in the rivers of KwaZulu-Natal.(vi) The reference state sites of the undisturbed headwaters were all rated as having a high ecological status exceeding a threshold value of 15, despite several structural differences in species composition between historic and present-day sites. This threshold value was based on the established European-derived diatom water quality indices and represents a benchmark expected of near-natural conditions.

The contrast in responses of a suite of dominant **pollution-tolerant** and **pollution-sensitive** diatoms, measured as species composition changes, was obtained from ecological profiling of river diatom communities impacted by human waste. The dominant pollution-tolerant diatom species were *Nitzschia paleaeformis* 85.5%, *Stauroneis kriegeri* 14.4% (acid mine drainage); *Navicula schroeteri* 46.1%, *Nitzschia clausii* 28.2% (sugar waste); *Nitzschia palea* 42.2%, *Nitzschia intermedia* 11.8% (pulp & paper waste); *Sellaphora pupula* 47.1%, *Nitzschia pal*ea 16.4% (sewage waste); *Nitzschia palea* var. *debilis* 44.4%,*Sellaphora pupula* 27.7% (industry waste). The low ecological status of these impacted river sites was reflected in a pollution index score of <10, irrespective of the diatom water quality index or the pollutant. The findings also showed that **pollution-sensitive** species were much reduced or eliminated by pollution. Substantial reductions in relative abundance were measured for *Brachysira vitrea (25.2-0%), Achnanthidium minutissimum (*19.2-0%), and *Encyonema cesatii* (17.7-0%) (Acid mine drainage); *Nitzschia gracilis* (18.1-0%) and *Fragilaria intermedia* (12.9-0%) (Sugar waste); *Cymbella turgidula* (48.4-4.2%) and *Navicula subrhyncocephala* (12.5-1.0%) (Pulp & paper waste); *Capartogramma crucicula (18.8-0%) and Navicula gastrum* (8.8-0%) (Sewage and industry waste).

The headwaters of rivers of two spatial zones (Zone 1 and 3) fulfilled the criteria for diatom reference conditions in KwaZulu-Natal. The metrics associated with these diatom communities were taken as benchmarks at the high end of a water quality condition gradient.

THE USE OF CONTEMPORARY AND HISTORIC DIATOM ASSEMBLAGES IN THE DERIVATION OF DIATOM REFERENCE STATE COMMUNITIES FOR RIVERS OF KWAZULU-NATAL, SOUTH AFRICA

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CHAPTER 1

THE CONCEPT OF REFERENCE STATE CONDITIONS FOR RIVERS

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1.2 The Concept of Reference State Conditions

1.3 Aims and Objectives

1.4 Geographic Setting of Study Area

1.5 Diatom Assemblages – Crucial Biological Quality Elements

1.6 Hypotheses and Philosophy of Research

1.7 Summary

THE CONCEPT OF REFERENCE STATE CONDITIONS FOR RIVERS

1.1 Introduction and Rationale

Pioneering work on biological surveillance of human impacts on rivers, using diatoms and other aquatic biota, was focused on classes of decomposable matter in the '*Saprobien system'* (Kolkwitz & Marsson 1908). This approach was formulated broadly on a system which categorised animal and plant species responses in relation to their tolerance to gradients of organic pollution (Mackenthum 1965). The system was however later discredited because of inaccuracies in the definition of the saprobic categories involved in self-purification of a river. It was termed "*ecologically inadequate*" (Lange-Bertalot 1979) and found to be problematic in concept (Cholnoky 1960a, Cairns *et al.* 1972, Cairns 1974, Round 1991) and therefore received little further serious attention post 1950, despite modifications to the original system.

The great potential of diatom communities as indicators of water quality and as potential measures of reference state conditions in rivers was first demonstrated by useful groundwork in diatom ecology by leading diatomologists (Patrick 1949, Cholnoky 1968a, Patrick 1973). Comparisons between impacted sites and non-impacted sites using inventories of diatom floras demonstrated the responses to gradients in stream pollution (Patrick 1949). The number of species recorded at an impacted location was contrasted against the number of species known to occur at 'healthy' sites. This structural attribute was taken as the measure of the altered community and hence the impacted stream condition. One of the key observations that arose from this work and previous research in other disciplines was the principle of stability of a community structure. It was noted that in stable unaffected communities there was a greater diversity of species (high species diversity coupled with low relative density of individual species) while destabilised communities, disturbed by pollution, resulted in a lower diversity with fewer species at higher densities (Lackey 1941, Bartsch 1948, Gaufin & Tarzwell 1952, Patrick *et al*. 1954, Patrick 1977).

Substantive advances were made in the development of other protocols for biological surveillance of river systems in several disciplines over the last quarter of the 20th century. Most of these approaches reduced biological information derived from river surveys to multi-metric data which was often aggregated into an index as a numerical expression of the condition of a river site (Karr & Chu 1997). There were also definitive outputs from several leading diatomologists who made meaningful contributions to the advancement of diatom taxonomy (Cholnoky 1968a, Krammer & Lange-Bertalot 1985,1986,1988,1991) and ecology (Descy 1979, Lange-Bertalot 1979, Cemagref 1982, Biggs 1989, Biggs 1990, Round 1993, Kelly & Whitton 1995, 1998; Hofmann 1996, Lenoir & Coste 1996, Pan *et al*. 1996, Kwandrans *et al*. 1998, Chessman *et al*. 1999). These approaches essentially estimated the degree of pollution based on the relative abundance of diatom species and their rated individual sensitivity to a variety of environmental factors (Lowe 1974, van Dam *et al.* 1994). Useful general reviews of the state of the art with respect to biological monitoring of rivers were also published in the latter part of the 20th Century (Round 1991, 1993; Karr & Chu 1997, Larsen 1997, Taylor 1997, John 1998, Kelly & Whitton 1998). There was also a phased shift away from surveillance using chemically-based classification systems alone such as the Saprobien system (Kolkwitz & Marsson 1908), the Halobien system (Kolbe 1932), and the Trophic status system (Naumann 1932), to the development of bio-indication derived from measures of the in-stream biota.

Diatom-based water quality indices were developed as measures of general water quality conditions e.g. Biological Diatom Index (BDI) (Lenoir & Coste 1996), the Generic Diatom Index (GDI) (Rumeau & Coste 1998), and the Eutrophication Pollution Index for diatoms (EPI-D) (Dell'Uomo 1996). Less attention had been given to refinement for different types of pollution with the exception of the Trophic Diatom Index (TDI) (Kelly & Whitton 1995, 1998). Most of these indices were based on the weighted average equation of the relative abundance of species (Zelinka & Marvan 1961). Varying methods of univariate analysis were also developed and were used essentially to test hypotheses (Clarke & Warwick 1997). Diatom metrics are quantitative measures of species-level structural attributes of a diatom assemblage e.g. Relative abundance and species richness (Porter *et al*. 2008). Each group of metrics was related to some aspect of community structure and collectively provided a basis for assessment of the condition of a river (Stevenson & Pan 1999). The need for and interest in Community Pattern Analysis led to the development of the more recent generation of computerised multivariate procedures to extract and interpret biological community response patterns in relation to pollution gradients (Clarke & Warwick 1997). This approach focussed more attention on the usefulness of inherent attributes of diatom assemblages and the scientific value derived from reference state communities.

1.2 The Concept of Reference State Conditions

The notion of comparing an existing present-day river condition with a near-natural historical state is not entirely new (Patrick 1949, Mackenthun 1965, Descy 1979). However objective methods have since been developed to characterise a type-specific water resource (e.g. headwaters of river systems) by defining and describing the biological elements that make up the target reference state communities in such reaches, free of human disturbance pressures. The most recent advances in biological protocols for river health assessment in the 21st century are now focused on characterising reference state conditions from which representative values of a biological quality element of interest (e.g. diatoms) can be derived (Wallin *et al*. 2003, Acs *et al*. 2004, John 2004). A fundamental principle of this approach is the comparison that can be made between the metrics of a diatom reference state community and those of an impacted community and how the latter may deviate from the expected near-natural conditions (Stevenson 2006).

"Let the Biota tell the Story"

The identification of natural reference state conditions has previously followed the more conventional '*a priori'* approach which is premised on establishing a correspondence between water quality of a river condition and a set of biological data (e.g. diatoms). A two step approach, involving an '*a priori'* classification of the physical attributes of near-natural headwaters based on geomorphology and geology combined with an '*a posteriori'* chemistry-free approach was the modus operandi followed in this research. The scientific logic relies on diatom responses, at the species level**,** to define the position of the sites along a hypothetical environmental gradient. Analysis of the diatom species composition responses therefore provides a classification which is free of assumptions as to the environmental factors that may influence the distribution of such diatom assemblages (Gerritsen *et al*. 2000). Such an approach of "*allowing the biota to tell their own story*" (Clarke & Ainsworth 1993), using exploratory multivariate procedures produces a more robust classification of river reference state sites (Gerritsen *et al.* 2000).

It has been advocated that the term "reference state", should be reserved for describing "*naturalness at one end of a biological gradient"* (Stoddard *et al.* 2006), thus implying the quintessence of biological integrity in the absence of human impacts. The assessment of ecosystem health changes in rivers of South Africa is driven by the imperatives of the National Water Act (Act 36 of 1998). The National Water Act (NWA), as promulgated in 1998, however does not speak to '*reference conditions*' per se but Section 137 Chapter 14 of the National Water Act (Act 36 of 1998) deals with the management of Water Resources including the prevention of the deterioration of surface waters and their rehabilitation to a 'good ecological status'. The imperatives of the NWA are now incorporated in the Implementation Manual of the South African River Health Programme (RHP), underpinned by the regulatory authority and custodian of water resources. These may therefore be regarded as national directives which give credibility to the most authoritative definition of a reference condition for rivers in South Africa.

"[1]*A reference condition is held to be the expected condition that reflects natural or least-impacted physical, chemical and biological characteristics of a site, river reach or river type, in the absence of anthropogenic stress***"** (DWAF 2008)**.**

Identification of reference conditions enables the degree of deviation from natural conditions, typically through human degradation, to be assessed. These are the foundations for developing biological criteria for the protection of aquatic ecosystems and evaluating impacts at monitoring sites (Dallas 2000; Dallas 2002). However, the level of deviation from a natural state is presented as an ecological and management narrative which is open to interpretation rather than evaluated against ecological class boundaries (Palmer *et al*. 2003).

Recent examples of this more relevant approach have been described in research papers in the last few years elsewhere in the world, notably those from Europe (Eloranta & Soininen 2002, Acs *et al.* 2004, Rimet *et al.* 2004, Kelly *et al.* 2008), from the USA (Fore & Grafe 2002, Stoddard *et al*. 2006), from Canada (Grenier *et al.* 2006, Lavoie *et al*. 2006) and from Australia (Chessman *et al*. 2007a). The absence of clearly defined "*Reference State Conditions*" for the headwaters of rivers (i.e. upstream of human interventions) was a previously recognized practical and theoretical weakness of several bio-monitoring protocols (Stevenson 2006).

The situation was no different in South Africa (DWAF 2008). Previous research and bio-monitoring protocols using diatom communities from South African rivers have not followed the more pragmatic reference state approach described above. This deficiency made it more difficult to interpret changes in the structure of impacted communities observed downstream. The use of diatoms, as primary producers, offers an additional benefit of improved confidence in river health assessments to be derived from simultaneous surveillance of biological measures at different trophic levels when attempting to describe pollution impacts (Hofmann 1996). Several prominent international river ecologists have in the past pointed out some of the weaknesses of only relying on secondary consumers, such as aquatic invertebrates, that are positioned at a higher trophic level and / or relying solely on chemical information as a substitute measure of biological condition of a river (Cholnoky 1960a, Round 1991, Karr & Chu 1997, Kelly 1998).

National regulatory directives, notably the USA Amended Clean Water Act (USA CWA 1972), the Australian and New Zealand Water Reform Framework (ANZECC & ARMCANZ 2000), the European Water Framework Directive (European Union 2000) and the National Aquatic Ecosystem Health Monitoring Programme (DWA&F 2008) have also

[1] See also similar definitions given by Uys (1994), the Australian Water Reform Framework (2000), EU Directive (Wallin *et al*. 2003), and the USA Clean Water Act (Stoddard *et al.* 2006).

stimulated a fundamental shift in emphasis from the initial focus solely on chemical and physical attributes of water quality to the wider use of biological assessment protocols. However, the added-value of using diatom information in water quality management has been overlooked until relatively recently in many parts of the world despite several scientific and technical advantages offered by diatoms (John 1998, Weilhoefer & Pan 2006, Walker & Pan 2006, Chessman *et al*. 2007a, 2007b; Raunio & Soininen 2007). Nevertheless, diatoms are now being increasingly introduced and accepted world-wide as part of regular bio-monitoring programmes (Kwandrans *et al*. 1998, Chessman *et al*. 1999, Kelly 2003, Acs *et al.* 2004, Tison *et al.* 2005, 2007; Potapova & Charles 2007, Porter *et al.* 2008, Kelly *et al.* 2008).

The utility of diatom assemblage attributes as effective and appropriate biological measures of stream condition has also been largely disregarded in South Africa over the last twenty years despite previous important historic taxonomic and ecological studies of river conditions (Cholnoky 1968a, Schoeman 1971, Archibald 1968, 1981). The National Water Resources strategy was that part of the Act that was designed to address the future condition of environmental water quality as part of a revised national river health programme (Palmer *et al.* 2003). It referred to bio-monitoring but said little about the utility of diatoms as water quality indicators in river health assessments.

Identification and Definition of Historical Reference State Sites

Several diatom research programmes, in more developed parts of the world, have reported the practical difficulties in locating suitable present-day candidate reference sites. Such difficulties were experienced in river systems of France (Tison *et al.* 2007), Finland (Eloranta & Soininen 2002), Hungary (Acs *et al*. 2004) and the United Kingdom where the lack of suitable reference sites has also been reported because of '*the long history of human settlement in river basins*' (Kelly *et al*. 2008). These limited options led to the exploratory use of diatoms extracted from historical plant specimens in museum collections (Nijboer *et al.* 2004, Yallop *et al*. 2004).

The situation in KwaZulu-Natal, a province of South Africa, is quite different and in some respects apparently unique. There are valuable and accurate historical records of diatom data derived from sites free of [2]human disturbance pressures in the headwaters of several rivers of the region. Furthermore the upper reaches of the largest rivers still lie within protected near-natural reserve areas even today. Permanent human habitation and activity is virtually non-existent in these headwater areas. This is ascribed to the extremes of climate, remote rugged terrain, inaccessibility by conventional means (no

[2] See also Appendix III for additional evidence of disturbance-free naturalness of headwater reaches

roads), and few resources for habitation (Thorington-Smith 1952) and to the implementation of a long standing conservation policy for these near-pristine protected areas.

The reference state selected for this investigation corresponds with undisturbed conditions in the headwaters that are truly free of human influences. The historic data set was generated in a period shown to be also consistent with pre-disturbance conditions. There is an intense scientific and administrative debate as to how reference conditions should be defined and identified for comparison with present-day conditions and against potentially degraded monitoring sites (Yallop *et al.* 2009)**.** One of the approaches recommended for establishing reference conditions is the use of historic data, providing that the context can be validated as a period free of human disturbance pressures (Wallin *et al.* 2003, Nijboer *et al.* 2004, Stoddard *et al* 2006, DWAF 2008). A context was therefore established for the interpretation of reference conditions from a broad based development trajectory for the Province of KwaZulu-Natal. The post World War II period (1945-1955) was taken as a historic threshold period prior to significant industrial and urban development in KwaZulu-Natal. It is based partially on evidence confirming that the historic period when the original diatom data was generated (Cholnoky, 1956, 1957, 1960) from the headwaters of KwaZulu-Natal Rivers was free of human disturbance. Substantive evidence of a historical, disturbance-free environment in the headwaters of KwaZulu-Natal Rivers during the 1950's is also derived from expert opinion and quantitative information**.** For example:-

"*The Drakensberg headwaters are the most pristine areas in South Africa, if not in Africa. The Royal Natal National Park was established as a protected area in 1906. The first of the remaining reserve areas in the Drakensberg was Giant's Castle, which was proclaimed in 1924-1925. Tourism in the 1950's had an absolutely minimal effect on the headwaters whilst agriculture has not been a factor at all" (Dr J Vincent pers. comm. 2011 ex Director of Museum Records in KwaZulu-Natal).*

A comprehensive post-World War II study of the Thukela catchment and its tributaries incorporating statistics and quantitative data on geology, vegetation, population, transport communications and industry revealed no such pressures on the headwaters of the Drakensberg (Thorington-Smith 1952). Reference is also made in Appendix III to some dilute water chemistry data and the natural vegetation for headwaters in the Drakensberg in the 1950's, confirming the absence of human impacts on the quality of water of the upper reaches of the rivers of the Thukela basin (Oliff 1960). Narrative extracts on a study of the plant ecology of the protected high altitude zone of the Thukela River basin are also presented in Appendix III indicating a disturbance-free environment for peripheral vegetation covering the sub-catchments of the headwaters of the largest river catchments in the Province of KwaZulu-Natal (Edwards 1967).

The philosophy that "*human community dysfunction will greatly increase if we do not accept that natural resources available to humans are finite"* (Hardin 1968) also has relevance to the future well-being and integrity of our precious headwater resources. It means that the per capita share of the ecological goods and services provided by these natural resources, in this finite world, must steadily decrease with tragic consequences for all if human populations continue to expand and consumption pressures increase. The once uncontaminated and little impacted headwaters of local rivers, here referred to as the "*freshwater commons,"* are now being subjected to the relentless pressures of local development and the more distant incremental daily bulk abstraction demands from external major urban conglomerates. This will ultimately threaten the overall integrity of biological structures and functions of rivers, resulting in the loss of ecological service functions and biodiversity, culminating in the potential collapse of previously balanced ecosystems in a scenario described as the "*Tragedy of the Commons"* (Hardin 1968). Note also the poor state of major South African Rivers as reported in the [3]National Spatial Biodiversity Assessment (Nel *et al.* 2007).

The concept of a high ecological status, as a basis for target reference state conditions in the headwaters of rivers, must therefore necessarily be developed from an assessment of different '*biological quality elements'.* One such key element is that of the diatoms, in particular because of their dominance amongst the microphytobenthos of a river system (Butcher 1932, Kelly 2004). It is crucial therefore to benchmark sites of high ecological status for diatom river reference state communities in KwaZulu-Natal Rivers.

Criteria for Selection and Definition of Reference State Sites

The criteria for selection and definition of a reference state in the headwaters of a river should be focused on conditions closest to a 'near-natural' baseline state as opposed to the alternative concept of the "*Best Attainable Condition*" (Stoddard *et al.* 2006) at an upstream control site. Key steps for establishing such river [4]**reference state conditions** using biota were recommended previously by a select panel of river ecologists from Australia and South Africa (Uys 1994). These recommended steps have not been implemented for diatoms to date in South Africa, and involved:-

- Use of an objective procedure to identify sites of good ecological status
- Use of biological elements to establish similarities / dissimilarities between sites
- Use of species level information

[3] See also recent findings of the National Spatial Biodiversity Assessment of rivers of South Africa (Nel *et al.* 2007) – Appendix III)

[4] Note: The River Health Programme Implementation document advocates the use of an '*a posteriori*' approach using multivariate procedures to identify reference states (DWA&F 2008).

- Use of the chemical and physical characteristics associated with these sites

1.3 Aims and Objectives

This investigation was focused on measuring and interpreting diatom community responses to conditions in headwaters of different categories of river to identify and benchmark type-specific reference state communities. The main research objectives of this investigation were therefore focused on:-

- Description, identification and characterization of diatom assemblages from type-specific 'near-natural' conditions **presently** existing in the headwaters of different categories of KwaZulu-Natal river systems

  (**Table 1**: **Resources A4, B3, C3**) Slide material collected between 2006 and 2009.
- Assessment of the utility of **historic** information retained by diatom assemblages described from samples taken in near-natural conditions from similar reaches of the same headwaters of rivers, prior to catchment development some 50 to 60 years ago.

  (**Table 1: Resources A1-A3, B1, B2, C1, C2**). This historic diatom data was extracted and re-worked from herbarium records and material, covering a wide range of rivers in the study area. Analysis of slide material from the Cholnoky components of the South African Diatom Collection was obtained from the original survey data collected in the period between 1954 and 1970 (Cholnoky 1956, 1957, 1960b, 1968b, 1970a).
- Documentation of diatom responses to different human impact pressures on rivers. (**Table 1**: **Resources D1, E1, F1, G1**).
- Comparison of the response of impacted diatom communities in small urban rivers with minimally disturbed communities, characteristic of the headwaters of small rivers. (**Table 1**: **Resources A3, A4; C3, D1**).
- Derivation of diatom reference state sites for type-specific conditions prevailing in KwaZulu-Natal river systems. (**Table 1**: **Resources A, B, C**).

1.4 Geographic Setting of Study Area

The study area includes the major part of the Province of KwaZulu-Natal which lies between Latitudes -26°.92S and -31°.08S and Longitudes 28°.79E and 32°.93E. The eastern limit is defined by the near-linear coastline of the Indian Ocean. The western limit of the study area is demarcated by the great escarpment of Southern Africa formed by the prominent basalt-capped Drakensberg mountain range that rises to just over 3400 m in the highest places.

Table 1. Matrix of Present Day and Historic data resources in relation to various river systems

A. Surveys of Headwaters of various rivers

Zone	Sub-region	Reference	River Systems	Historic Resources	Present Day 2006-2009
Zone 3	**Montane Uplands**	Cholnoky 1956	Thukela River (Source Zone)	**A1**	
		Cholnoky 1957	Thukela River (Catchment)	**A2**	
		Cholnoky 1960b	KwaZulu-Natal Zone 3 Rivers	**A3**	
		Present (2006-09)	**Zone 3 Rivers : Montane Uplands**		**A4**
Zone 2	**Interior Midlands**	Cholnoky 1957	Selected rivers	**B1**	
		Cholnoky 1960b	KwaZulu-Natal Zone 2 Rivers	**B2**	
		Present (2006-09)	**Zone 2 Rivers : Interior Midlands**		**B3**
Zone 1	**Coastal Lowlands**	Cholnoky 1960b	KwaZulu-Natal Zone 1Rivers	**C1**	
		Cholnoky 1968b	St Lucia Rivers	**C2**	
		Present (2006-09)	**Zone 1 Rivers : Coastal Lowlands**		**C3**

B. Human Impact Studies

Zone	Activity	Reference	River Systems	Historic	Recent
Zone 1	Sewage/Industry	**Archibald 1998/99**	Mbilo /Amanzimnyama Rivers		**D1**
Zone 3	Acid Mine Drainage	**Archibald 1998**	Tshoba River 1998		**E1**
Zone 1	Sugar Waste	Cholnoky 1970a	Nonoti River 1971	**F1**	
		Archibald 1971	Nonoti River 1971	**F1**	
Zone 1	Pulp&Paper Waste	**Archibald 1998/1999**	Lower Thukela River 1999		**G1**

See also Appendix 1 - for global positioning of sites

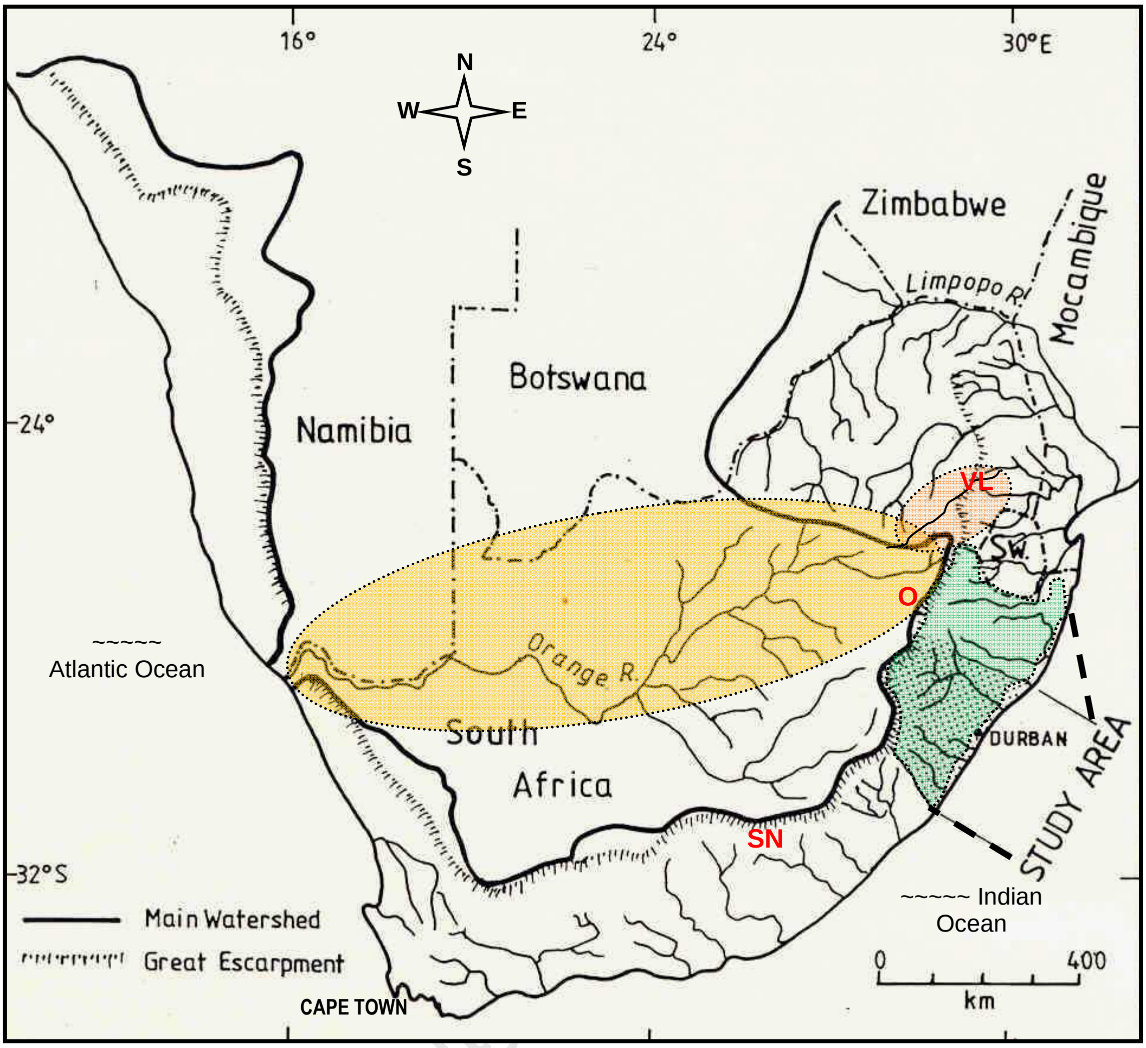


Figure 1 **Study Area (green) incorporates the Rivers of the Province of KwaZulu-Natal on the Eastern seaboard of South Africa.**
The Orange River (orange) incorporates the main river basin and its Vaal tributary**.**
SN - Source of Sundays River : **O** - Source of Orange River : **VL** – Source of Vaal River : **Sw** – Swaziland [Figure 1 adapted from Cooper 1991]

This extensive mountain range is positioned roughly on a curvi-linear north-east to south-west axis and marks the divide between the headwaters of the ten largest rivers of the province and that of the Orange River, South Africa's largest river system. The Orange River basin traverses the entire country from east to west and the river flows westwards from the highest points in Lesotho before discharging into the Atlantic Ocean (**Figure 1**).

The small and relatively short Lebombo range in the north-eastern corner of the province extends from the coastal sand dunes around Lake St Lucia into the neighbouring territory of Swaziland. It separates the expansive coastal flood plain of the Pongola from its interior (**Figure 1.1**).

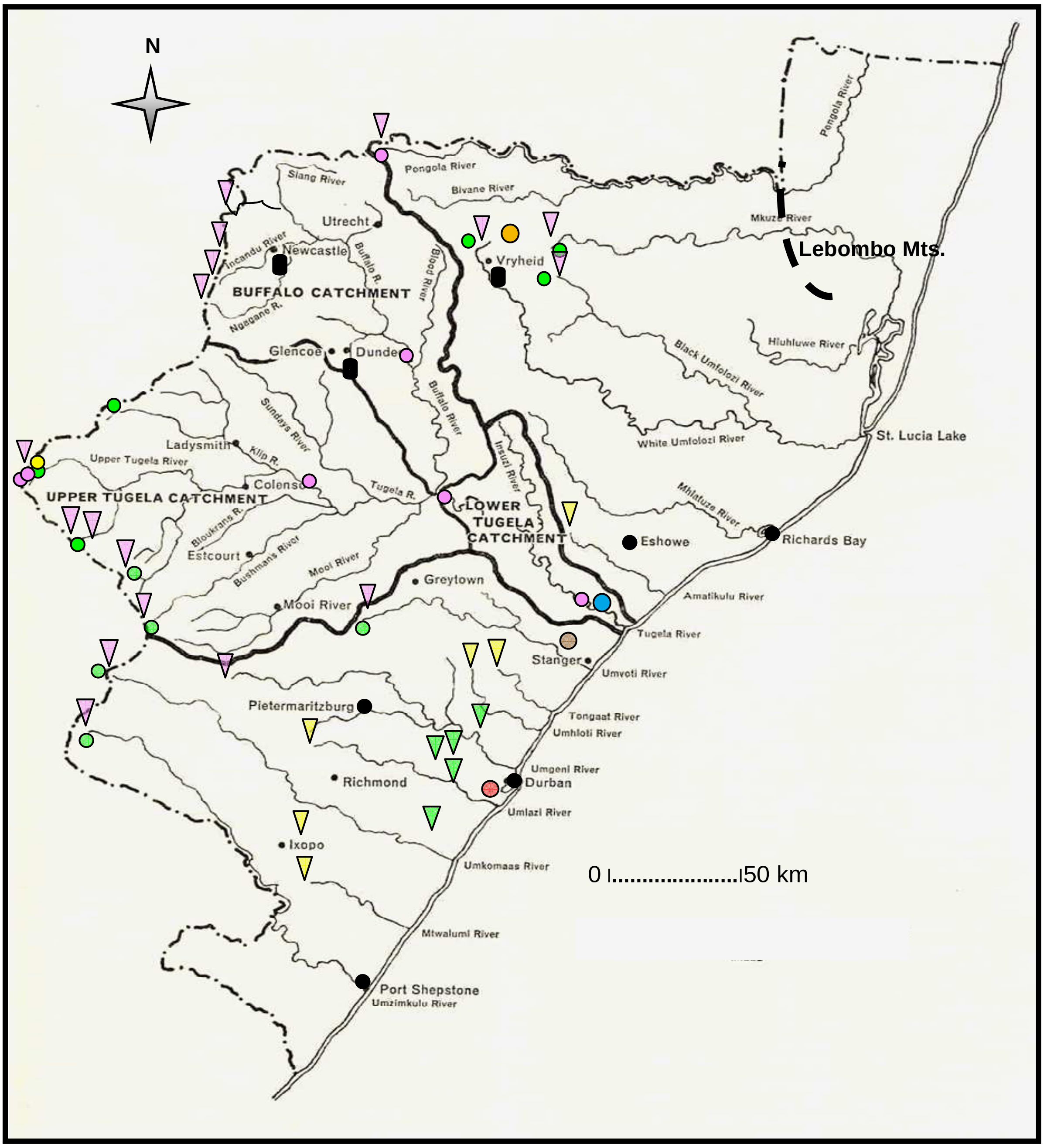


Figure 1.1 Orientation of main river systems relative to major towns and cities of KwaZulu-Natal, showing locations of historic and recent sampling areas.

(Figure adapted from Cooper 1991)

Cholnoky sampling areas	○	(1956)	○	(1957)	○	(1960)
Sampling Areas (2006-2009)	▽	(Zone 3)	▽	(Zone 2)	▽	(Zone 1)
Human Disturbance Pressures						

○	Acid Mine Drainage	○	Pulp & Paper Effluent
○	Sugar Mill Waste	○	Sewage and Industry Waste
▮	Coal Triangle		Newcastle-Vryheid-Dundee
●	Cities: Durban (-29°.865S,31°.028E) Pietermaritzburg (-29°. 599S) 30°.383E)		

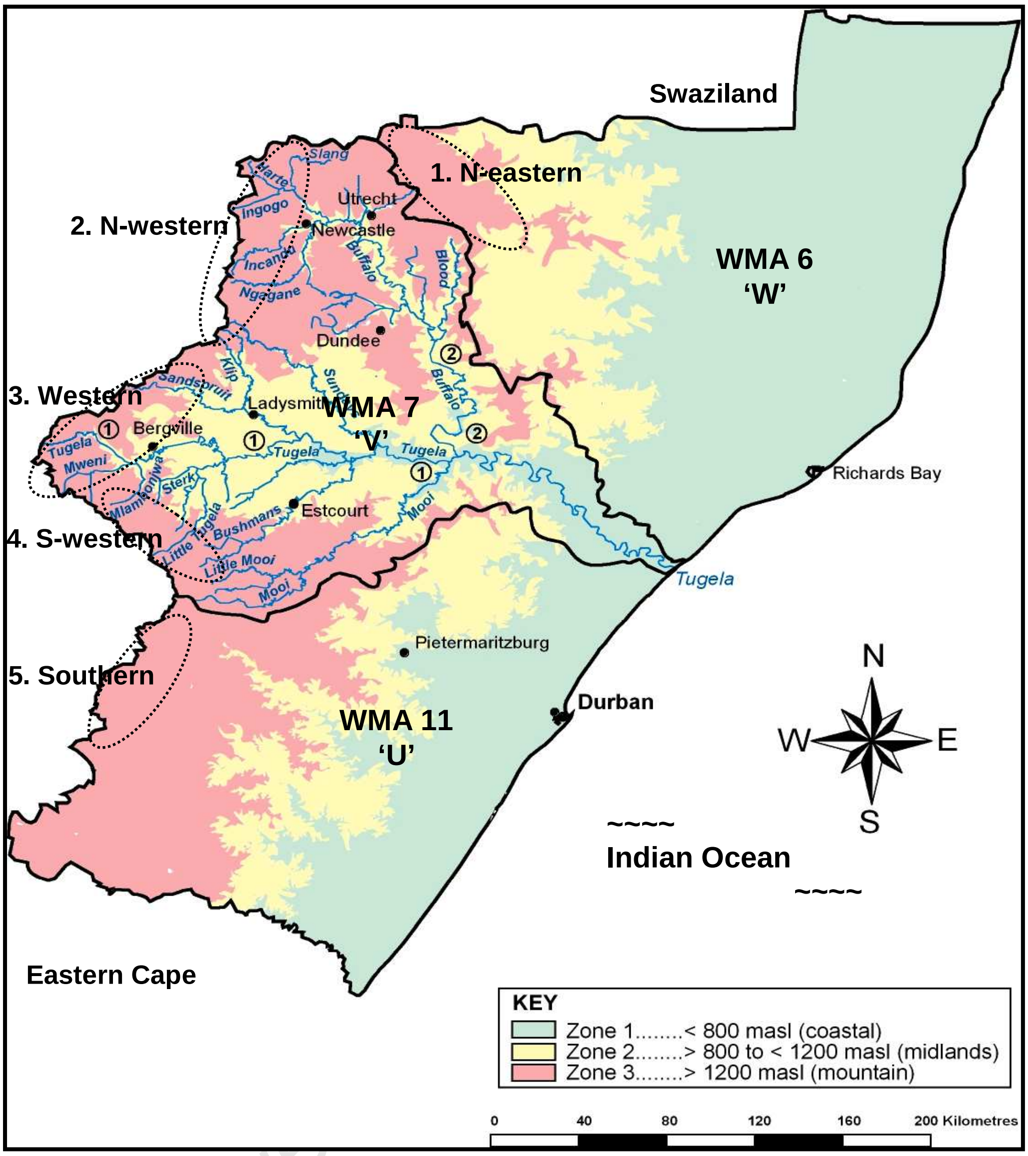


Figure 1.2 **Overlay of contoured zonation on three Water Management Areas of KwaZulu-Natal showing the two basins of the Thukela River (Basin1-Main River and Basin2-Buffalo Tributary) and sub-regional headwaters of Rivers in Zone 3.** (Archibald 1996)

Sub-regions
(1) North-eastern headwaters (Pongola, Mkuze and Mfolozi)
(2) North-western headwater tributaries of the Buffalo River tributary (Thukela)
(3) Western headwaters of main Thukela River
(4) South-western headwater tributaries of the Thukela River
(5) Southern headwaters (Mkomazi, Mgeni, Mzimkulu)

Approximately 80 rivers in the province drain eastwards into the sea via estuaries, coastal lagoons and river mouths along the eastern seaboard of South Africa between Port Shepstone in the south and St Lucia in the north (Begg 1978). The importance of these relatively short rivers is accentuated because these systems collectively generate nearly 60% of the Mean Annual Runoff of the country (van der Molen 2000). The centrally placed Thukela (Tugela) River Basin is the largest in KwaZulu-Natal and drains a catchment area of about 29 000 km^2 (Cooper 1991).

This river basin forms the central Water Management Area (**WMA7-Drainage Region 'V'**) of the province (**Figures 1.1, 1.2**).Two other Water Management Areas span the province of KwaZulu-Natal. One extends to the north of the Thukela Basin and incorporates the Pongola, Mkuze, Mfolozi and Mhlatuze Rivers (**WMA6-Drainage Region 'W'**) including all the large natural lakes of the province such as Kosi, Sibayi and St Lucia, - a World Heritage Site (**Figure 1.1**). The Pongola River is the most northerly of the large rivers in KwaZulu-Natal and forms the boundary with the adjacent province of Mpumalanga and Swaziland. It finally discharges into Maputo Bay in Mocambique. The third Water Management Area lies to the south of the Thukela River and includes the headwaters of the large Mgeni, Mkomazi, and Mzimkulu Rivers (**WMA11-Drainage Region 'U'**) (**Figures 1.1, 1.2**).

1.5 Diatom Assemblages – Crucial Biological Quality Elements

"*A biological quality element that responds precisely and monotonically to gradients of human disturbance is not only a useful measure of biotic integrity but is an attribute that can be used practically to determine deviations from reference conditions***"** (Stevenson & Smol 2003). Metrics derived from diatom assemblages in rivers, free of human disturbance pressures, have been used successfully to assess riverine environmental conditions from many parts of the world (John 1998, Kelly 1998, 2004; Acs 2004, Eloranta & Soininen 2002, Porter *et al*. 2008, Potapova & Charles 2003, 2007). Diatom assemblages are valuable and crucial biological elements in assessments of river 'health' because they retain intrinsically relevant environmental information at the base of the food chain where there is a **direct correspondence** with water quality, particularly in near-natural headwaters (Hynes 1963, Kemp 1963, Leland & Porter 2000).

Human disturbances should therefore be measurable against a 'reference state' as indicated by the diatom communities (Taylor 1997).There would be an expectation of marked changes in algal attributes and species composition under such conditions (John 1998, 2004; Weilhoffer & Pan 2006, Chessman *et al.* 2007a). Great importance is therefore attached to the attributes associated with the assemblages as crucial elements constituting the diatom '*reference state'* communities (Porter *et al*. 2008). The measurement of the relative abundance of species also provides key insights of the structural stability of the biota of an aquatic system. Early recognition of the crucial role of diatom assemblages, as biological quality elements, also came from previous research on diatoms from rivers in South Africa (Cholnoky 1960a, 1968a). Similar findings have been made in other parts of the world by several other advocates of the pivotal role of diatoms in river 'health' assessments, notably by leading diatomologists (Round 1991, John 1998, Kelly 1998, 2004; Stevenson & Smol 2003).

Ad hoc short-term studies of river diatoms have also been made previously in several parts of South Africa between 1970 and 1980 relating water quality conditions to species composition (Schoeman 1976, 1979a).The response of diatom communities to changing salinity gradients in the Sundays River, Eastern Cape Province was the first comprehensive spatial analysis of an entire river system in South Africa (Archibald 1981). Other studies resulted in some informative findings on the relationship between water quality changes and diatom species composition (Cholnoky 1958, Archibald 1972). The spin-off from all this early research on diatoms led to the establishment of the comprehensive South African Diatom Collection which now contains a legacy of historic taxonomic records held in preserved material and on diatom slides. More recently the correspondence between water quality and diatom communities was tested in selected river systems of the provinces of the Eastern Cape, Western Cape and Mpumalanga (van der Molen *et al.* 1998, 2000). The relationship between water quality changes and the response of diatoms in some other river systems in the interior of South Africa has also been investigated while the efficacy of European-derived diatom water quality indices, in particular that of the IPS diatom index, has also been tested (de la Rey *et al.* 2004, Taylor 2004b, Taylor *et al.* 2005b, 2006, 2007c, 2007d).

However no meaningful work on diatom communities of KwaZulu-Natal Rivers has been carried out since the 1955-1960 period. The historic, mostly taxonomic surveys, of river diatoms were not designed to provide a comprehensive interpretation of conditions for benchmarking diatom reference state communities in KwaZulu-Natal (Cholnoky 1956, 1957, 1960b). Hence this diatom material with the associated taxonomic and analysis record sheets have remained dormant in the South African Diatom Collection for nearly 60 years. The collection contains valuable literature and approximately 26 000 diatom slides, including those from the study area. It is presently housed at the University of the Northwest in Potchefstroom under the custodianship of the South African Institute for Biodiversity and is regarded as the largest of its kind in Africa (Harding *et al.* 2005).

1.6 Hypothesis and Philosophy of Research

A general supposition was made that the headwaters of rivers, free of human disturbance pressures, would be expected to engender diatom assemblages of high ecological status, conforming to an aquatic environment with a high degree of naturalness, associated with a reference state condition.

- Furthermore, the structure of a diatom community (as revealed by collective species level responses and attributes of an assemblage) would be expected to vary with stream condition and provide reliable diagnostic information for the identification of diatom reference states under near-natural conditions.

- A chemistry-free diatom-based approach was expected, theoretically, to provide an objective method for the derivation of such diatom reference state communities.
- A further supposition was made that a diatom assemblage is a biological quality element that intrinsically responds to gradients in the environmental condition of a river.

The autecological attributes, at a species level, would be expected to provide a collective response signal from river conditions exposed to varying degrees of human impact different to that observed at type specific reference state sites. A set of key questions and expectations arise out of the suppositions presented above in the quest to define diatom 'reference' state conditions in KwaZulu-Natal Rivers. *"It is necessary to establish a biologically-derived baseline corresponding to river conditions where there has been little or no human influence"* (Karr & Chu 1997)

[a] ***How do diatom assemblages vary between headwater sites in KwaZulu-Natal Rivers and what are the attributes of diatom species assemblages recorded from the least impacted river sites of the region***?

A high ecological status of the near-natural headwaters of various river categories was expected to produce diatom assemblages with a species composition that reflected uncontaminated river water. (Chapter 6)

[b] [5]**Which are *the dominant diatom species representative of reference state conditions in local rivers, and how do these compare with those from headwaters in other rivers of the world?*** *(Chapter 6)*

The ubiquity and sub-cosmopolitan nature of many river diatoms implies that the autecology of the dominants might also be applicable and relevant across continents while still making allowances for endemism. The autecology of key dominant species from uncontaminated waters was expected to be similar to that found in other parts of the world and therefore similar dominant species would be expected to occur locally in least disturbed river reaches. The relative abundance of the dominant species is regarded as a critical quantitative indicator of the ecological status of a river (Cholnoky 1968a, Round 1991).

[c] ***How do variations in the response patterns of diatom communities relate to gradients over time in geology/ water quality at a small spatial scale of river zones***? (Scenario A2 Chapter 5)

Diatom assemblages drawn from uncontaminated headwaters with similar environmental features would be expected to be similar and conversely assemblages

[5] See Table 6.7 for comparison of dominant reference state taxa from other parts of the world

would be expected to differ between headwaters with different environmental characteristics. Diatoms in different undisturbed headwaters of rivers of a region would be expected to respond to gradients in elevation, geology and water quality between Montane Uplands and Coastal Lowland sites.

[d] ***What are the differences in the attributes of diatom assemblages between sites in near-natural conditions (reference state sites) and those at sites impacted by human activity****?* (Scenario Chapter 5)

There was an expectation that the environmental gradients resulting from the most severe human impacts to which rivers are subjected would be measureable from changes in the attributes of diatom species composition as specific indicators of different types of pollution.

1.7 Summary

Some key concepts have been introduced in this chapter and several key issues are addressed in greater detail in subsequent chapters.

- Previous research on diatoms identified that a shift in the structural characteristics of an assemblage could be related to the sensitivity of diatoms to changing gradients in water quality. The relative abundance of species in an assemblage is promoted as a more meaningful measure in diagnosing reference site communities than floral inventories of diatom species alone.
- Diatom community analysis of a near-natural river condition (reference state sites), at the species level, will provide an added-value measure of biological integrity at an additional key trophic level at the base of the food chain.
- A two step '*a priori'* and an '*a posteriori'* chemistry-free diatom-based approach is promoted as a valid objective protocol to define and delineate reference state sites along environmental gradients, at an appropriate geographic scale.
- The simultaneous availability of **two key resources** from the same region, namely that of near-natural **present-day reference site conditions** in the protected headwaters of river catchments, together with the existence of valid **historic diatom data** from the same headwaters, is considered to be a unique circumstance in the quest to identify reference state communities**.**
- Analysis of historic diatom assemblages, which retain intrinsic historic environmental information of river conditions, will reveal historical water quality conditions and the historical ecological status of a river. The real possibility of identification of diatom 'Reference State' communities for rivers of the study area may be derived from the confirmation of a pre-existing high ecological status of a candidate site, thus justifying the use and scientific value of unique historic diatom data.

CHAPTER 2

THE DELINEATION OF SPATIAL ZONES FOR CLASSIFICATION OF HEADWATERS IN RIVERS OF KWAZULU-NATAL

2.1 Introduction

2.2 Geological Features and Formations in Headwaters

2.3 Spatial Frameworks for Definition of Reference State Sites

2.3.1 Eco-regions

2.3.2 Headwater Spatial Zones

2.4 Morphometric Characteristics of River Basins

2.5 Hydrological Characteristics of Rivers

2.6 Discussion

THE DELINEATION OF SPATIAL ZONES FOR THE CLASSIFICATION OF HEADWATERS IN RIVERS OF KWAZULU-NATAL

2.1 Introduction

Geological characteristics and geomorphological features assume two important dynamic functions in the differentiation of the headwaters of river systems. Firstly, a description of the geomorphology of an area gives some understanding of the underlying form of structural features in a river basin at a regional scale. Geomorphic characteristics of rivers also provide an indication of river profiles and in-system features and give some explanation of the consequent hydraulic characteristics of the drainage channel (Chessman *et al.* 2006). The diversity in fluvial geomorphology of headwaters has also been shown to create an expectation of differences in the responses of biological communities (Fryirs 2001).The second function is the bearing and influence that geology has on the correspondence between lithology, soils and the ionic composition of uncontaminated, near-natural headwaters of rivers and streams (De Villiers 2005). These two functions are related but have different dynamics and time frames. The former process is measurable on a geological time scale, the latter is related to shorter time scales associated with weathering, seasonal fluctuations of precipitation, flow regimes and more immediate biological responses. The weathering of rocks and soils and the consequent dissolution of chemical constituents that provide essential chemical constituents of river waters has been linked fundamentally to river basin geology (Biggs 1990, Leland & Porter 2000). A decisive conclusion reached after an intensive study of the headwater stream dynamics of a near-natural forested ecosystem of Hubbard Brook in the United States was that "c*omparisons of biogeochemical data from natural systems with those that have been manipulated by man provide important information about the functional efficiency or 'health' of an ecosystem*"(Likens *et al.* 1977). Such an acute insight gives scientific expression to the principle that in the headwaters 'the *valley rules the stream"* (Hynes 1975). Sections in this chapter build on this maxim and outline the influence of elements of the macro-environment on the naturalness of the headwaters of river basins.

A brief account is given of aspects of geomorphology, geology and the hydrology that are pertinent to the objectives of describing the natural geological reference state template against which diatom communities are most likely to conform. These environmental elements underpin the geological and water quality template of reference state conditions and add substance to the rationale behind the classification that "a *reference state condition should be inferred from river reaches which have had little or no artificial modification or alteration by man"* (Fryirs 2001). It was deemed logical to confine the spatial scale to smaller headwater zones within a region, such that three relatively small headwater spatial zones were identified for local rivers. Type specific conditions of the headwaters in each spatial zone were by definition not comparable across the country or longitudinally downstream because the geological templates in each zone are different hence engendering different diatom responses even in the absence of human disturbance. The great diversity of headwater catchment characteristics across South Africa does not allow for applications in regions of the country outside the province. A comparison of deviations between monitoring sites and type specific reference conditions in headwaters is only relevant within each spatial zone of the Province of KwaZulu-Natal (See also Chapter 8).

2.2 Geological Features and Formations in the Headwaters

The fundamental geological features of the province are presented in a map of KwaZulu-Natal (van der Eyk *et al*.1969) (**Figure 2**). The geological systems in the province appear as two distinct components namely, a younger northern-eastern extremity of recent sediments of the Pongola flood plain separated by the Lebombo Range from the older central hinterland stretching westwards and southwards, incorporating the remainder of the river basins of the province **(Figures 1.1, 2)**. The Pongola River basin with its distinctive flood plain is the most geologically differentiated of all other river systems in the study area (Archibald *et al.*1969). The geology of the higher lying headwaters of the Pongola River, unlike other major rivers, is formed from granites with small unique outcrops of the Witwatersrand and Dominion Reef systems in the central area of the basin. The flood plain occupies the most north-eastern part of the region and it is built from Tertiary and Recent sediments nearer to the coast. A band of Cretaceous rocks and a strip of Stormberg rhyolites and basalts border the Lebombo mountain range extending into Swaziland to the north. The high-lying components of the headwaters of the other major rivers of this sub-region (Mfolozi, Mkuze) are located within the Ecca series, in the vicinity of Vryheid. The highest terrain of the headwaters, in the younger north-eastern sector, reaches altitudes in excess of 2000 m.a.s.l. and is characterised by the Drakensberg volcanics and basalt capping of the Stormberg series (**Figure 2**).

A distinct coastal basement complex, consisting mainly of granites, is exposed in a relatively narrow band which runs on both sides of the **axis of tensional folding** in the southern sector. This band starts from a southern coastal shore position and runs obliquely northwards across the province to a position about 40 km inland before major faults disrupt the pattern to the north of Eshowe (**Figure 2**). The identifiable major units of the hinterland geology show up as bands of outcrops running almost parallel to the coastal margin. Such a symmetrical spatial arrangement was originally described as being caused by the "*Natal monocline"* (King 1940).

This interpretation was however later discredited and the geological feature which is unique and peculiar to the eastern seaboard was shown to have resulted from extensive tensional folding on a north-south axis (Maud 1961). The headwaters of 60 small rivers were created between Eshowe in the north and Port Shepstone in the south by this folding which occurs at about the 800 m contour. Distinctive and relatively steeply graded river profiles result from this structural feature (**Figures 1.1, 2**). The coastal area extends in a narrow section south of Durban to Richards Bay and thereafter expands into the broader Pongola coastal flood plain in the extreme north-east corner of the province.

Broad bands of progressively younger outcrops of the Karoo Supergroup (as constituted by the Dwyka, Ecca, Beaufort and Stormberg series) occur at increasing altitude and distance from the coast. These features also run the length of the province, almost parallel to the coastal margin **(Figure 2**). The Thukela River basin extends between Latitudes -27^{o}.41S and -29 ^{o}40S and between Longitudes 28^{o}.96E and 31^{o}.44E and is differentiated into the main Thukela channel draining the headwaters of a south-western sub-catchment and the north-western branch of the Buffalo sub-catchment (**Figures 1.1, 1.2**).The upper Thukela headwaters drain eastward through a succession of sedimentary strata of the Karoo Supergroup.

The geology of these headwater streams is varied and includes basaltic lava of the higher Drakensberg, Stormberg and Beaufort series, and old granite with beds of Table Mountain Sandstone (TMS). Most of the headwater tributaries of the middle Thukela Basin are underlain by carboniferous sandstones and shales of the coal-rich seams of the Ecca series within the coal triangle (**Figure 1.2**). Towards the south-east, older sandstones overlay basement complex granites and gneisses, with the sequence largely reversed near the coast, as a result of the tensional folding (**Figure 2**). The north-western headwaters of the upper Buffalo River sub-catchment, to the west of Newcastle, are dominated by rocks of the Ecca series (**Figures 1.1, 2**).

The geological structure of the headwaters of river basins to the south-west of the Thukela basin is relatively simple. The strata are almost horizontally disposed with rocks of the Beaufort series forming the high-lying western parts of the larger river basins (e.g. the Mgeni and Mkomazi Rivers). These are followed in normal sequence by outcrops of the successively older and lower-lying Ecca, Dwyka, Table Mountain Sandstone (TMS) series and granite rocks which all become exposed in irregular belts almost parallel to the coastline (Kemp *et al*. 1976) (**Figure 2**). The headwaters in the southernmost parts of KwaZulu-Natal are built on the Stormberg series which give way in succession to shales and sandstones of the Beaufort and Ecca series and older Dykwa formations.

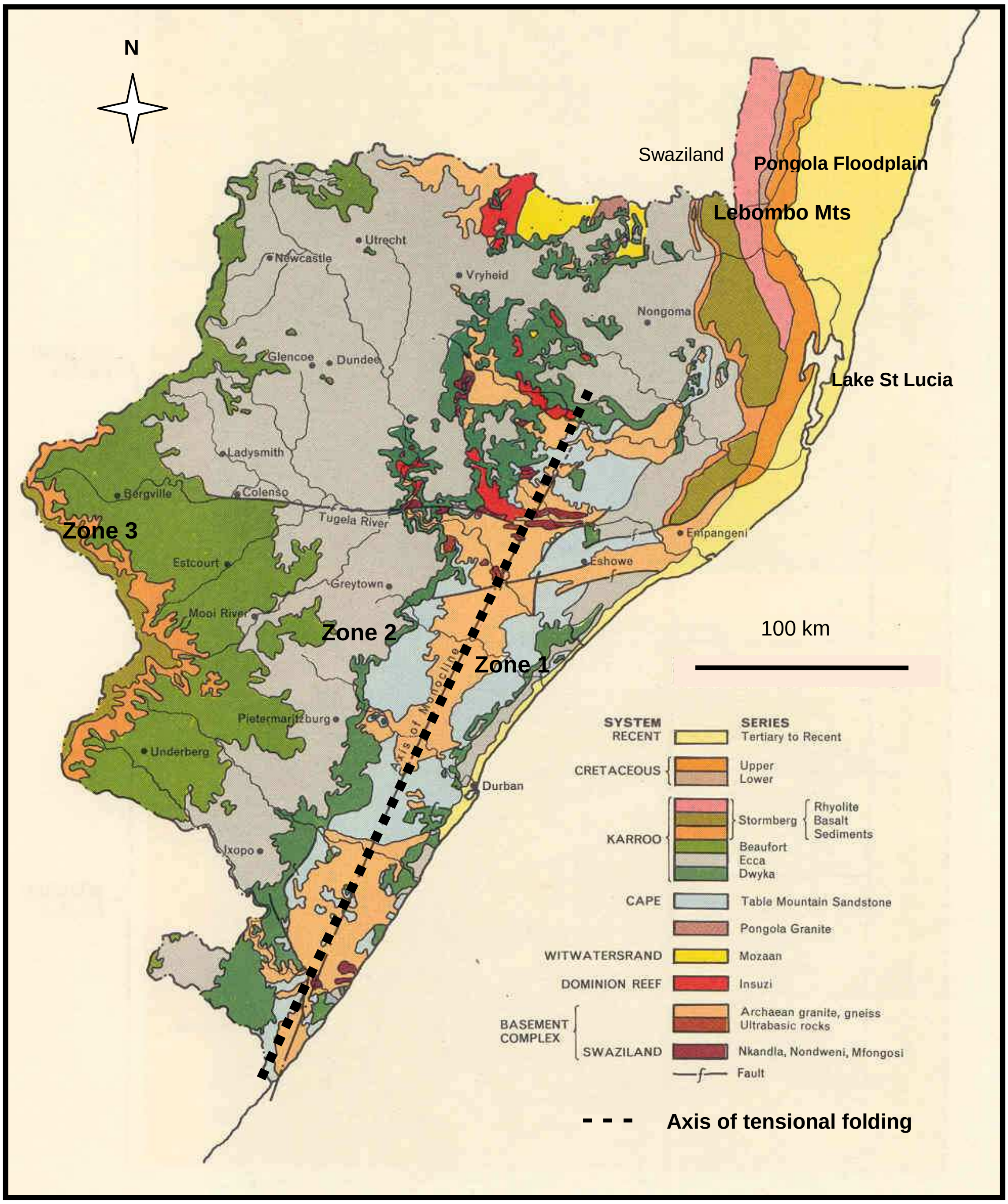


Figure 2 **The orientation of the main geological formations of the Province of KwaZulu-Natal showing the coastal axis of tensional folding**

(After van der Eyk *et al*. 1969)

A Table Mountain Sandstone formation is evident in the headwaters of rivers draining the Coastal Lowlands Typically the headwaters of these rivers are dominated by older granites and Natal Cretaceous Series at the coast (**Figure 2**). An analysis of the percentage outcrops of dominant geology and lithology of the headwaters of three river categories shows distinct differences between the North-west (Pongola, Mkuze, Mfolozi) and the South-west (Thukela-Mzimkulu) (**Table 2.1**) There is also a general trend (gradient) in percentages of the series of a younger Karoo System from the headwaters of large rivers through to the older Table Mountain Sandstone and granites of the headwaters of small rivers in the Coastal Lowlands (**Table 2.1**).

2.3 Spatial Frameworks for the Definition of Reference State Sites

Several diatom studies have demonstrated the advantages of limiting the spatial scale of a study area when attempting to define diatom reference state sites over large areas (Kutka & Richards 1996, Pan *et al*. 2000, Tison *et al*. 2005, 2007, Philibert *et al.* 2006, Potapova & Charles *et al.* 2003, 2007). This strategy minimises inherent variability of physiographic features and regional geochemistry and reduces the possibility of water quality differences and consequent poor correspondence with diatom responses. It was physically impossible and scientifically inappropriate to extend a research programme of this kind on a large scale to incorporate the diversity of river networks and geological variations of South Africa.

The operating philosophy promoted in the most recent documentation of the South African River Health Programme also recognised differences of scale and the advantages of using smaller scale eco-regions in developing reference conditions for river management on a national level (Kleynhans *et al*. 2005, 2007). Small spatial units, therefore, have been proposed as logical entities for the definition of reference state conditions, precisely because the physiographic and natural features are less variable.

2.3.1 Eco-regions

The concept of eco-regions was developed in the United States. The classification shows progressively increased environmental detail at each level and is premised on the existence of relatively homogenous geographical areas sustaining common biotic and abiotic components (Omernik 1987). A similar approach has been developed at a national level in South Africa (Kleynhans *et al.* 2005, 2007) (**Figure 2.1**). The coverage of the Level I and Level II eco-regions proposed for the country was compared with the proposed classification of discrete spatial zones derived from the unique natural geomorphological features of the province, as conceived for this investigation.

The Level I and II eco-regions, as defined for South Africa, however, show a measure of incongruence and lack of physical correspondence between amorphous-shaped eco-region units and the geology of the predominantly east-west orientated, well defined discrete river basins. Three of the nationally defined eco-regions, No^{s} 14, 16 and 17, cut across the river basins in parts of the province while N^{o}13 has no rivers with headwaters in the eco-region (**Figure 2.1**).

Table 2.1 Dominant lithology and outcrops (%) in the headwaters of rivers originating in three spatial zones in KwaZulu-Natal

Spatial Category ▼	River System ▼	Source Altitude m.a.s.l.	River Length km	Dominant Geological Series	Dominant Lithology	% Headwater Outcrops Geological Series: Recent	Beaufort	Ecca	Dwyka	TMS	Granite
Zone 3	*Source > 1200 m.a.s.l. - Montane Uplands rivers traversing three zones*										
	1 Pongola	1850	320	Bushveld Complex	Pongola Granite						100
	2 Mkuze	1230	306	Ecca	Shales			100			
	3 Mfolozi	1250	395	Ecca	Shales			100			
	4 Thukela	3109	405	Stormberg	Basalt		100				
	5 Mvoti	1479	197	TMS	Sandstone		18	82			
	6 Mgeni	1829	232	Beaufort	Beaufort		100				
	7 Mkomazi	2650	298	Stormberg	Basalt		100				
	8 Mzimkulu	2440	329	Stormberg	Basalt		70	30			
Zone 2	*Source 800 -1200 m.a.s.l. - Interior Midlands rivers traversing two zones*										
	1 Amatikulu	760	96	Basement granite	Granite						100
	2 Tongati	747	50	Basement granite	Granite					58	42
	3 Mdloti	854	81	Basement granite	Granite					30	70
	4 Mlazi	1220	145	TMS	Shales		10	90			
	5 Lovu	1200	135	TMS	Shales		50	50			
	6 Mpambinyoni	962	100	TMS	Shales				12	25	63
	7 Mtwalume	985	85	TMS	Shales			50	33	17	
	8 Mzumbe	933	84	TMS	Shales				17	26	57
Zone 1	*Source < 800 m.a.s.l - Coastal Lowland rivers traversing one zone*										
	1 Nonoti	488	38	TMS	Sandstone					100	
	2 Ohlanga	324	28	TMS	Sandstone				60	40	
	3 Mbilo	550	35	TMS	Sandstone					100	
	4 Mhlatuzana	800	50	TMS	Sandstone					38	62
	5 Isipingo	328	27	Basement granite	Granite				100		
	6 Amanzimtoti	274	12	Basement granite	Granite			3	38	34	35
	7 Little Amanzimtoti	165	15	Basement granite	Granite						100
	8 Mahlongwana	430	23	Basement granite	Granite						97
	9 Mzinto	520	37	Basement granite	Granite				3		97
	10 Mtentweni	340	20	TMS	Sandstone						100

(Brand 1967, Archibald 1969, Kemp 1976) TMS - Table Mountain Sandstone (m.a.s.l = metres above sea level)

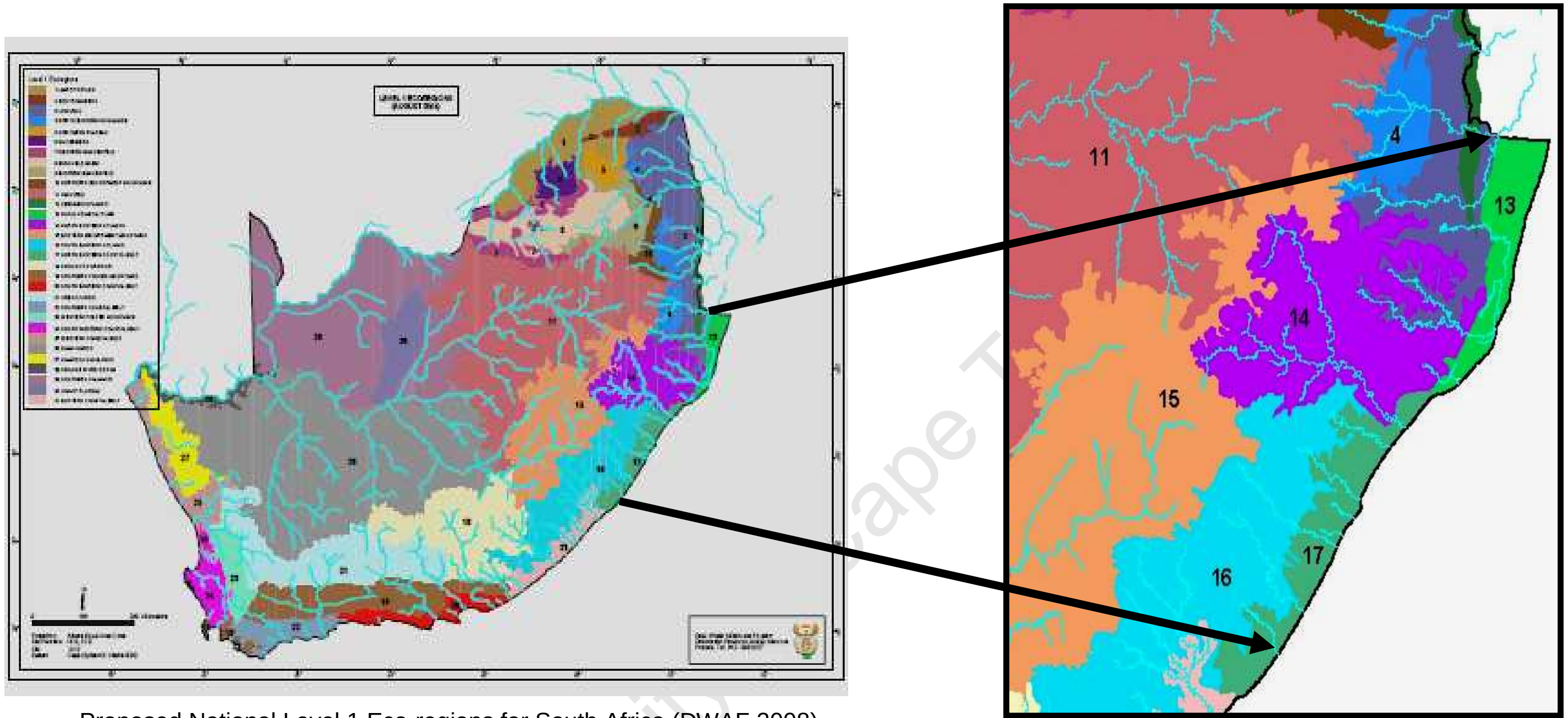
This system has units that have been delimited and defined using descriptors in a national context to make it consistent with the country-wide scheme (Kleynhans *et al.* 2005) and therefore it is less congruent with discrete regional river basins incorporated in the study area. A smaller sub-regional scale was therefore conceived and defined as more appropriate for this study, as evidenced also by findings in similar studies (Kutka & Richards 1996). The classification and delineation of discrete spatial zones was expected to incorporate logical headwater river groupings. It was expected that there would be a better correspondence between the responses of indicator organisms (i.e. diatom assemblages), as candidate target reference state communities, to geological templates of the headwaters of specific river groupings (Metzeling *et al*. 2002, 2006).This investigation was therefore necessarily focused on a discrete and independent set of river headwaters as the key elements representing the entire 'population sample' of the network of east-flowing rivers of the study area. Data from these surveys would allow inferences about the diatom attributes of [6]reference state sites of this entire resource from the characterization of the least impacted communities of rivers in the Province of KwaZulu-Natal. Note also the findings of the National Spatial Biodiversity Assessment (NSBA) of rivers (Nel *et al.* 2007) and the South African River Health Programme which showed the need to manage and protect the headwater tributaries.

2.3.2 Headwater Spatial Zones

The definition of type-specific reference state sites in river systems of the study area is more closely suited to delineation of spatial zones and classification of headwaters, especially where distinct river habitats are evident along the gradient of a river profile. Longitudinal zonation of a river is of prime interest to hydrobiological investigations of a river basin and several distinct habitats have been described in previous river studies of the region (Oliff 1960, Brand *et al.* 1967, Kemp 1969). Differences in altitude (hence water temperature) current speed, river substrate and the gradient in total dissolved solids are factors which influence the responses of the micro-flora, particularly the diatom communities (Oliff 1960, Hynes 1963, Kemp 1969). The need, therefore, for an '*a priori'* chemistry-free classification of 'near natural' sites as potential reference sites dictated that these sites would most likely be located in the headwaters of different categories of these rivers.

These are the locations where river condition is expected to be closest to a near-natural state in terms of its geomorphic and geologic characteristics (Fryirs 2001) and peripheral vegetation.

[6] See also Appendix III for information on natural vegetation of headwaters



Proposed National Level 1 Eco-regions for South Africa (DWAF 2008)

Figure 2.1 Proposed Level 1 Eco-regions covering the study area incorporating KwaZulu-Natal river basins (Kleynhans *et al.* 2005)

13 - Natal Coastal Plain ~ **14 -** North-eastern Uplands ~ **16 -** South-eastern Uplands ~ **17-** North-eastern Coastal Belt.

[Department of Water Affairs and Forestry (2008) NAEHMP : RHP Inception Phase – Record of Decision Report.

Justification for such categorisation of the rivers was based on geological and geomorphological structural features peculiar to the region and the location of the headwaters in spatial zones within a west-east orientation of all the KwaZulu-Natal river basins (**Figure 2**).

Zone 1 Rivers : Eastern Headwaters of the Coastal Lowlands (< 800 m.a.s.l)

The eastern Coastal Lowlands were created naturally by the presence of the distinct axis of tensional folding giving rise to small, short lowland rivers with their headwaters located at altitudes less than 800 m.a.s.l. There are about 60 of these short rivers of less than 75 km in length in this spatial zone. The headwaters of these coastal rivers lie within the mist belt in which grassland and coastal evergreen forest dominate the natural vegetation lining the water courses. These short rivers traverse and drain only one spatial zone and make up the greatest number of rivers in KwaZulu-Natal. The steep hinterland and lack of a coastal plain means that many small rivers have formed independent linkages to the coastline via fresh-water dominated lagoons (Cooper 1991).These small rivers are least important as potable resources because of the low mean annual runoff and because of their low elevation in the coastal zone relative to urban infrastructure. However most of the smaller lagoon systems associated with these rivers have a high recreational potential and ecological value as refuges for juvenile marine fish species along the east coast.

An expansive population of more than eight million people occupies a continuous urban conglomerate stretching several kilometers to the north and south of the City of Durban. Sewage contamination of these smaller rivers is common therefore between the Mpambinyoni, 55 km to the south, and the Tongati, 35 km to the north of Durban. These small urban rivers are regarded as the 'environmental workhorses' because many receive human and industrial waste. The natural flow regime and lagoon mouth dynamics of such small rivers is therefore often altered through flow augmentation from urban inter-basin transfers and cross-catchment return flows. The eastern spatial zone incorporating the **Coastal Lowlands** was defined as **Zone 1** (**Figure 2.2a**).

Zone 2 Rivers : Central Headwaters of the Interior Midlands (800 -1200 m.a.s.l)

The boundaries of the interior plateau of the midlands were set between the 800 - 1200 m contours giving rise to a spatial zone containing the medium sized rivers with their headwaters within these limits. The 1200 m contour was demarcated as the upper boundary of the few rivers in spatial Zone 2 where this contour coincides with an upper summer temperature limit of 15˚C above which trout do not thrive in KwaZulu-Natal Rivers (Kemp 1969). The rivers of these Interior Midlands range in length from 50 - 145 km. (**Table 2.2, Figure 2.2b**). There are ten medium-sized rivers that traverse two spatial zones to reach the

Indian Ocean and all are still typically free-flowing systems. The mean annual run-off volumes are relatively small and therefore construction of large main-channel dams has not been economically viable. The central spatial zone of the **Interior Midlands** was defined as **Zone 2** (**Figure 2.2b**).

Zone 3 Rivers : Western Headwaters of the Montane Uplands (> 1200 m.a.s.l)

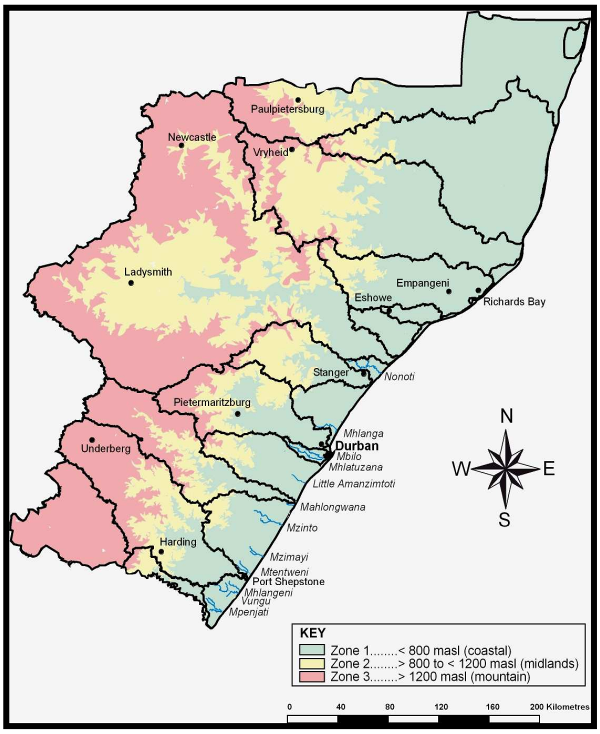
The third natural spatial zone is defined by the barrier created by the Drakensberg Mountains in the west giving rise to the largest rivers of KwaZulu-Natal with their headwaters located at altitudes > 1200 m.a.s.l. The river reaches above this contour in this spatial zone support an important commercial trout industry, the success of which depends on the maintenance of the near-natural ecological features of these rivers. Most of the headwater sites of the ten largest rivers of the province are located at the highest points in the Drakensberg mountain range. The true source of the Thukela and the Mkomazi Rivers, for example, is found in the springs of Mont-aux Sources and Sani Pass respectively at high altitudes between 3000–3200 m.a.s.l. These rivers are also the longest (> 250 km) and traverse all three spatial zones culminating in morphologically and functionally different estuarine systems of high ecological value (e.g. St Lucia Estuary on the Mfolozi River; Richards Bay – a port on the Mhlatuze River, and the Thukela River mouth). The western **Montane Uplands** spatial zone was defined as **Zone 3** (**Figure 2.2c, Table 2.2**).

2.4 Morphometric Characteristics of River Basins

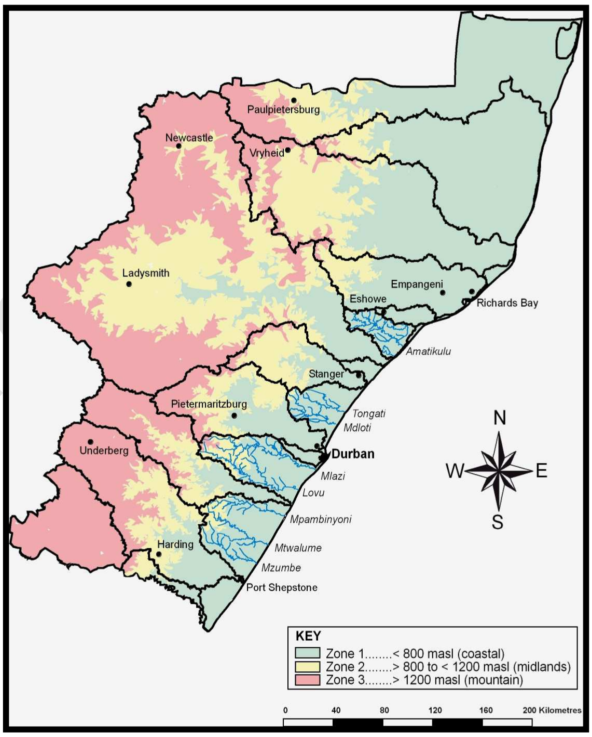
The eastern seaboard of South Africa has natural freshwater resources made up essentially of a network of rivers and some coastal freshwater lakes in the north-east.

A large subterranean aquifer is present in the north-east and this emerges as surface water in a chain of natural freshwater lakes including the world heritage site of Lake St Lucia, now referred to as the iSimangoliso Wetland Park to the north of the town of Richards Bay. The entire province except for the north-eastern corner is traversed by 10 large rivers, 10 medium and about 60 small river systems most of which are located to the south of Durban (**Figures 1.2, 2.2**).

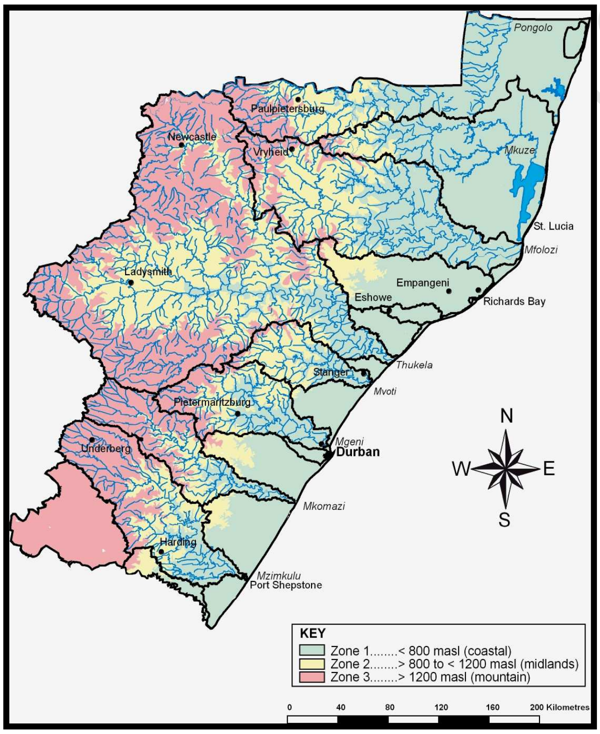
The high elevation of the escarpment (>3000 m.a.s.l) and the relatively short distance to the east coast (<450 km) produces a steep hinterland in comparison with the western side of the subcontinent where the Orange River drains to the west coast over a thousand kilometres (**Figure 1**). This prominent geomorphological divide creates relatively short east-flowing rivers with steep gradients in the upper reaches and a relatively high velocity for the entire length (Cooper 1991).



[a] Spatial Zone 1 Coastal Lowlands: Small Rivers



[b] Spatial Zone 2 Interior Midlands : Medium Rivers



[c] Spatial Zone 3 Montane Uplands : Large Rivers

Figure 2.2 Network of rivers within the delineation of the three spatial zones used in the classification of the headwaters of rivers in KwaZulu-Natal.

[(Archibald 1996) Maps by RA Singh]

River profiling in KwaZulu-Natal is therefore quite complex because the region is bounded inland by the Drakensberg escarpment and the ground falls away steeply to the coast in a series of erosion terraces (Kemp 1969). River courses are narrow and river basins are laterally restricted with deep divides resulting in poorly developed flood plains (Cooper 1991).

Such rivers have been classified as young relatively immature, under-developed systems (Kemp 1969). Most of these large rivers pass through narrow deeply incised coastal bottle-neck gorges at the coast in the absence of distinct flood plains, as exemplified by the Oribi Gorge on the Mzimkulu River **(Figure 3, Chapter 3).** Similar constricted coastal gorges are also found in the lowland valleys of the Mkomazi, Mgeni, Mvoti and Thukela Rivers (**Figure 2.2c**). Relevant data have been presented to show the differences in morphometric, geological and hydrological characteristics of the three river categories (**Tables 2.1, 2.2**). The catchment area of the ten largest rivers ranges over an order of magnitude from 2773 - 29 000 km^2 while river length ranges from 405 - 197 km. The medium size river catchments range from 22 - 956 km^2 with the corresponding length of these rivers ranging from 50 - 145 km. The smallest catchment areas range from 18 - 113 km^2 while the length of the associated rivers ranges from 12 - 50 km (**Table 2.2**).

2.5 Hydrological Characteristics of Rivers

The water resource management strategy of the Province of KwaZulu-Natal is controlled nationally within three Water Management Areas, although the distribution and user functions have been devolved to a local Catchment Management Advisory Forum. The Province of KwaZulu-Natal is considered to be the water-rich region of South Africa because the river systems produce about 60% of the mean annual runoff of the country (van der Molen 2000). This is sustainable due to the magnitude and reliable pattern of the average annual rainfall. The average annual rainfall varies between 1250 mm in the mountains to 650 mm in the drier plateau of the Interior Midlands. It then increases again to 900 mma^{-1} in parts of the Coastal Lowlands (Kemp 1963).The natural flow regime of all KwaZulu-Natal Rivers is subject to regular seasonal fluctuations, with high summer flows (October to March) followed by low winter base flows in the drier winter months (April to September). The consistency of low flows in the winter provided a more stable aquatic environment which favoured a more reliable sampling programme.

Water Management Area WMA6 – (Drainage Region 'W')

The major rivers servicing this area are the Pongola (M.A.R. 1 127x10^6 m^3 a^{-1}), Mfolozi (M.A.R. 729x10^6 m^3 a^{-1}), and the Mkuze (M.A.R. 295 x10^{6}m^{3}a^{-1}) all of which are located to the north of the Port of Richards Bay (**Table 2.2**, **Figure 1.2**). The headwaters of the Pongola River are located in the most north-westerly ranges of the Drakensberg Mountains, to the north of Utrecht. The river flows northwards after traversing the Lebombo Mountains and the wide coastal plain in the north-eastern extremity of the province and finally finds its way into Maputo Bay in Mozambique. The headwaters of the Mkuze and Mfolozi Rivers drain a large area of the coalfields of the north-western part of the province before discharging into the northern and southern sections of Lake St Lucia respectively. It is a characteristic of this water management area that there are few small and medium sized rivers, other than the Hluhluwe River which drains the Mfolozi-Hluhluwe game reserve and subsequently discharges into Lake St Lucia.

Water Management Area WMA7- (**Drainage Region 'V'**)

The Thukela River is the largest of all the rivers with a catchment area of 29 000 km^2 (M.A.R 4 $595x10^6 m^3 a^{-1}$) (**Table 2.2, Figure 1.2**). However the discharge ratio of the entire river system is not necessarily the highest because a large part of the river basin incorporates arid areas of the Interior Midlands which is drained by major tributaries (e.g. Buffalo and Sundays) some of which have a much lower mean annual runoff.

Water Management Area WMA11 – (**Drainage Region 'U**')

The Mgeni River and its tributaries represent one of the most reliable freshwater resources in the province because of an elevated position in relation to the large urban conglomerates of Pietermaritzburg and Durban (**Table 2.2, Figures 1.1, 1.2**). A chain of large dams have been constructed along the Mgeni to sustain the industrial, commercial and domestic needs of 80% of the population of the province, namely Midmar ($230x10^6 m^3$),Albert Falls ($293x10^6 m^3$) and Inanda ($250x10^6 m^3$).

Annual rainfall in the high ground of these large river catchments in the southern area of KwaZulu-Natal (e.g. Mzimkulu and Mkomazi) varies between 900 - 1000 mm but this reduces to 750 mm in the midlands, some 50 - 80 km from the coast. About 80% of the total rainfall occurs in the summer months between October and March (Kemp 1976). Many of the smaller Zone 1 Rivers are located in this area. The smallest river system is that of the Little Amanzimtoti River located to the south of Durban. The river is only 15 km in length and the mean annual runoff is given as $2x10^6\ m^3 a^{-1}$ (**Figure 2.2a**).

The data presented provide a good indication and comparison of the range in natural hydrological characteristics between the largest and smallest rivers of the province (Begg 1978, Cooper 1991) (**Table 2.2**).

2.6 Discussion

The relevance of spatial zones, geology and geomorphology

There is good justification for limiting the spatial scale of the study area to headwater spatial zones to reduce the inherent variability of the physiographic features, regional geochemistry and water quality of rivers when attempting to define diatom reference state sites.

Table 2.2 Morphometric and hydrological characteristics of a selection of KwaZulu-Natal Rivers

Spatial Category ▼	River System ▼	Source Altitude m.a.s.l	Catchment Area km^2	River Length km	Mean Annual Runoff $m^3 x10^6 a^{-1}$	Discharge Ratio $m^3 x10^6 a^{-1} / km^2$
Zone 3	*River Source : > 1200 m.a.s.l originating in Montane Uplands : Large rivers traversing three zones*					
1	Pongola	1850	10243	320	1127	0.110
2	Mkuze	1230	8982	306	295	0.033
3	Mfolozi	1250	10645	395	729	0.068
4	Thukela	3109	29000	405	4595	0.158
5	Mvoti	1479	2773	197	468	0.169
6	Mgeni	1829	4863	232	682	0.140
7	Mkomazi	2650	4183	298	1036	0.248
8	Mzimkulu	2440	6562	329	1478	0.225
Zone 2	*River Source : 800 -1200 m.a.s.l originating in Interior Midlands : Medium rivers traversing two zones*					
1	Amatikulu	760	814	96	201	0.247
2	Tongati	747	422	50	75	0.178
3	Mdloti	854	474	81	117	0.247
4	Mlazi	1220	956	145	68	0.071
5	Lovu	1200	785	135	112	0.143
6	Mpambinyoni	962	548	100	52	0.095
7	Mtwalume	985	553	85	60	0.108
8	Mzumbe	933	549	84	71	0.129
Zone 1	*River Source : < 800 m.a.s.l originating in Coastal Lowlands :Small rivers traversing one zone*					
1	Nonoti	488	180	38	44	0.244
2	Ohlanga	324	105	28	26	0.248
3	Mbilo	550	67	35	5.1	0.076
4	Mhlatuzana	800	113	50	8.5	0.075
5	Isipingo	328	49	27	6.4	0.131
6	Amanzimtoti	274	33	12	1.5	0.045
7	Little Amanzimtoti	165	18	15	1.2	0.067
8	Mahlongwana	430	92	23	12	0.130
9	Mzinto	520	149	37	22	0.148
10	Mtentweni	340	50	20	15	0.300

Updated (Cooper 1991) (m.a.s.l. = metres above sea level)

A fundamentally more pragmatic system of **spatial zonation of the headwaters** of rivers draining the province of KwaZulu-Natal was conceived and is presented as the basis on which a typology for potential reference state sites can be defined. A similar approach was adopted in Australia for a relatively small river system (Fryirs 2001).This formed the basis for linking geomorphic features to fluvial biodiversity in a subsequent study of the same catchment. Differences in river geomorphic features ("*river style*") were reflected in differences in water quality which in turn were attributed to changes in the responses of diatom communities (Chessman *et al.* 2006).

The rationale for classification of headwaters of river basins by spatial zones was based on the geomorphic and geological features of river systems that are unique and peculiar to the eastern seaboard of South Africa. The position, orientation and elevation of the Drakensberg mountain range produced a drainage system of relatively short rivers that flow eastwards along a steep profile. The larger rivers have little opportunity to coalesce and therefore remain as discrete longitudinal basins which have been restricted laterally ending in coastal 'bottleneck' gorges that facilitate steep-sided river outlets to the sea (**Figure 2.2c**).The steep hinterland and tensional folding also means that the headwaters of the many small rivers are confined to the Coastal Lowlands and, in the absence of coastal flood plains, these have formed independent drainage systems and smaller outlets to the sea (Cooper 1991). Similar morphometric features of short steeply profiled rivers also prevail in the coastal zone of New South Wales to the south of Sydney in Australia (Chessman *et al.* 2006).

- The geomorphology of the eastern seaboard of South Africa, in general, and the line of tensional folding in particular provided for the creation of the three physical spatial headwater zones of three different categories of river, namely (i) the Montane Uplands, (ii) the Interior Midlands and (iii) the Coastal Lowlands.
- The basement rock of the headwaters of the three categories of river is located in geologically distinct areas, namely (i) the Montane Uplands are dominated by basalts (ii) the Interior Midlands are dominated by shales and (iii) the Coastal Lowlands are dominated by granites and sandstones (**Table 2.1**).

  Given the influence that geology has on water quality in river systems, there is an expectation that the water quality of the headwater sites will differ naturally. Sub-regional water quality characteristics of surface waters are also closely related to geology and soils of drainage basins (Wetzel 1975). It has been demonstrated from investigations of both local river systems and those in other parts of the world that the lithology of different geological formations has a distinctive influence on the chemical composition of river waters (Hynes 1975, Likens 1977, Kemp 1969, Biggs 1990, Leland & Porter 2000, De Villiers 2005). Differences in water quality of these headwaters are therefore expected to engender distinctive distributions and responses of the diatom communities.
- The hydrological features of the river systems provide a comparison of the dimensions of the three categories of river in the study area. The marked seasonality of the flow regime in all river systems had an important bearing on the development of a sampling strategy to avoid periods of high flow during the summer wet season from October to March.

The headwaters of the large rivers originating in the Montane Uplands have a typically steep profile giving rise to rapid flows in the torrential zone. This is contrasted with the relatively shallow profile of the headwaters of rivers originating in the Interior Midlands and the Coastal Lowlands giving rise to smaller slower flowing streams.

Furthermore the hydrological regime of smaller river systems is also disturbed by human interventions nearer the coast. These rivers experience inter-basin flow augmentation from return flows out of wastewater treatment discharges that alter the normal seasonal flow patterns in the lower reaches of these smaller rivers. The larger rivers, on the other hand are subjected to flow regulation by impoundment of the main channel. However none of these impoundments are located in the upper most headwater reaches where the potential reference sites of the headwaters of the local rivers are located.

CHAPTER 3

FIELDWORK AND ANALYTICAL METHODS

3.1 Introduction

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FIELDWORK AND ANALYTICAL METHODS

3.1 Introduction

Diatom Assessment Protocols are well developed and there is a wealth of information on various best practices for retrieval, extraction and analysis of diatoms. Discussions with many colleagues revealed that personal preferences and circumstances often dictate choice of method for given situations. The methods selected for parts of this investigation were derived essentially from the collective experience of early mentors (Cholnoky 1968a, Archibald 1968, 1981; Schoeman 1971, Schoeman & Archibald 1976) and additionally from literature and updated manuals that have appeared in recent years. All these information sources provide a comprehensive selection of methodologies and techniques detailing similar critical steps for the extraction and investigation of diatom material from rivers irrespective of the purpose of the diatom investigation.

The outputs from some of these initiatives appeared about the time of the commencement of this thesis and there were also useful recent guidelines on diatom methodologies (Kelly *et al*. 2001, D.A.R.E.S. 2004, Chessman *et al*. 2007b). Similar guidelines have now also been published and incorporated in documentation of the South African National Aquatic Ecosystem Health Monitoring Programme (SA NAEHMP), (DWAF 2008) after the commencement of this investigation. The most comprehensive guidelines for diatom research in rivers of South Africa are also incorporated in a method's manual which draws on insights from years of '*hands on*' experience with early South African diatomologists (Taylor *et al*. 2005a Taylor *et al.* 2007a, 2007b).

3.2 Fieldwork Procedures

The approaches adopted in this research have also been influenced by the insights provided in reviews of other biological sampling strategies (Mackenthun 1965, Cairns *et al*. 1972, Cairns *et al* 1973, Cairns 1974, Cairns & Pratt 1986) and a review of the utility of diatoms in river water-monitoring studies (Round 1991).

3.2.1 Diatom Sampling in Rivers

Site selection and spatial coverage

The headwaters of each river were designated as the key elements of the 'target population' of different categories of river in KwaZulu-Natal. A systematic discrete sampling strategy was considered to be most appropriate to fit a principal study objective which was to describe the criteria associated with a reference state condition in these headwaters (Larsen 1997). It was unrealistic to sample the headwaters of every headwater tributary because "*the investigator is faced with a dilemma –"whether to have a large but known or a small but unknown error, and whether logistic expediency or statistical rigour should be the aim. Random sampling has a single advantage over systematic – it permits valid estimates to be* *made of the standard error because every possible sample has an equal and independent probability of being selected, yet it does not necessarily produce the best estimate of the parameters"* (Cassie 1969).The numerous challenges to the development of an adequate sampling design ultimately also depends on making inferences on the entire resource by characterizing a sub-sample. "*Systematic sampling will almost always produce an estimate with less error because the sampling area is covered more evenly. However the error itself cannot be estimated because all possible samples do not have an independent probability of being selected" (Cassie 1969).*

First order headwater tributaries of the upper Thukela River gave the widest possible replication of least-impacted sites and covered the largest number of representatives of tributaries in the upper catchment areas (**Table 1**: Resources A2, A3, A4 : Zone 3 Rivers). There was less opportunity for sampling sites in reaches that could be described as '*free of human impact'* in the headwaters of the Interior Midlands (**Table 1**: Resource B1,B2,B3 - Zone 2 Rivers) and the Coastal Lowlands (**Table 1**: Resource C1, C5 - Zone 1 Rivers). The location of the headwaters of many of the rivers in these two spatial zones was often exposed to some form of intensive cultivation of agricultural land and/or urban development with the implication that most sites may have experienced human disturbance pressures.

Seasonality

The sampling strategy aimed to reduce the variability in species composition responses by standardizing on the preferred substrate and by removing effects of seasonal fluctuations in the river flow regime. Surveys of river sites between 2006 and 2009 were always conducted during the winter low flow period between April and September thus avoiding variations in diatom responses to the major wet season influences. Experience has shown that mid-winter low flow periods are the optimal "windows of opportunity" to sample perennial rivers because the concentrations of various constituents of a river water are expected to stabilize under a relatively constant mid-winter flow regime. No sampling was carried out within three weeks after a rain event to preclude the real possibility of sampling habitats exposed to scouring and displacement of the pre-existing populations.

Micro-habitat substrates

Diatom investigations have in the past not given sufficient attention to the fact that microphytobenthos growths can be spread between different micro-habitats of a river, each with its own characteristic flora (Round 1991). Relatively recent diatom research on the relationship between water quality and diatoms in some South African rivers showed that the models derived from epilithic diatom communities were better than that derived from the epipelon (van der Molen 2000).

It was only rarely necessary to sample the epipsammon or epipelon of soft submerged sandy substrates and / or silty material respectively in the sediment-filled coastal reaches of rivers, where no hard surfaces existed.

Hard surfaces - Cobbles ('Stones-in-current' habitat)

This investigation concentrated on the epilithic diatom component typically associated with hard surfaces of submerged cobbles or stones with a diameter roughly between 150-250 mm (**Figures 3, 3.1**). The upper surface of the cobble was always submerged in flowing water (15-20 cm deep) to ensure that the diatom growth reflected the characteristics of a stable physico-chemical environment at the site of interest. The epilithic component is comparable to the 'stones-in-current' habitat - one of the discrete habitats used for the assessment of invertebrate responses in South African rivers i.e. the 'SASS' invertebrate protocol (Chutter 1972, 1998, Kleynhans *et al*. 2007) (**Figures 3, 3.1)**.

The diatom film was removed from the upper surface of five hand-sized cobbles by firmly scraping or brushing off the material with a small stiff bristled brush into a plastic tray. This exercise was repeated five times from different transects within a 50 m reach of the river or stream while making sure the cobbles were removed randomly from the wadeable part of the stream. The surface scrapings were collected in a tray and subsequently poured into pre-cleaned 100 ml Pyrex jars (**Figure 3.1**). These scrapings were preserved with ethanol in the field (in preference to formalin) for further acid treatment and analysis in the laboratory (Section 3.3) (Taylor *et al*. 2007a). It was an essential part of the procedure to check that the sample contained sufficient living diatoms in a population to avoid dead 'drift' material generated from upstream. A diatom sample was split into two components – Fresh material was always also used for image analysis of living diatoms whilst the preserved component was used for preparation of permanent slides for species composition analysis.

Soft Substrates – Samples from Marginal Sediments

Soft marginal sediments were only sampled in the absence of suitable hard surfaces. Collections were made on rare occasions from soft substrata as a back-up resource. Living diatom material was extracted from the top 5-10 mm of several randomly selected areas within a 10 m river reach using a turkey baster or alternatively a 50 ml plastic syringe. The material was plated into suitable transparent Petri dishes in the laboratory to separate and extract the live diatom components from the sediments for image analysis of species and their specific plastid configurations using the '*light and cover slip*' method (Round 1991, 1993).

[a] Headwaters with riffles and ponds

[b] Cobbles in clear winter flow

[c] Deeply incised coastal valley (Oribi Gorge)

[d] Headwaters of Bushmans River

Figure 3 Mzimkulu River [a] – [c] and Bushmans River at Giant's Castle [d] showing upland and lowland physical features of river sites

[Images - CGM Archibald 2008]

3.2.2 River Water Sampling

In-situ field measurements were made of pH, conductivity, dissolved oxygen and water temperature in the river reach of interest using an Eijkelkamp18.28 multi-parameter analyser fitted with the necessary probes capable of the required precision for these measurements. Measurements of oxygen concentration and conductivity values were also made on 1 litre water samples that were returned to the laboratory.Details of the physical characteristics of each site were recorded with spot photography of downstream and upstream features **(Figures 3, 3.1, 3.2**).

3.3 Laboratory Analytical Methods

Comparison of similar and other approaches described by several diatom researchers in other parts of the world demonstrated the wide range in personal preference for methodologies of treatment and subsequent extraction and counting of the diatoms.

[a] Near-natural headwater reach

[b] Unshaded, steep mountain torrent zone

[c] Low flow, clear stream and cobbles

[d] Diatom film removed from upper surface

Figure 3.1 Mlambonjwa tributary of the Thukela River (Cathedral Peak) showing characteristic features of a typical sampling area in a headwater reach
[Images CGM and BJ Archibald 2008]

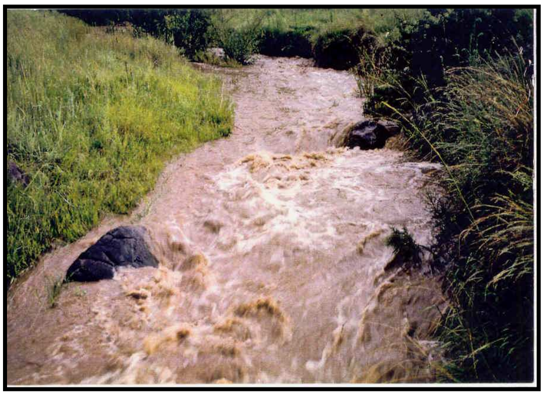
3.3.1 Analysis of Diatom Samples

Two methods were used for cleaning and separating the diatom material by acid digestion. The shorter and safer method was used more extensively, after the first batches of samples were processed in 2006 (Taylor *et al.* 2007a).

The HCl and $KMNO_4$ Method (Taylor *et al.* 2007a)

The organic material within and surrounding the diatom material in a sample was first oxidized with strong acids in order to obtain cleaned material for analysis at the appropriate magnification and for long term storage. The sample was exposed to a mixture of hot HCl and $KMNO_4$ for at least 2 hours. This was recommended as a better practice because it takes into account the human safety factor in frequent exposure to chemicals while processing large batches of samples continuously over several hours (Taylor *et al*. 2007a).

[a] 30 minutes before a storm event



[b] 20 minutes after flooding

Headwater tributary of the Thukela River showing rapid transformation of the flow regime and changes in turbidity after a short-lived intense storm event in the Montane Uplands

[c] Mzimayi River: A small Zone 1 river

[d] Mzumbe River: a medium-sized Zone 2 river

Figure 3.2 Transformation of the flow regime and turbidity [a - b] after a storm event and the ultimate siltation of a river bed in the coastal reaches of rivers [c - d]. [Images CGM Archibald 2006]

The raw diatom material was treated with the acid / permanganate mixture (5 ml of each) sufficient to cover the diatom material, irrespective of the method of collection in the field. The mixture was heated in appropriate 150ml Pyrex beakers and boiled gently for at least two hours on a hot plate, within a fume cupboard.

The acid-cleared samples were washed 5 successive times in distilled water by centrifuging at 2500 r.p.m. for ten minutes to remove traces of acid in the diatom material. The supernatant was discarded after each wash until the final stage when the cleaned sample (diatom pellet) was made up to 2 ml. A drop of the slightly turbid diatom material was then distributed on glass coverslips, covered to prevent dust, and allowed to air dry on a solid undisturbed surface to avoid formation of concentric rings of diatoms.

The H_2SO4 / HNO_3 method (Swatman 1941, adapted by Welsh (1964)

The method followed by early diatomologists in South Africa was first described by Swatman (1941) and later more comprehensively by Welsh (1964). This method was originally used to process the first batch of samples for this study but was discontinued because it was more time consuming and more importantly required handling of more dangerous chemicals. The method was used previously for the substantive taxonomic studies of river diatoms in South Africa and Lesotho respectively (Archibald 1968, 1981, Schoeman 1971)

Image Analysis and Diatom Identification

An Olympus BX51 System microscope, fitted with three objectives (x100, x400 and x1000 oil immersion) and a JVC Digital Color Video camera was used for all microscopic investigations of the diatom material. The microscope was linked directly to a *Packard-Bell-Pentium-D* X-treme Computer loaded with the *Analy**SIS*** software (Analy**SIS** 2001) – a commercially available Soft Imaging System. This package contains features for micro-measurements and storage of images on an internal database. It allows for adjustments in the presentation of the image. Medium-power images (x400) were captured with an *Olympus PLAN 40x/0.65 Ph 2 Objective*. The configuration of the chloroplast of some species was a useful additional diagnostic tool in the preliminary identification of some species. This was done in the manner advocated for ecological studies to improve identification at the species level (Cox 1996).

Several well known and widely used authoritative local and international taxonomic works were constantly consulted in the identification of the diatoms to species level for all samples. These included some classic works on diatom taxonomy (Hustedt 1930, 1959, 1961; Patrick & Reimer 1966, Krammer & Lange-Bertalot 1985; 1986, 1988, 1991) - a four part series on the *Süsswasserflora von Mitteleuropa* (Lange-Bertalot & Krammer 1989; Krammer 1982, 1986, 1992) and (Cox 1996). Use was also made of additional reference books and manuals that have been published as aids to the identification of diatoms from other habitats or from other regions of the world (Round *et al*. 1990, Gell *et al*. 1999, Metzeltin & Lange-Bertalot 2002, Metzeltin & Garcia-Rodriguez 2003).

The most authoritative works used for identification of the diatoms from local rivers included published literature on rivers of KwaZulu-Natal (Cholnoky 1956, 1957, 1960b, 1968b, 1970a) and from other taxonomic studies on diatoms from various South African rivers (Archibald 1968, 1981; Schoeman 1971, 1979a; Schoeman & Archibald 1976). A more recent publication of an illustrated guide to common diatom species found in South African rivers was also used to identify diatoms (Taylor *et al*. 2007b), inclusive of taxonomic records from South Africa already captured in the Omnidia database (Lecointe *et al.* 1993).

Identification to species level is scientifically more informative and meaningful especially where several key species with different autecological ratings are included in a large taxon (e.g. *Nitzschia* or *Navicula*). Identification to genus level alone may not provide sufficient diagnostic power in the final analysis of a group of samples when inputs are required for the calculation of certain of the existing water quality indices.

The diagnostic value of metrics such as species richness, species diversity and evenness indices also relies on species level information for more accurate assessment of human impacts on rivers. Identification to genus level only, was therefore considered inappropriate and to have no real significance in hydrobiological research (Cholnoky 1968a, Schoeman 1971, Round 1991). "*It is dangerous to compare streams simply on genera recorded, and using generic identification in water quality studies is even more dubious*" (Round 1991).

Diatom counts

A variety of counting methods have been used by several different diatomologists However, the procedure used in this investigation essentially followed previous best practices (Cholnoky 1968a, Schoeman 1971, Archibald 1968, 1981). All drew on the experiences and procedure originally described by Thomasson (1925). Counts of 400-500 individuals were considered sufficient for statistical analysis (Cholnoky 1968a). It was subsequently shown that counts of 200 individuals / slide led to variations greater than 6% whereas counts between 400 and 800 produced variations of less than 2% between two counts (Schoeman 1971). It has also been shown that there was no substantive loss of information affecting the TDI index value after several hundred cells have been counted (Kelly 1998).

It is a logical argument that counting 800 cells requires nearly double the effort in time with minimal additional gain in valuable information. Counting effort, therefore, ranged between 400 and 500 individuals/count for all slides in this investigation. This range was consistent with the outputs reported from other diatom researchers most of whom exceeded the minimum of 200 individuals /count recommended as a lower limit (Bate & Newall 2002). The relative abundance of each individual species that contributed to the count of a diatom assemblage was calculated using the Thomasson analysis procedure (Thomasson 1925), as modified by Cholnoky (1955).

Relative density of species = (Number of individuals of a species / Total number of individuals in the count) x 100/1.

The aggregate % of the dominants and subdominants made up the diatom component most likely to influence the water quality index score and usually exceeded 80% of the sample count. Species with relative density scores of less than 1% were aggregated and recorded as '*other species'.* This aggregated component rarely exceeded 2% of the total sample count and was treated as part of the '*error range'* of a sample count (Cholnoky 1968a, Schoeman 1971) *'Other species*' were captured in floral lists developed in scans of a slide prior to counting.

3.3.2 Analysis of River Water

Water Quality Variables

Water samples were analysed for constituents and variables which were expected to have a distinct influence on the distribution of diatoms. Particular attention was given to generating information on those attributes listed and recommended as quality elements for the assessment of the ecological status of a river site (Wallin *et al.* 2003). The analyses of all micro-nutrients (e.g. soluble phosphorus and nitrogen fractions) were reported at concentrations which were low enough to be expressed in $\mu g \ell^{-1}$ (i.e. a physical *mass per unit volume)*, and at the appropriate level of accuracy and precision for limnological investigations of rivers. Total dissolved solids, and the major cations and anions were expressed as $mg\ell^{-1}$ since these macro-nutrients were naturally more abundant than the micro-nutrients.

However, molarity expressed as $mmol\ell^{-1}$ (chemical mass per unit volume) is a more meaningful expression of the concentration of dissolved material vis-á-vis osmo-regulation and its effect on the distribution of diatoms in river water (Cholnoky 1963, Schoeman 1971, Wetzel 1975). The use of units that express the amount of chemical mass per unit volume, rather than its physical mass per unit volume, is a more useful measure when comparing waters of different ionic concentrations as experienced by living diatoms in situ (Day 1990). However the chemical results for this investigation were not reported in this manner and the conventional approach using physical *mass per unit volume* was followed for consistency.

Procedures for collection, handling and preservation of water samples in the field followed the recommendations in the South African Bureau of Standards manual (SABS ISO 1994). Pre-cleaned acid-washed 1 litre polyethylene bottles were used to collect the water samples which were semi-submerged in ice-cooled water in cooler boxes i.e. without freezing or addition of preservatives. Water samples were returned to the laboratory for analysis within 48 hours of collection.

All the analyses of water samples, collected in the 2006-2009 period, were undertaken by qualified staff at the CSIR (KwaZulu-Natal) Regional Laboratory which carries a National Laboratory Accreditation certification for the constituents of interest. The analyses of constituents under consideration, in general, followed well documented procedures (e.g. Golterman 1969, APHA 1995) incorporating modifications and updates on these particular methods. Project-specific physico-chemical data was obtained from records generated during the execution of the fieldwork on the rural river sites.The soluble fraction of a water sample was analysed after being passed through pre-rinsed 47mm Millipore filters with a 0.45µ pore size to ensure uncontaminated filtered water for further analysis of the soluble components of the sample. The following constituents were considered pertinent to the objectives of this investigation.

Physical Measures	pH, Total Alkalinity, Conductivity (Total Dissolved Solids) Temperature
Oxygen regime	Dissolved oxygen, Chemical Oxygen Demand
Nutrient regime	Total Soluble Phosphorus (TSP), Total Kjeldahl Nitrogen (TKN), Nitrate-Nitrogen (NO_3-N), Ammonium Nitrogen (NH_4-N), Soluble silicon
Macronutrients	Calcium, Magnesium, Chloride and Sulphate

The bulk of the general water quality data (as per variables listed above) for river sites, was obtained from a National Water Quality database managed by the Resource Quality Services Division of the Department of Water Affairs (M.Silberbauer - pers. comm. 2008) and from the Ethekwini Municipal database (C Fennemore - pers. comm. 2009) . This data provided a basis for a general assessment of the prevailing chemical quality of the rivers of this province (Chapter 4). However, several disjunctions were observed in the data set at any given site although this database appears to have a comprehensive spatial and temporal coverage of the rivers in the study area. Missing data was noted in a time series and also for different variables of interest to the project. Secondly, the location of sites was not always consistent with the objectives of the study because the national network was not designed to characterize the natural water quality of aquatic ecosystems (Palmer *et al*. 2003). Data were scrutinized for irregularities and in most cases the median values of the data sets from the winter low flow period were considered appropriate for interpretation of the prevailing water quality conditions of selected rivers in the province.

Conductivity and Total Dissolved Solids (TDS)

Conductivity and TDS are measures of the total amount of soluble material (mostly cations and anions but also organic material in some more polluted waters) in a sample of water.

This measure is often made as a substitute when low Total Dissolved Solids values are likely to be common in chemically dilute waters in the mountain headwaters of rivers (Dallas & Day 1993).

The TDS concentration provides a useful indication of the potential osmotic pressure of river water. Furthermore TDS values can usually be interpreted from conductivity values which are reliably and easily measured in the field. TDS and conductivity correlate closely for a given type of water and a conversion factor was originally estimated for river conditions in KwaZulu-Natal (Kemp 1969).

$$\text{TDS mg}\ell^{-1} = \text{Conductivity value (mSm}^{-1}\text{) x 6.6}$$

[Note : $1\text{mSm}^{-1} = 10\mu\text{S cm}^{-1}$ (Day 1990)]

This conversion factor for TDS was confirmed after wider application in South African rivers (Dallas & Day 1993) but the accuracy and applicability decreases in more acid and /or alkaline waters (Day 1990).

Dissolved Oxygen

The oxygen content of the running water in the headwaters of rivers is seldom, if ever, depleted because the steep river gradients and turbulent waters ensure good aeration in the torrent-zone. Measurements were made with a portable Eijkelkamp18.28 multi-parameter analyser in addition to using the well documented Winkler method in the laboratory (Golterman 1969).

Nutrients

The soluble fractions of plant nutrients were determined by automated colorimetric analysis using an Auto Analyser III (SABS 1994).

Cations and Anions

Calcium and magnesium in a water sample was determined using an Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES). Chloride and soluble silicon were determined by colorimetric analysis using a UV-Vis detector. Sulphate was determined by reaction of sulphate with Barium to produce $BaSO_4$ followed by measurement using a turbidimeter.

3.4 Historic Diatom Material

A historic condition is '*a state interpreted from historical records'* Stoddard *et al*. (2006). Such historic diatom records of rivers in KwaZulu-Natal are contained in the South African Diatom Collection and these represented a unique historic resource. The availability of this resource represented an important difference from experiences in other countries in which there is an absence of suitable present-day candidate reference state sites and seemingly little in the way of historic diatom material of **near-natural conditions** as well (Eloranta & Soininen 2002, Ács *et al*. 2004, Nijboer *et al.* 2004, Yallop *et al.* 2004, Kelly *et al*.

2008). Historic diatom material (slides, reports and some record sheets) were used in this research programme to identify and evaluate the condition of several potential candidate reference sites at that time.

The unique historic data set of diatoms from KwaZulu-Natal Rivers was investigated for potential candidate reference sites. This data set was generated from work undertaken of a wide coverage of the rivers of the Province of KwaZulu-Natal in the mid-1950's (Cholnoky 1956, 1957, 1960b, 1970a). No other such detailed historic material and records of river biota exists for rivers in South Africa. However the aims and objectives of the original surveys were different from the present research and therefore a pre-screening of the material was necessary. A total of [7]570 historic sites were initially screened for suitability using several criteria to eliminate inappropriate sites, namely:

- samples taken outside of the winter low flow period (April - September)
- samples taken from substrates other than cobbles or rock surfaces
- samples taken downstream in the proximity of point source pollution impacts
- sites that were known to be located downstream of an obvious diffuse source, such as informal settlements or intense cultivation
- genuine main channel river or headwater tributary sites were retained in the historic data set

The historic Zone 3 sites retained in the data set were all in headwater reaches of major rivers and coincided with the present day sampling of a 50-100m reach rather than to that of a discrete sampling site. However, Zone 2 sampling reaches were found to be problematic in that the **historic areas** in this zone were not in the headwaters of Zone 2 Rivers of the Interior Midlands. These historic sites were typically occupied by indigenous folk who practised cattle-rearing and subsistence farming for generations, invalidating the concept of reference conditions. Furthermore the **present-day sites** in the appropriate headwaters for Interior Midland Zone 2 Rivers were almost all exclusively exposed to different land-uses within private farmland. This also invalidated the classification of present-day Zone 2 sites as candidate reference sites. The data from the pre-selected sites was captured in the Omnidia data base and various diatom indices and metrics were generated from the options presented in the programme (Lecointe *et al.* 1993). This information was used for further assessment of sites as potential candidate reference sites using a suite of diatom water quality indices to assess the ecological status of a site.

[7] (See also Appendix I for details of sites retained for further analysis)

3.5 Diatom Attributes and Metrics

Species Richness

Diatom species lists (species richness) were compiled from historic records on the relative abundance of species in an assemblage (Cholnoky 1956, 1957, 1960b). The naming of species mostly followed the concepts provided in taxonomic works (Krammer & Lange-Bertalot 1985, 1986, 1988, 1991), taking note of name changes made in more recent times (Round *et al*. 1990) and those recorded in an updated version of the original Omnidia database (Lecointe *et al.* 1993).

Relative Abundance of Species or Relative Density (%) in an Assemblage

The primary consideration in the examination of diatom material on a slide was given to the more common and abundant forms (dominants >5%, subdominants 3-5% of the total count at a site,) as it these that characterize the community responses and less attention was given to the rare species (Schoeman 1971, Round 1991, 1993).

Species Diversity and Evenness

Diversity and Evenness are two common measures that were obtained by computations derived from the analysis of counts on the species composition from each of the river sites. Results for some diversity indices were explored in this investigation taking note that the 'diversity principle' under certain circumstances may be misleading (Archibald 1972, van Dam 1974, 1982; Hughes 1978, Stevenson 1984, Round 1991).

Diatom Water Quality Indices

The application and selection of an appropriate diatom water quality index for classifying water quality conditions has been questioned because of reservations about the autecology of some species, about the accuracy of identifications, and about counting effort and possible limitations to the use of abundance weighted indicator values (Denys 2004, Besse-Lototskaya *et al*. 2006.). Nevertheless Omnidia is a commonly used programme which has been refined over the last 15-20 years from various large scale diatom studies in Europe (Lecointe *et al*. 1993). It was used primarily for the calculation of diatom water quality indices in this investigation. Several diatom metrics and biotic indices are also produced per sample entry. The most commonly used diatom indices were generated from the relative abundance analysis of the diatom communities worked through on a site by site basis. The software utilises formulae, such as the weighted average equation (Zelinka & Marvan 1961) for calculation of the diatom water quality indices.

The IPS (Index of Pollution Sensitivity) was assessed to be the most appropriate for application in South African rivers in the absence of a formal South African Diatom index (Taylor 2004a, 2004b) and was used extensively in this investigation. Values from other indices were generated for comparison using the same input per sample from the Omnidia software. The Trophic Diatom Index (TDI) Kelly & Whitton (1995), the Biological Diatom Index (BDI) Lenoir & Coste (1996), the Generic Diatom Index (GDI) Rumeau & Coste (1988) and the (Eutrophication Pollution Index (EPI-D) Dell'Uomo (1996) are all diatom-based indices for which values were produced simultaneously in the Omnidia output.

3.6 Statistical Software and Procedures

Multivariate procedures

Literature searches and a comprehensive review of at least 50 papers on diatoms have shown that there are many useful software programmes each with a suite of multivariate techniques that are frequently used for diatom community analysis. Some shortcomings in various procedures and routines have been described quite recently (Kent 2006). However, the stated misgivings are offset by the reality that there not many alternatives at this stage to some of the tried and tested procedures and routines devised over the last 20 years (Hill & Gauch 1980, ter Braak 1986, ter Braak & Prentice 1988, Clarke & Ainsworth 1993). Statistical routines to handle multivariate analyses are numerous and varied in their ease of application, efficiency of data processing and in their output displays. The main applications used for various purposes in this investigation included:

'PAST' (**PA**laeontological **ST**atistics) is an easily operated statistical program with a good electronic explanatory manual on each routine (Hammer *et al.* 2001). It offers both univariate and multivariate routines. However the graphics require considerable enhancement and are not as good as the outputs from CAP.

CAP 4 The bulk of the data processing for this investigation was handled with closely linked but independent programs developed by Pisces Conservation Ltd. The most recent versions of *CAP4, ECOMII, SDR4 and QED1.1.3.450* have been produced after continual improvement to routines (Henderson & Seaby 2008). Version 4 of The **C**ommunity **A**nalysis **P**ackage was found to be the most user-friendly, in terms of input requirements, data manipulations and output displays. CAP4 includes Agglomerative Cluster Analysis, ordination procedures such as Non-metric Multidimensional Scaling (NMDS), Principal Components Analysis (PCA) and Reciprocal Averaging (RA).

Detrended **Cor**respondence **Ana**lysis (DECORANA) as originally devised (Hill & Gauch 1980) was used if necessary to improve upon Reciprocal Averaging (Correspondence Analysis CA) outputs. The program also includes items such as SIMPER and ANOSIM for the assessment of group similarities and a test of the significance of groups respectively.

CAP4 is a user-friendly programme with which to explore, compare and analyse community structure. The routines offer a very extensive range of methods, together with ECOM II, to determine the influence of environmental variables on diatom communities and compositional gradients, if required (Henderson & Seaby 2008).

SDR4 *Species Diversity and Richness 4* is a programme designed for professional ecologists. The methods offer calculation of various Diversity Indices, estimates of total Species Richness and the study of patterns of abundance. It provides a powerful suite of methods to explore, compare and analyse community structure. The SDR4 was first released in 2006.

The use today of multivariate procedures for community analysis is a '*sine qua non',* yet the uncertainties of employing more complex mathematical techniques in freshwater ecology have been highlighted in the past (Fryer 1987) leading to reservations as to the efficacy of some applications even in recent times, as expressed in similar reservations more than a decade ago. "*Multimetric biological monitoring should combine biological insight with statistical power. Regional biology and natural history, not a search for statistical relationships and significance, should drive both sampling design and analytical protocol. Although statistics can and should be used to validate metric choices and predictions while building a multimetric index, excessive dependence on the outcome of statistical tests can obscure meaningful biological patterns"* (Karr & Chu 1997).

CHAPTER 4

WATER QUALITY TEMPLATES FOR REFERENCE STATE CONDITIONS IN RIVERS OF KWAZULU-NATAL

4.1 Introduction

4.2 Aims

4.3 Methods

4.4 Results

4.4.1 The Geological Template and River Water Quality

4.4.2 Water Quality Characteristics of Rivers in KwaZulu-Natal

4.4.3 Reference State Water Quality Templates

4.5 Discussion

WATER QUALITY TEMPLATES FOR REFERENCE STATE CONDITIONS IN RIVERS OF KWAZULU-NATAL

4.1 Introduction

The water chemistry of uncontaminated headwaters of a river is primarily influenced by the lithology of the drainage basin and by weathering processes (Kemp 1969, Hynes 1975, Biggs 1990, Dallas & Day 1993, Leland & Porter 2000, de Villiers 2005). The Total Dissolved Solids (TDS) derived from the weathering of rocks and soils and the consequent chemical constitution of the water, described here as a geological template, has been shown to be a strong determinant of the response of diatom assemblages (Biggs 1990, Leland & Porter 2000, Porter 2008). The chemical reactions and release of minerals through natural solubilisation results in a dilute solution of salts downstream but this seldom approaches saturation with respect to any dissolved salt in local rivers (Kemp 1969) (**Table 4.1**).The geological template, represented by the abundance of particular ions in different geological rock types, together with the climate (temperature and rainfall) are the main factors listed as defining the water quality template, characterised by the type and amount of soluble substances contained in the headwaters of rivers (Tordoffe *et al.* 1985, Dallas & Day 1993, Day & King 1995). Data is presented in this chapter of the characteristics of a water quality template as it pertains to the headwaters of rivers within sub-regions of the study area. A water quality template describes the particular suite of physico-chemical characteristics peculiar to the near-natural environmental attributes of a sub-region to which diatom assemblages will conform (Leland & Porter 2000).

It is to be expected that "*temporal and spatial variability is inherent in any water quality measure chosen to represent the near natural state of ecological systems*" (Stoddard *et al.* 2006). Furthermore spot measurements of water quality variables reveal information of the physico-chemical condition of a river only at a given point at a given instant of time (Kemp 1969). A reference state will also therefore exhibit a distribution in physico-chemical values over several similar sites rather than being defined by one unique data set. River flows also show marked seasonal variation in close association with the rainfall patterns of a region. Stable low flows are therefore experienced in rivers during the dry winter period on the eastern seaboard of South Africa. Only data pertaining to such periods of low winter flow (April - October) were considered for all the river systems throughout this investigation. The diversity of geological features and gradients in the chemical quality of the headwaters of rivers of the study area may be expected to give rise to sub-regional differences and expectations of varying composition of diatoms, even in the absence of human disturbances.

The significance of assessing variations in water quality in the headwaters of rivers derives therefore from these direct integrated responses of the diatoms to the variability of natural chemical gradients (Phillibert *et al.* 2006).

4.2 Aims

The main objectives were focussed on establishing and describing the water quality template of rivers, under typical winter low flow conditions, by :-

- describing the influence of the geological template on the water quality of rivers in the study area and documenting the present-day and historic water quality characteristics of near-natural headwaters.
- describing water quality templates which are consistent with reference state conditions in the minimally disturbed headwaters of various sub-regions.

4.3 Methods

No additional water quality monitoring of the rivers of KwaZulu-Natal was undertaken for the duration of this investigation. However several independent sets of physico-chemical data of rivers in KwaZulu-Natal were scrutinised and provided information from which templates of a stream typology were described.

Present-day Data Sets

The national laboratories of the Resource Quality Services, Department of Water Affairs, provides a water quality analysis service with a large database for a comprehensive set of river water quality variables i.e. major salts, nutrients, trace metals and physical attributes. The extensive database was interrogated for recent data pertaining to reaches of the rivers under consideration for this investigation (Silberbauer pers.comm. 2008). However large national water quality data bases are generally set up with other long term national objectives in mind and therefore sampling points are not always in the most appropriate position for a specific study. A much smaller data set on the hydrochemistry of large KwaZulu-Natal Rivers focused more on the specific relationship between headwater lithology and water quality variables (De Villiers 2005). This data set was published originally in units of chemical mass per unit volume (µmolsℓ^{-1}) but the data was converted into units of physical mass per unit volume (mgℓ^{-1}) for more direct comparison and consistency of reporting for this investigation (M. Silberbauer - pers.comm. 2010). The data provided additional insights into the present-day water quality characteristics of several sites in the headwaters of some of the larger Zone 3 Rivers of the region.

Spot measurements of water quality were also obtained at most of the sites that were sampled during the investigation between 2006 and 2009. However these spot measurements do not provide any indication of the range in values nor do these account for the antecedent variability of a constituent or variable at the point of sampling.

Historic data sets

The historic physico-chemical data is representative of the conditions prevailing in the headwaters of rivers of the study area where there was no formal development some 40-50 years ago. Several water quality surveys of the headwaters were undertaken throughout the province in the late 1960's and 1970's (Brand *et al.* 1967, Archibald *et al.* 1969, Kemp 1976). Other spot measurements of physico-chemical data were obtained from earlier ad hoc bio-monitoring and chemical surveys of some rivers of the study area (Kemp 1962, 1963, 1967, 1969). Some spot measurements of water quality were apparently the only records made at the time of the earliest diatom surveys (Cholnoky 1956, 1957, 1960b, Oliff 1960).

4.4 Results

The influence of local geology on water quality in rivers is described from results derived from data which provided a basis for expected differences in water quality characteristics in the near-natural headwaters of different river categories.

4.4.1 The Geological Template and River Water Quality

The geological series and the associated lithologies relevant to the river basins of the study area have been described previously (**Figure 2, Table 2.1).** An analysis of major inorganic solutes from 20 sites in unpolluted headwaters of selected rivers of KwaZulu-Natal included all the principal geological series of the study area. Each concentration of a major solute was expressed as a percentage of the total sum of the concentrations of all major cations and anions (**Table 4.1A**) (Kemp 1963). The original tabulated presentation was re-arranged according to spatial zones to demonstrate the gradients in the major solutes arising from the dominant geological series (**Table 4.1B**). More recent confirmatory evidence has also been published of the influence of geology on differences in the hydrochemistry of some of the headwaters of large rivers to the North-west (e.g. Pongola and Mkuze Rivers) and of the headwaters of rivers in the South-west (e.g. Thukela, Mkomazi, Mgeni and Mzimkulu Rivers) of KwaZulu-Natal (De Villiers 2005) (**Table 4.2**).

Total Dissolved Solids (TDS) values were lowest in the headwaters of the Montane Uplands spatial zone (Zone 3 Rivers) where shales and sandstones, associated with the Ecca and Beaufort Series prevail. The lithologies of the Ecca and Beaufort Series are similar thus yielding very similar percentages of cations and anions in river waters draining these formations (Kemp 1963, 1969) There is an order of magnitude increase in TDS values in headwaters of Zone 2 Rivers and Zone 1 Rivers where the highest values were recorded in association with Dwyka formations, nearest to the coast (**Table 4.1**). This finding matched a similar condition described for ground waters of the area (Bond 1946).

Table 4.1 The influence of lithology on the chemistries of uncontaminated headwaters of rivers in KwaZulu-Natal.

Geological Series	Lithology	Conductivity# mSm^{-1}	TDS $mg\ell^{-1}$	Ca	Mg	Na	K	M:D Ratio	CO_3	SO_4	Cl	SiO_2
				(Expressed as percentages of the Total Concentration of Cations and Anions) (Mean values n = 20)								
[A.] Chronological Order of Geological Series												
Tertiary-Recent	Surface quartzites	26.5	175	18.0	2.2	14.1	1.5	0.77	31.0	6.1	22.5	4.6
Beaufort	Shales and sandstones	5.8	38	8.7	4.2	8.0	1.9	0.76	41.0	6.0	6.9	23.3
Ecca	Shales and coals	7.9	52	7.3	4.7	10.1	1.1	0.93	44.1	7.6	6.2	18.9
Dwyka	Shales and tillite	47.6	314	8.3	7.3	19.4	0.7	1.29	34.2	2.8	23.0	4.2
Table Mountain Sandstone	Sandstones, quartzites	12.7	84	4.3	4.7	19.9	2.2	2.46	20.7	8.1	23.8	16.3
Basement Granite	Granite and gneisses	20.3	134	6.8	5.3	21.2	1.0	1.83	29.5	5.4	21.0	9.8
(Kemp 1963) : See also Figure 2 for Geological Map and Series in KwaZulu-Natal												
[B.] Gradients across Spatial Zones												
Zone 3 : Headwaters of Montane Upland Rivers												
Beaufort	Shales and sandstones	5.8	38	8.7	4.2	8.0	1.9	0.76	41.0	6.0	6.9	23.3
Ecca	Shales and coals	7.9	52	7.3	4.7	10.1	1.1	0.93	44.1	7.6	6.2	18.9
Zone 2 : Headwaters of Interior Midland Rivers												
Dwyka	Shales and Tillite	47.6	314	8.3	7.3	19.4	0.7	1.29	34.2	2.8	23.0	4.2
Zone 1 : Headwaters of Coastal Lowland Rivers												
Tertiary and Younger	Surface quartzites	26.5	175	18.0	2.2	14.1	1.5	0.77	31.0	6.1	22.5	4.6
Table Mountain Sandstone	Sandstones, quartzites	12.7	84	4.3	4.7	19.9	2.2	2.46	20.7	8.1	23.8	16.3
Basement Granite	Granite and gneisses	20.3	134	6.8	5.3	21.2	1.0	1.83	29.5	5.4	21.0	9.8

Rearranged from Kemp (1963) Note: #Conductivity mSm-1 = [TDS$mg\ell^{-1}$ / 6.6] (Kemp 1969, Dallas & Day 1993)

M:D Ratio of Monovalent to Divalent Cations

The surface waters associated with the Table Mountain Sandstone and Granites in the coastal areas also differ from Montane Upland Ecca and Beaufort Series in that the former have higher proportions of sodium and chloride. Only a very small part of the naturally occurring chloride concentrations of headwaters can be traced to the igneous rocks and sedimentary formations (Kemp 1969) (**Table 4.1**).Carbonates and bicarbonates, however are the predominant and principal anions in the headwaters of most of the Zone 3 Rivers draining rocks associated with the Karoo System lithologies in the South-west of the province (Kemp 1963, 1969, De Villiers 2005). (**Tables 4.1, 4.2**).

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Table 4.2 Summary statistics of the hydrochemistry of headwaters of Zone 3 rivers originating in the North-west and South-west of KwaZulu-Natal

Water Quality Variables ▼	Units ▼	North-Western headwaters of Large Zone 3 Rivers #					Interquartile	South-western headwaters of Large Zone 3 Rivers *					Interquartile
		Maximum	75^{th} Percentile	Median	25^{th} Percentile	Minimum	Range	Maximum	75^{th} Percentile	Median	25^{th} Percentile	Minimum	Range
pH		8.05	7.83	7.51	7.37	7.24	**7.37-7.83**	7.39	7.26	7.08	6.99	6.86	**6.99 - 7.26**
Total Dissolved Solids	$mg\ell^{-1}$	856	325	236	220	96	**220 - 325**	74	66	62	53	48	**53 - 66**
HCO_3^-	$mg\ell^{-1}$	58.4	0.35	0.15	0.1	0.1	**0.10 - 0.35**	48.3	43.9	36.9	33.9	32.3	**33.9 - 43.9**
NO_3^-	$mg\ell^{-1}$	0.682	0.620	0.496	0.434	0.31	**0.434 - 0.620**	5.828	4.030	1.364	0.837	0.372	**0.837 - 4.030**
PO_4^{3-}	$mg\ell^{-1}$	0.011	0.009	0.009	0.009	0.009	**0.009 - 0.009**	0.320	0.231	0.031	0.009	0.009	**0.009 - 0.231**
Ca^{2+}	$mg\ell^{-1}$	52.0	24.3	17.3	16.5	6.4	**16.5 - 24.3**	8.1	8.0	7.1	6.1	5.7	**6.1 - 8.0**
Mg^{2+}	$mg\ell^{-1}$	51.4	14.2	13.4	12.5	4.5	**12.5 - 14.2**	3.6	2.9	2.6	2.4	2.0	**2.4 - 2.9**
Ca/Mg Ratio		2.14	1.39	1.26	1.22	1.01	**1.22 - 1.39**	3.08	2.81	2.65	2.38	1.97	**2.38 - 2.81**
Na^+	$mg\ell^{-1}$	140.2	59.7	27.7	16.7	11.7	**16.7 - 59.7**	4.5	3.4	3.1	3.1	2.4	**3.1 - 3.4**
K^+	$mg\ell^{-1}$	3.1	2.8	2.3	1.5	0.7	**1.5 - 2.8**	1.6	0.6	0.4	0.4	0.3	**0.4 - 0.6**
M:D Cation Ratio		2.29	1.32	1.09	0.99	0.38	**0.99 - 1.32**	0.57	0.43	0.38	0.35	0.33	**0.35 - 0.43**
Cl^-	$mg\ell^{-1}$	176.4	67.6	15.6	12.2	10.7	**12.2 - 67.6**	3.7	1.1	0.9	0.8	0.4	**0.8 - 1.1**
SO_4^{2-}	$mg\ell^{-1}$	30.6	18.0	10.3	9.6	2.4	**9.6 - 18.0**	8.0	2.2	1.5	1.1	0.5	**1.1 - 2.2**

Data Source: (De Villiers 2005) **M:D Cation Ratio** = Ratio of Monovalent (Na^+ + K^+) to Divalent cations (Ca^{2+}+Mg^{2+})

(# Rivers with headwaters in North-west - Pongola, Mkuze, Mfolozi, Mhlatuze, Blood) (* Rivers with headwaters in the South-west -Thukela, Mkomazi, Mzimkulu)

Generally, calcium, magnesium and sodium are the predominant cations in these rivers (Kemp 1963, 1969; De Villiers 2005) (**Tables 4.1, 4.2**). The M:D ratios of Monovalent (Na^+ + K^+) to Divalent cations (Ca^{2+} + Mg^{2+}) also confirm that calcium and magnesium are the dominant cations in the headwaters of rivers originating in the Southwest together with carbonate anions, a characteristic of the dissolution of igneous rocks (Dallas & Day 1993) (**Table 4.1**). Sulphate is prevalent principally where the oxidation of pyrite occurs commonly and naturally in igneous rocks of the eastern regions of the province, such as the coal fields of the upper Mfolozi catchment near Vryheid (Kemp 1969) (**Figure 1.1**). The phosphate concentration of rivers in KwaZulu-Natal is always naturally low in near-natural headwater reaches of the major rivers and seldom exceeds100 $\mu gP\ell^{-1}$ as soluble phosphorus (Kemp 1969, De Villiers 2005) (**Table 4.2**).

4.4.2 Water Quality Characteristics of Rivers in KwaZulu-Natal

Comparative data on the ranges of water quality characteristics of the upper reaches of the main rivers of each spatial zone in the study area is presented from analyses generated between 1967 and 1976 (**Table 4.3).** More recent data for some rivers in KwaZulu-Natal were obtained during the period of investigation (2006-2009) (**Table 4.4).**

The historic data shows that most of the headwaters of the Zone 3 Rivers in the Southwest retained waters with low TDS concentrations and, as would be expected low conductivity values (Brand *et al.* 1967, Archibald *et al.* 1969, Kemp 1976) **(Table 4.3**). The reported historic conductivity values ranged between 1.4-8.2 mSm^{-1} (TDS 11-55 $mg\ell^{-1}$) for the Southwest headwater sites of the larger Zone 3 rivers (**Table 4.3**). However, the headwaters of large rivers draining the North-eastern region (e.g. Mkuze and Black Mfolozi Rivers) showed a greater range in TDS concentrations (11.2-1366 $mg\ell^{-1}$) **(Tables 4.2, 4.3)**.

Naturally high concentrations of sulphate have also been measured regularly in the headwaters of rivers in the North-eastern sector of the study area such as the Pongola, Mfolozi and Mkuze Rivers (**Tables 4.2, 4.3**). By contrast, only low concentrations of sulphate have been recorded in headwaters of the large rivers draining the South-western parts of the study area **(Table 4.4).** Naturally elevated concentrations of chlorides have been recorded in the small rivers near the coast and this has been ascribed partly to aerosols of marine origin (Kemp 1969, Archibald & Muller 1987, Allanson *et al.* 1990, De Villiers 2005) (**Tables 4.2, 4.3**).The soluble silicon concentration does not vary systematically with TDS concentrations and is somewhat random in a range of 12-16mg $SiO_2\ell^{-1}$ in local river waters (Kemp 1969). There is a natural abundance of this element in the rivers of the study area (**Table 4.4**). Uncontaminated headwaters, such as those in the Montane Upland regions, have a pH range of 6.8 – 8.3. Deviation from these near-natural limits is often taken as an indication of potential mineral pollution when extremely low or high pH values prevail.

Table 4.3 Summary statistics of water quality variables for the headwaters of KwaZulu-Natal rivers originating in different spatial zones (1967-1976)

Winter Means (n= 10)

Water Quality Variable ▼	Units ▼	Zone 1 Coastal Headwaters				Zone 2 Midlands Headwaters				Zone 3 - Montane North-west Headwaters				Zone 3 Montane South-west headwaters			
		Maximum	Median	Minimum	**Range**	Maximum	Median	Minimum	**Range**	Maximum	Median	Minimum	**Range**	Maximum	Median	Minimum	**Range**
pH		8.12	7.50	6.72	**6.72 - 8.12**	8.10	7.55	7.38	**7.38 - 8.10**	7.95	6.65	5.25	**5.25 - 7.95**	7.81	7.62	7.00	**7.00 - 7.81**
Temperature	℃	18.5	15.0	11.0	**11.0 - 18.5**	19.8	14.8	7.5	**7.5 - 19.8**	16.8	13.9	12.8	**12.8 - 16.8**	13.5	10.1	3.9	**3.9 - 13.5**
Dissolved Oxygen	$mg\ell^{-1}$	10.8	9.6	4.6	**4.6 - 10.8**	10.8	9.65	8.9	**8.9 - 10.8**	10.3	8.5	5.4	**5.4 - 10.3**	11.4	10	9.6	**9.6 - 11.4**
Dissolved Oxygen	% saturation	129.0	97	45	**45 - 129**	114	103	93	**93 - 114**	111	95	61	**61 - 111**	113	107.5	98	**98 - 113**
BOD	$mgO\ell^{-1}$	5.9	1.1	0.5	**0.5 - 5.9**	4.2	1	0.3	**0.3 - 4.2**								
Kjeldahl-N	$ugN\ell^{-1}$	1500	400	10	**10 - 1500**	9300	400	10	**10 - 9300**	1100	10	0.1	**0.1 - 1100**	1600	100	10	**10 - 1600**
Inorganic Nitrogen	$ugN\ell^{-1}$	925	210	23	**23 - 925**	2080	560	108	**108 - 2080**	510	166	50	**50 - 510**	520	80	40	**40 - 520**
Soluble Phosphorus	ugPℓ-1	403	47	13	**13 - 403**	160	78.5	19	**19 - 160**	57	20	13	**13 - 57**	80	20	10	**10 - 80**
Total Alkalinity	$mgCaCO_3\ell^{-1}$	120.5	74.9	15.8	**15.8 - 120.5**	167.3	32.2	17.7	**17.7-167.3**	258.9	41.2	18.6	**18.6 - 258.9**	34.4	29.3	13	**13 - 34.4**
Conductivity	mSm^{-1}at 25℃	114.4	29.8	10.0	**10 - 114.4**	78	11	9.3	**9.3 - 78**	249.1	59.5	9.1	**9.1 - 249.1**	8.2	4.6	1.4	**1.4 - 8.2**
TDS	$mg\ell^{-1}$	512	171	65	**65 - 512**	422	109.5	48	**48 - 422**	1366	456	63	**63 - 1366**	55	49	11	**11 - 55**
$^{\#}CO_3^{2-}$ (calculated)	$mgCaCO_3\ell^{-1}$	72.0	45.0	9.0	**9 - 72**	100.4	19.3	10.6	**10.6 -100.4**	155.3	24.7	11.2	**11.2 - 155,3**	20.6	17.6	7.8	**7.8 - 20.6**
Ca^{2+}	$mgCa\ell^{-1}$	21.3	9.9	2.4	**2.4 - 21.3**	28.9	5.15	2.7	**2.7 - 28.9**	120.7	7.6	3.8	**3.8 - 120.7**	8	5.2	1.4	**1.4 - 8.0**
Mg^{2+}	$mgMg\ell^{-1}$	26.1	11.2	1.1	**1.1 - 26.1**	21.6	3.1	2.2	**2.2 - 21.6**	36.5	3.7	3.4	**3.4 - 36.5**	4.8	3	0.6	**0.6 - 4.8**
Ca/Mg ratio		2.6	1.0	0.6	**0.6 - 2.6**	1.8	1.4	1.0	**1 - 1.8**	3.3	2.1	0.9	**0.9 - 3.3**	2.8	1.83	1.1	**1.1 - 2.8**
Na^{+}	$mgNa\ell^{-1}$	140.1	37.6	14.1	**14.1 - 140.1**	94.0	17.0	5.0	**5.0 - 94.0**	156.4	44.1	5.2	**5.2 - 156.4**	5	3.1	1.1	**1.1 - 5.0**
$K1^{+}$	$mgK\ell^{-1}$	4.6	1.5	1.2	**1.2 - 4.6**	3.8	1.0	0.5	**0.5 - 3.8**	6.6	3.2	0.7	**0.7 - 6.6**	0.8	0.4	0.2	**0.2 - 0.8**
M:D ratio		4.0	2.7	1.7	**1.7 - 4.0**	4.3	1.6	0.7	**0.7 - 4.3**	21.2	0.6	0.6	**0.6 - 21.2**	0.7	0.4	0.3	**0.3 - 0.7**
Cl^{-}	$mgCl\ell^{-1}$	255.8	59.8	18.7	**18.7 - 255.8**	122.5	21.3	3.4	**3.4 - 122.5**	12.1	3.8	0.1	**0.1 - 12.1**	3.8	1.5	1.1	**1.1 - 3.8**
SO_4^{2-}	$mgSO_4\ell^{-1}$	29.5	9.1	1.0	**1 - 29.5**	25.3	4.7	0.1	**0.1 - 25.3**	1020	289.2	1.0	**1 - 1020**	1.5	1	0.1	**0.1 -1.5**
Soluble silicon	$mgSi\ell^{-1}$	14.3	13.7	11.5	**11.5 - 14.3**	24.2	17.8	12.5	**12.5 - 24.2**								

(Brand *et al*. 1967, Archibald *et al*. 1969, Kemp *et al*. 1976) [$^{\#}CO_3^{2-}$ = 0.6x Alkalinity (Kemp 1971a)]

M:D cation ratio = Ratio of monovalent to divalent cations)

Table 4.4 Physico-chemical characteristics of sites from the headwaters of Zone 3 Rivers in KwaZulu-Natal (2006 - 2009)

Water Quality Variable ▼	River ► / Units ▼	North-east headwaters: Pongola	Mkuze	Mfolozi	Range	South-west headwaters: Thukela Tributaries: Bushmans	Thukela Tributaries: Mlambonjwa	Thukela: Main	Mvoti	Mgeni	Mkomazi	Mzimkulu	Range
pH		7.51	8.03	7.11	**7.11 - 8.03**	7.8	7.37	6.8	7.30	7.40	7.10	6.60	**6.6 - 7.8**
Temperature	℃					10.4	11.6	10.6	10.4	9.5	11.2	7.9	**7.8 - 11.6**
Kjeldahl-N	$ugN\ell^{-1}$			757		420	126	180		344	310	300	**126 - 420**
Inorganic Nitrogen	$ugN\ell^{-1}$	100	188	510	**100 - 510**	90	80	120	500	349	220	420	**80 - 500**
Soluble Phosphorus	$ugP\ell^{-1}$	10	12	20	**10 - 20**	10	6	50	70	80	30	20	**6 - 80**
Total Alkalinity	$mgCaCO_3\ell^{-1}$	50.0	106.7	35.8	**35.8 - 106.7**	60.0	34.2	12	38	26	34.4	29.2	**12 - 60**
Conductivity	mSm^{-1} at 25℃	9.2	96.4	14.7	**9.2 - 96.4**	9.1	9.3	2.7	11.1	5.0	5.5	3.8	**2.7 - 11.1**
*TDS	$mg\ell^{-1}$	60.7	636	97.2	**60.7 - 97.2**	60.1	61.7	17.1	73.2	33.0	36.3	25.1	**17.1- 73.2**
#CO_3^{2-} (calculated)		30.0	64.0	21.5	**21.5 - 64.0**	36.0	20.5	7.2	22.8	15.6	20.6	17.5	**7.2 - 36.0**
Ca^{2+}	$mgCa\ell^{-1}$	6.0	62.5	8.9	**6.0 - 62.5**	8.0	10.3	1.9	5.9	4.7	8.1	6.3	**1.9 - 10.3**
Mg^{2+}	$mgMg\ell^{-1}$	5.0	28.5	5.2	**5.0 - 28.5**	7.2	4.6	0.6	4.2	2.5	3.0	2.1	**0.6 - 7.2**
Ca/Mg ratio		1.2	2.1	1.7	**1.2 - 2.1**	1.1	2.2	3.2	1.4	1.9	2.70	3.0	**1.1 - 3.2**
Na^+	$mgNa\ell^{-1}$	5.6		5.2	**5.2 - 5.6**	4.9	3.1	3.0	0.59	0.2	3.1	2.7	**0.24 - 4.9**
K^+	$mgK\ell^{-1}$	0.7	3.2	2.2	**0.7 - 3.2**	0.4	0.4	0.8	1.0	0.6	0.32	0.4	**0.32 - 1.1**
M:D cation ratio		0.57		0.58	**0.57 - 0.58**	0.35	0.23	1.5	0.59	0.65	0.32	0.37	**0.23 - 1.5**
Cl^-	$mgCl\ell^{-1}$	7.0	17.4	12.9	**7.0 - 17.4**	3.0	1.5	1.2	10.0	7.0	2.1	2.4	**1.2 - 10.0**
SO_4^{2-}	$mgSO_4\ell^{-1}$	5.3	288.4	8.7	**5.3 - 288.4**	4.6	2.0	2.0	6.2	1.1	1.3	1.3	**1.1 - 6.2**

[* TDS $_{calculated}$ = Conductivity x 6.6 (Kemp 1969), # CO_3^{2-} $_{calculated}$ = Alkalinity x 0.6 (Kemp 1963)]

M:D cation ratio = Monovalent to Divalent Cation ratio

The near-natural fresh waters of the headwaters of the rivers are characteristically free of organic pollution and are almost always naturally saturated with oxygen **(Tables 4.3, 4.4)**. The oligotrophic (nutrient poor) and chemically dilute condition of the present-day upland headwaters is borne out by a more recent investigation of the linkage between lithology and hydrochemistry in an independent study of some of the key rivers of the province (De Villiers 2005) (**Table 4.2**) and from present-day data (**Table 4.4**).

4.4.3 Reference State Water Quality Templates

The suite of physico-chemical characteristics peculiar to the near-natural attributes of the headwaters was used to describe a river water quality template for various sub-regions of the study area. The range of values that collectively describe such a water quality template for some headwaters of the rivers of the Province of KwaZulu-Natal was derived from several data sets (**Tables 4.2, 4.3, 4.4)**.

4.5 Discussion

The main purpose of describing different water quality templates is to '*enable type specific reference state conditions to be better defined by a sub-regional template of water quality elements which in turn is used as an anchor of the classification system*" (Wallin *et al*. 2003). An additional guideline, that has been given for water quality elements to achieve a high ecological status of a reference state condition in a river recommends that "*concentrations of these water quality elements should not reach levels outside the established ranges to ensure the functioning of the type-specific ecosystem"* (Wallin *et al*. 2003).

Characteristics of water quality templates of rivers in KwaZulu-Natal.

It is necessary to describe a reference state water quality template which reflects minimally disturbed headwaters of a river from which the corresponding diatom reference state communities may be defined. An *a priori* general classification of river water templates of KwaZulu-Natal was based on the attributes of two natural chemical series of waters in the rivers of the study area (Kemp 1971) termed '*chlorided'* waters *and 'sulphated'* waters (Kemp 1969). The significance of this finding is the distinction that can be made between the sub-regional water quality templates of the headwaters of different spatial zones, namely:-.

- **A Template for chemically dilute Zone 3 headwaters of rivers originating in the Western and South-western Montane Uplands spatial zone.**

The classification of river water quality templates is more realistically based on the TDS concentrations rather than on specific differences in chemical composition The concentration of the Total Dissolved Solids (TDS) in rain water is usually very low (<10 mgℓ$^{-1}$) and the ionic concentrations of the headwaters of Montane Uplands of Zone 3 Rivers has also been shown to remain low over many decades. Uncontaminated near-natural waters in the south-western Montane Uplands Rivers usually do not have an electrical conductivity greater than 25 mSm^{-1} (Kemp 1963).

The lowest conductivity measurements reported nationally for South African Rivers range between 0.9-3.6 mSm^{-1} giving an equivalent TDS range of 6-24 $mg\ell^{-1}$ (Dallas & Day 1993). The geological influence on the TDS of rivers and streams draining igneous or metamorphic rocks has also been shown to produce low TDS values (10-75 $mg\ell^{-1}$) in uncontaminated reaches (Kemp 1969, Dallas & Day 1993, De Villiers 2005). This is true of the water quality encountered in the Montane Uplands reaches of rivers where the headwater sites of all large river systems in the South-western parts of the study area are located (De Villiers 2005).

The analytical results of the river waters of the study area were standardized by using a purely chemical scheme based on molar concentrations of the acids and bases dissolved in the water (Kemp 1971). The molar percentage of each major solute in uncontaminated waters was thus related to that of carbonic acid. The molar spectra, derived in this manner, for the Montane Uplands Rivers, show the dominance of carbonates in the headwaters of these rivers. Gradients in chemical ratios exist in many of the headwaters of the large rivers, the analytical results from which show the Monovalent **:** Divalent cations ratios (M:D ratio) to be less than 1.5 in most chemically dilute oligotrophic Zone 3 River waters.

- ■ **A Template for high ionic concentration, Zone 3 headwaters of rivers originating in the North-western and North-eastern Montane Uplands.**

The sulphated water series is less prevalent in local rivers, and is not well represented among the **unpolluted** river waters of the study area (Kemp 1971). The exception to this condition was found in the North-eastern coal mining ‘triangle’ in the area around Vryheid where sedimentary lithologies (Ecca shales) are more prominent (**Figure 1.1)**. Generally, the headwaters of most rivers in the province do not contain more than a few $mg\ell^{-1}$ of sulphate in the absence of pollution. However, the TDS values of the headwaters of Zone 3 Rivers of the North-eastern area can increase **naturally** to concentrations in excess of 100 $mg\ell^{-1}$ where erodible shale and sandstone bedrock is commonly found in the Ecca Series. The river water quality template of the North-western sub-region is also characterized by naturally elevated TDS concentrations derived from relatively high concentrations of sodium, chloride and sulphate in the rivers of the area. However pollution of headwaters of some tributaries of larger rivers in the North-eastern interior of the province may also be caused by sulphates that find their way into river water from acid mine drainage of coal mine dumps (Kemp 1967, Archibald & Taylor 2007).

- **A Template for high ionic concentrations in Zone 1 headwaters of rivers originating in the Coastal Lowlands.**

The river basins of the Coastal Lowlands are dominated by granites or recent tertiary formations and are also close enough to the coast to be exposed to wind-blown aerosols containing salts of marine origin (Bond 1946, Archibald & Muller 1987, Allanson *et al*. 1990), a phenomenon sometimes referred to as 'cyclic salt' (Dallas & Day 1993). This combination produces a water quality template characterized by TDS concentrations which are naturally elevated and higher than the chemically dilute waters of the Montane Uplands. The molar spectra of the Coastal Lowlands show carbonates are much reduced while sodium and chloride percentages are markedly higher (Kemp 1969).

Pronounced gradients in organic contamination of rivers of the coastal region are evident in 'extreme environments', all of which are downstream of human disturbances e.g. sugar mill waste, pulp & paper waste, sewage and industry waste (Chapter 7). However there is no evidence of such contamination in the headwaters of the rivers of the region.

Observations from other parts of the world

Several findings recorded in recent international diatom literature give credence to the interpretation that TDS, pH and alkalinity gradients are important discriminating factors explaining much of the variance in diatom community responses (Potapova & Charles 2003, Charles *et al.* 2006, Grenier *et al*. 2006, Lavoie *et al.* 2006, Philibert *et al.* 2006, Chessman *et al.* 2007, Lavoie *et al.* 2009). The importance of gradients in pH and alkalinity has also been reported as the main discriminating factor that influences the variation in diatom responses to water quality, irrespective of spatial entities or stream type (Grenier *et al*. 2006, Charles & Potapova 2006). Furthermore the range in ionic concentrations (measured as TDS) and the range in pH values together with the buffering capacity that is strongly influenced by differences in alkalinity, were also held to be important factors in the distribution and response of diatoms (Cholnoky 1960a, 1963, 1968a, 1970b).

The capacity for osmo-regulation, as a selective differential uptake mechanism of dissolved substances by diatoms, has been presented as a plausible rationale for explaining diatom distributions along changing ionic gradients (Cholnoky 1963, Lowe 1974, van Dam 1994, Porter 2008). A high permeability of the diatom cell protoplasm is a physiological attribute that preferentially favours diatom growth in brackish waters irrespective of the nature of the chemical constituents of that water (Cholnoky 1963, Porter 2008).The 'halobien concept' (Kolbe 1932) focused specifically on the chloride ion concentration as a key influence but the concept was questioned by findings from the inland soda lakes of Hungary (Cholnoky 1963).The ability of diatoms to withstand high variations in molarity of an aquatic medium was thus demonstrated in these Hungarian lakes, in the absence of chloride ions.

This led to the supposition and belief that molarity (chemical mass per unit volume), irrespective of the chemical constituents, was one of the main explanatory variables influencing the response of diatoms to gradients of ionic concentration of the ambient medium (Cholnoky 1963).

The nature of the ionic content of freshwater is influenced essentially by the composite of the eight common major ions but it is the total ionic concentration (molarity) that is important vis-á -vis how osmo-regulation influences the distribution of diatoms (Wetzel 1975). Monovalent ions such as Na^+ and K^+ together with the divalent Mg^2+ ions are relatively conservative as is the Cl^- ion. However Ca^{2+} and SO_4^{2-} ions are reckoned to exert some influence on the distribution of diatoms because species with the highest optima for SO_4^{2-} ions are often found in high conductivity waters (Potapova & Charles 2003) e.g. Acid Mine Drainage (Chapter 7). Gradients in the ratio of monovalent to divalent cations (M:D) are also influential in affecting diatom growth patterns (Wetzel 1975, Potapova & Charles 2003). An M:D cation ratio of less than 1.5 (i.e. a predominance of the divalent Ca^{2+} and *Mg^{2+} cations)* is also reported favourable for growth of species such as *Tabellaria flocculosa* (Roth) Kutzing in oligotrophic environments (Wetzel 1975).

Relatively low calcium concentrations prevail in the rivers of the eastern seaboard primarily because there are very few limestone formations. Calcium–rich gypsum is found rarely only in the Interior Midlands of the Thukela River valley away from any headwaters. The concentration of sodium is twice that of the world average (Kemp 1969), and it has been suggested that this was because most of the sedimentary rocks of the region were formed under marine influences and therefore may contain remnants of (connate) seawater (Bond 1946).

The "Saprobien system" (Kolkwitz & Marsson 1908) sought to explain the distribution and composition of living organisms (e.g. diatoms) in their responses to phases of self-purification (organic pollution gradients) without remedial measures. The waste products were erroneously assumed to be homogenous and therefore the gradients in nutritional stimulants provided by nutrients, which were also contained in these wastes, were overlooked (Cholnoky 1960a).The importance of nutrient content, with reference mainly to soluble nitrogen and phosphorus, was first mooted when gradients in trophic status were termed eutrophic, mesotrophic and oligotrophic to explain the response and distribution of diatoms to nutrient contamination gradients (Naumann 1932). *The phosphate concentration of rivers in KwaZulu-Natal is always naturally low in uncontaminated reaches of major rivers (*Kemp 1969*)* because only natural traces of phosphate are taken up from sedimentary rocks and from the apatite of small areas of igneous rocks.

Summary of key issues

- Water quality templates that conformed to reference conditions were described for rivers originating in two spatial zones giving rise to different sub-regions in KwaZulu-Natal, based primarily on differences in lithology and as a consequence that of Total Dissolved Solids concentrations and changes in molarity.

  Zone 3 Headwaters

  - Circum-neutral, low alkalinity, chemically dilute Zone 3 waters in rivers originating in the Western and South-western Montane Uplands derived from headwater basalts.
  - Alkaline, high ionic concentration headwaters of rivers originating in the North-western and North-eastern Upland sub-region predominantly derived from Ecca shales.

  Zone 1 Headwaters

  - Alkaline, naturally high ionic concentrations in Zone 1 headwaters of Rivers originating in the Coastal Lowlands derived from granites and Table Mountain sandstone and to a lesser extent from cyclic salt of marine origin.

A reference water quality template for the headwaters of Zone 2 Rivers did not fulfil the criteria for river conditions 'free of human disturbance'. The Zone 2 headwater sites were found to have undergone nearly irreversible transformation of land-use.

- Temporal and spatial variability is inherent even in near-natural river waters and therefore water quality templates of reference state conditions are expected to exhibit a range of values for each water quality variable within a pool of sites.
- Periods of low flow were associated with less variability with a consequent reduction in the range of values for water quality constituents, thus facilitating a better correlation between water quality templates and diatom responses under reference state conditions.
- Chemical gradients were recognised in the differences between the water quality templates of the Montane Uplands and Coastal Lowlands river waters leading to the expectation of different diatom communities in minimally disturbed waters of the headwaters of both these spatial zones.
- The classification of water quality conditions prevailing in the headwaters was based essentially on the TDS of river waters while also accounting for properties relating to alkalinity, pH value, organic content and nutrient content as a basis for describing the essential elements of the water quality template of KwaZulu-Natal Rivers.

CHAPTER 5

THE DERIVATION AND BENCHMARKING OF DIATOM REFERENCE STATE SITES IN RIVERS

5.1 Introduction

5.2 Aims

5.3 Methods

5.3.1 Definitions and Principles

5.3.2 Classification of River Sites

5.4 Results

5.4.1 Assessment of Headwater Sites

5.4.2 Ecological Status of Candidate Reference Sites

5.5 Discussion

THE DERIVATION AND BENCHMARKING OF DIATOM REFERENCE STATE SITES IN RIVERS

5.1 Introduction

The reference condition approach has been incorporated into several biomonitoring programmes such as the updated USA Clean Water Act (1972), Australian Water Reform Framework guidelines ANZECC & ARMCANZ (2000), the European Water Framework Directive (2002) and the South African River Health Programme (DWAF 2008). An '*a priori approach*' based on geology and geomorphology of the province was the first step to establish the stream attributes which validates the ecological basis for identifying small scale homogenous spatial zones such as the headwaters of three different categories of KwaZulu-Natal rivers. This step defines the undisturbed abiotic conditions from which the biological quality elements associated with a reference condition can be determined.

The subsequent use of an '*a posteriori' approach*, as a second step, to classify reference sites based on biological elements (e.g. diatoms) using multivariate analysis is a procedure recommended by the River Heath Programme (DWAF 2008). It makes no prior assumptions about the similarity of biological assemblages at different sites. Rather, the structure of diatom assemblages is used to group sites that have similar taxonomic composition, thus providing an objective way of identifying reference sites with similar assemblages" (DWAF 2008) in the manner applied successfully in Canadian rivers (Lavoie *et al.* 2006)"

The classification of local river systems within three spatial zones was based primarily on the geomorphology of the river basins that provided a necessary logical '*a priori'* foundation for identifying candidate reference sites at a relatively small scale (Stoddard *et al*. 2006). The geographic characteristics common to established eco-regions covering the study area were too broadly based on general landscape and climatic features. This necessitated refinement in scale to three spatial zones incorporating the sub-regional headwaters of each category of river system (Chapter 2). Correlation with the variance in diatom distribution, as influenced by broad landscape features at an eco-region scale, has been reported as low and the efficacy of this approach has also been questioned (Pan *et al*. 2000, Weilhoefer & Pan 2006, Metzeling *et al.* 2006). Furthermore the need to identify and classify the near natural river reaches in the region dictated that the best available 'pool of sites' would most likely be identified by '*a priori'* groupings of the headwaters of rivers in the study area.

Comparison between headwaters and lower reach sites of a long river that traverses more than two spatial zones is inappropriate. Changes in the geological templates longitudinally downstream in KwaZulu-Natal Rivers (Figure 2, Table 2.1) leads to natural increases in the dissolution of chemicals (Kemp 1969). Some more recent research has shown that diatom responses may conform to gradients in alkalinity (Kelly *et a*l. 2008) or ionic composition (Potapova & Charles 2003) for unimpacted streams. These changes in water quality are reflected in different diatom assemblages with increasing 'distance' from the reference condition even in the absence of human disturbances. The reference condition approach adopted in this thesis is however premised on **type specific** conditions (as recommended in EU Directives (Wallin *et al.* 2003) rather than **site specific** conditions. This implies that comparison with the deviations from reference conditions is only valid if the **type specific** conditions are being compared **within the same spatial zones.**

5.2 Aims

The overall objective was to identify clusters of similar river sites that support diatom assemblages belonging to communities engendering a high ecological status, associated with the headwaters of a river that could account for **a reference state condition**.

The main aims of this part of the investigation were therefore:

- Determination of potential groups of candidate reference sites in the headwaters of rivers using cluster analysis to initially distinguish **candidate reference state** sites with similar diatom communities, and confirmed by NMDS ordinations of these sites.
- Identification of candidate diatom reference state sites in rivers, within specific spatial zones, by adopting a chemistry-free, diatom-based approach to interpret, '*a posteriori',* the ordination of sites in relation to unmeasured environmental gradients.
- Classification of the ecological status of candidate reference sites using a suite of water quality indices.

Note: [The metrics and attributes of the target reference state assemblages derived from the communities associated with these sites are described in Chapter 6.].

5.3 Methods

Data from diatom assemblages was generated from analysis of samples obtained from historic and present-day river surveys. Many of the samples, that were common to both sampling programmes, were located at sites in similar reaches but these were not necessarily obtained from the same microhabitat or from precisely the same position in the headwaters of key rivers because the narrative description of the position of historic sites was not always precise or referenced with co-ordinates (**Appendix 1**).

An '*a posteriori'* chemistry-free ordination of sites, based on the collective autecological characteristics of individual diatom taxa, is a diatom-based strategy that has been used elsewhere when researchers were pursuing similar objectives (Lavoie *et al*. 2006, Grenier *et al*. 2006, Weilhoefer & Pan 2006).

The methodology used in this investigation followed a logical step by step process. :-

- A set of headwater river sites was classified '*a priori'* within each of three spatial zones, each defined by sub-regional geomorphological features of the study area (**Chapter 2**).
- Historical and present-day data from the headwater sites of Zone 2 Rivers were, however, shown to be inadequate for the derivation of reference state sites because the results from the initial screening did not meet requirements and expectations of sites free of human disturbance.
- Physico-chemical attributes of the near-natural headwaters of Zone 1 and Zone 3 rivers were used to describe the sub-regional water quality templates in relation to the lithologies of these headwaters (**Chapter 4**).
- Diatom-based clustering and ordination of sites was achieved from information derived from the diatom community structure using species-level abundance scores.
- Ecological quality status was finally determined from the documented autecology of species using a spectrum of diatom water quality indices. These outputs confirmed the selection of a high ecological status for present-day and historic candidate reference state sites.

This comprehensive approach classifies potential reference state sites, in terms of ecological integrity, using multivariate clustering and ordinations of the diatom communities. It makes no prior assumptions about the similarity / dissimilarity of diatom assemblages at different sites or of the environmental factors that may explain the distribution of the diatom assemblages. Rather, diatom assemblage data are used to group sites that have similar taxonomic composition, thus providing an objective way of identifying potential candidate reference state sites in each spatial zone. Investigation of these biological communities is often also focused on inferring species-environment relationships. However this requires a second set of corresponding environmental data, such as the physico-chemical attributes of water quality elements. The requirements for the latter posed some logistical problems because of the naturally high spatial and temporal variability of the water quality variables. The development of median values from an antecedent series of data prior to a diatom survey is a prerequisite to address this issue because of "*the fallacy of trying to relate temporal ecological data to chemical values measured at the same time*" (Round 1991). The water quality data measured in recent surveys only provided confirmatory evidence of the prevailing water quality conditions at the time of sampling. Furthermore no adequate long term time series of historic water quality data was available. This was problematic because it is the range in variable values given by a time series (e.g. pH range) which is more scientifically meaningful than the absolute value of spot chemistry readings taken at the time of sampling (Cholnoky 1960a).The historic and present-day data of diatom communities were collected and stored in matrices as qualitative presence / absence records and as quantitative scores at the species level for each site. The quantitative scores of diatom assemblages at the species level were expressed as individual counts or as percentage relative abundance of the total species composition at a site.

Principles and operating criteria dictated the choice of the most appropriate multi-variate procedures (Clarke & Warwick 1997, Henderson & Seaby 2008). Hierarchical agglomerative clustering using Bray-Curtis similarity measures has been widely used in ecological studies because it separates groups of sites with distinct community structure. Furthermore "*strongly grouped cluster analysis is best used in conjunction with ordination"* such that agreement between the two outputs improves the robustness of the interpretation (Clarke & Warwick 1997). Hierarchical Agglomerative Cluster Analysis (ACA) using Ward's method and the Bray Curtis similarity measure was preferred and recommended for the initial distinction of discrete groups of sites because the outputs may relate better to Non-metric Multidimensional Scaling (NMDS) (C Morris – pers. comm. 2010). Ward's method is based on minimising variance within clusters and maximizing variance between clusters and therefore the scale that is associated with the outputs represents the degree of variance between groups. Analysis of Similarities (ANOSIM) was used as a test of the significance of similarity between defined groups. Such a test determines whether the difference between groups is meaningful versus being obtained randomly by chance (Clarke 1993).

One of the prime objectives of indirect gradient analysis is to summarise the main variation in the species data using ordinations of all the samples (sites), without recourse to environmental data. Non-metric Multidimensional scaling (NMDS) was the ordination technique most frequently favoured and used to display the interrelationships on a continuous scale because it gives a graphical representation of the similarity between samples (sites) in two-dimensional space (Clarke & Warwick 1997, Henderson & Seaby 2008). However the procedure does not identify or display the most important variables (species) associated with the samples (sites). A covariance–based Principle Components Analysis (PCA) was also applied to quantitative data because the procedure identifies those variables (species) which closely define the similarity between samples (sites) (Henderson & Seaby 2008) (See also Chapter 6). However, in many instances diatom community data is prone to data matrices retaining several zeros and therefore methods involving Principle Components Analysis (PCA) may not always be appropriate unless the bias attributed to dominant species (high scores) allows a requirement for exclusion of rare species (low scores) (Clarke & Warwick 1997).

5.3.1 Definitions and Principles

Certain key operating principles and definitions were used from various directives and manuals as guidelines to describe conditions necessary to fulfil target criteria for the derivation of candidate sites of high or good ecological status, as recommended in the REFCOND documentation (Wallin *et al.* 2003) and other pertinent literature (European Union 2000, Davies & Jackson 2006, Stoddard *et al.* 2006, SA National Aquatic Ecosystem Health Monitoring Programme (DWA&F 2008).

(i) A **water body type** is defined as "*a discrete and significant element of surface water such as a river or stream"* (Wallin et al. 2003).

The headwaters of the rivers, upstream of human activities in the study area, were taken as the water body type having the greatest potential in the quest for reference state sites. The referencing of **type-specific** conditions was a specific objective of the investigation as opposed to describing site-specific conditions which have rather more limited application (Stoddard *et al.* 2006).

(ii) **Ecological status** *is an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface water* (Wallin *et al*. 2003*).*

The ecological status of the reference state sites was determined from species level data of diatom assemblages, as the discernible biological quality element, using a range of water quality indices (Section 5.4.4). A normative definition of *ecological status* or condition has also been published (European Union 2000).

(iii) "*There are no or only very minor anthropogenic alterations to the values of the physico-chemical and hydromorphological quality elements for the surface water body type from those normally associated with that type under undisturbed conditions". "The values of the* ***biological quality elements*** *for the surface water body should reflect those normally associated with that type under undisturbed conditions and show no or only very minor evidence of distortion"* (Wallin *et al.* 2003).

*(iv) The r**eference state site** is the expected condition that reflects natural or least impacted physical, chemical and biological characteristics of a site, river reach or river type, in the absence of anthropogenic stress* (DWAF 2008).

Operational principles were also used for guidance in the determination of reference state sites.

■ ***Principle #1:*** "**Reference state conditions** *should be established for a water body type together with quality elements which in turn are represented by parameters indicative of the status of the quality elements"* (Wallin *et al* 2003).

"**Reference condition** *is a term that should be reserved for referring to the 'naturalness of the biota (structure and function) and that naturalness implies the absence of human disturbance or alteration"* (Stoddard *et al.* 2006).

- ***Principle #2:*** *"The large natural variability in river basins, resulting from differences in attributes of climate, landform, geology, soils and vegetation, predetermines the need for smaller scale regional reference conditions. The process by which reference conditions are derived may vary from one biotic component to another"* (Stoddard *et al.* 2006).
- ***Principle #3:*** "*Reference conditions for each water body type can be a state in the present or in the past "* (Stoddard *et al.* 2006).
- ***Principle #4:****"Reference conditions enable the degree of deviation from natural conditions (typically human degradation) to be ascertained. These conditions are the foundation for developing biological criteria for the protection of aquatic ecosystems and evaluating impacts at monitoring sites"* (Dallas 2002).

5.3.2 Classification of River Sites

"*The presence or absence of diatoms within a community is not a random occurrence of species in time and space but rather reflects a patterned response to the external environment*" (Levandowsky 1972). A diatom assemblage represents a collective integrated response of the component species to the ambient aquatic environment. This biological signal is manifested in assemblage attributes, the metrics of which are measures derived directly from the river conditions in some space-time interval.

The approach in this research was to minimise external factors affecting community variability between sites by focussing on the discrete headwaters of the rivers in winter. It is necessary to identify and classify a pool of representative candidate reference sites for each category of river so that an acceptable precision and reliability is achieved in establishing such diatom reference sites for a region (Tison *et al.* 2005, 2007). A fundamental goal of this approach is benchmarking a reference state diatom community, which is defined as being free of human disturbance and of high ecological status.

Near-natural reference sites therefore should be representative of broadly homogenous smaller scale eco-regions or spatial zones. Multiple candidate reference sites within each of the river spatial zones would be expected to improve reliability of criteria used in this classification scheme. Some similar studies have shown that benthic diatom communities are spatially influenced by localised habitat features and therefore sampling a pool of sites that focuses on similarities of dominant river habitats within a spatial zone may also improve the accuracy of defining such sites (Pan *et al.* 2000, Stoddard *et al*. 2006).

This led to the first cut in the differentiation of the headwaters of rivers of the eastern seaboard being grouped into three spatial zones as described previously in **Chapter 2**. A '*sampling site'*, from which biological information was extracted using the structure of diatom communities, was defined as "*the basic sampling unit separated in space or time from other sites"* (ter Braak 1986) (**Appendix 1**).

5.4 Results

5.4.1 Assessment of Headwater Sites

Several scenarios were explored in the process of identifying candidate reference sites from the headwaters of rivers, mainly using the multivariate techniques referred to above, by allowing *" the biota to tell their own story*" (Clarke & Warwick 1997).

[SCENARIO A1] ASSESSMENT OF HISTORIC SITES FROM THREE DIFFERENT SPATIAL ZONES

An initial comparison was made of the pool of **historic diatom data** derived from all available headwater sites from the three spatial zones. The outputs from Agglomerative Cluster Analysis produced two major groupings, Group 1 and Group 2, each of which was further subdivided into subgroups (**Figure 5.1**). Subgroups 1A and 1B both contain a mix of sites from the headwaters of all three spatial zones at a high level of variance from Group 2. Disparate groups of Zone 1 headwater sites fell into separate smaller subsets of the larger Subgroups 1A and 1B. However the 'within group' similarities of the bulk of Zone 3 headwater sites produced Group 2, distinct from headwaters of rivers in other spatial zones. Group 2 sites were differentiated into the high altitude headwater sites of the main Thukela River (Subgroup 2A - Z3MX sites) indicating some variance (dissimilarity) from the bulk of Zone 3 sites making up Subgroup 2B, all drawn from headwaters of main upper Thukela River (**Figure 5.1**). An NMDS ordination of the same historic diatom count data from rivers in the study area reinforced the pattern produced initially by the site groupings in the dendrogram in that there was a clear separation of the group of sites (Group 2B) drawn from the main Thukela River at one end of a condition gradient (Axis1) (**Figure 5.1.1**). A second gradient (Axis 2) was evident with the separation of the high altitude sites (Z3MX1-Z3MX5 sites in sub-Group 2A) from sites in subgroup2B. The smaller distinctive subset of high altitude sites, were differentiated along an unmeasured environmental gradient (Axis 2) from those positioned along the main gradient (Axis1). Sites within this subgroup 2A were differentiated from the main cluster of Z3 Upland sites in Group 2B (red triangle) (**Figure 5.1.1**). The more distant positioning of several sites in Groups 1A and 1B on Axis1 corresponds with the 'mixed clustering of sites' in the dendrogram (**Figure 5.1.1**).

[SCENARIO A1] ASSESSMENT OF <u>HISTORIC</u> SITES FROM THREE DIFFERENT SPATIAL ZONES

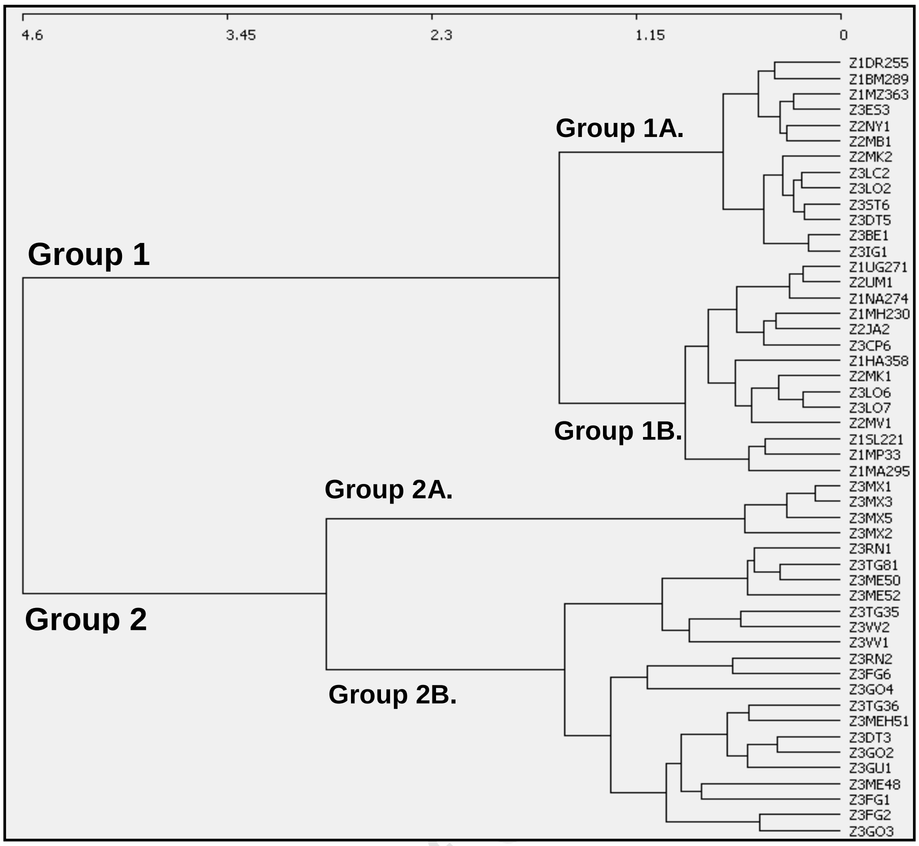


Figure 5.1 **Agglomerative Cluster Analysis of historic diatom community data from headwater sites in rivers originating in three spatial zones (Z1, Z2, and Z3) using Ward's method and Bray-Curtis similarity measure.** [Resources: A1-A3, B1, C1 (Cholnoky 1956, 1957, 1960b)]

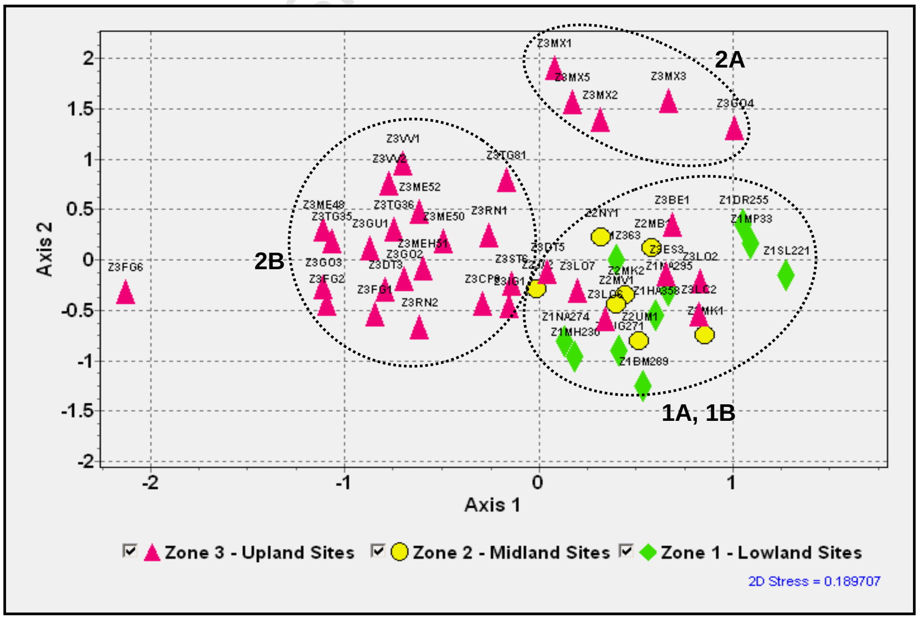


Figure 5.1.1 **NMDS ordination of historic diatom data (log_{10}[x+1] transformed) showing the positioning of sites from rivers originating in all three spatial zones (Z1, Z2, and Z3) using Bray-Curtis similarity measure.**

[SCENARIO A2] ASSESSMENT OF <u>PRESENT- DAY</u> SITES BETWEEN TWO SPATIAL ZONES WITH DIFFERENT BEDROCK GEOLOGY

A comparison was made between groups of **present-day** headwater sites of rivers originating in the Coastal Lowlands (Zone 1) and the headwaters of rivers originating in the Montane Uplands (Zone 3). The sites in the headwaters of these spatial zones were mostly located in areas dominated by different geological bedrock. The headwaters of the former were predominantly made up of granite or from sedimentary rocks of the Table Mountain Sandstone formation. Headwaters of the latter were made up of basalt / volcanic cappings of the Stormberg series including the shales of the Ecca and Beaufort Series (**Figure 2**, **Table 2.1**). Outputs from the Agglomerative Cluster Analysis showed a distinct separation of Group 1B (> 90% Zone 1 sites) and Group 2 (exclusively Zone 3 sites) (**Figure 5.2**). A disparate set of Zone 1 sites and Zone 3 (Group 1A) showed greater 'within group affinity' than with the respective larger groups of Group1B and Group 2. The latter was further separated into high altitude sites (Subgroup 2A) and the larger Group 2B headwater sites of large Zone 3 Rivers.

An NMDS ordination of diatom community data showed a similar differentiation of these two main groups, Group1B and Group2 (**Figure 5.2.1**). A distinctive triad of high altitude sites (encircled triad - red triangle) was also isolated in this plot at one end of an environmental gradient (Axis1). A less distinct condition gradient (Axis2) separated Group 1B, represented predominantly by Zone 1 sites, from a smaller Group 1A with a mixed representation of Zone 3 and Zone 1 sites. However Group1B (Zone 1) and Group2 (all Zone 3 sites) were ordered along a more distinctive condition gradient (Axis1).

The ANOSIM statistic ('R'= 0.53 ; p = 0.001),which was used to compare the two main groups, is relatively high in the range of +1 to -1 thus emphasizing that the most similar samples are retained separately within each of the 2 distinctive groups. The 'Stress factor' (as recorded in **Figure 5.2.1**) is itself also relatively low implying the ordination of the samples (sites) relative to one another is reliable.

Scenario	Comparison of Groups	ANOSIM Sample Statistic 'R'	*p* Value	Randomisation	No.of Samples	No.of Variables
A2	Z1 Coastal vs Z3 Montane	0.53	0.001	1000	44	208

Seaby & Henderson (2006)

[SCENARIO A2] SITES FROM GEOLOGICALLY DIFFERENT SPATIAL ZONES

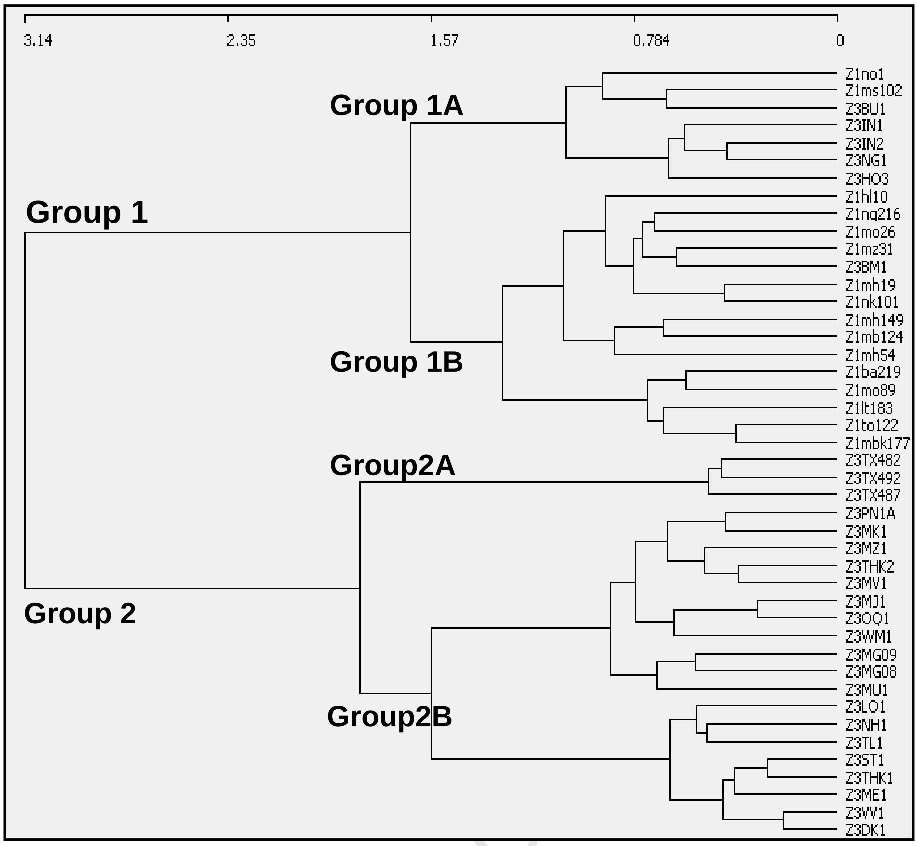


Figure 5.2 **Agglomerative Cluster Analysis dendrogram of diatom community data from headwater sites of rivers originating in the Coastal Lowlands (Z1) and Montane Uplands (Z3). Clustering using Ward's method and Bray-Curtis similarity measure**. [Present-day Resources A4, C3 (2006-2009)]

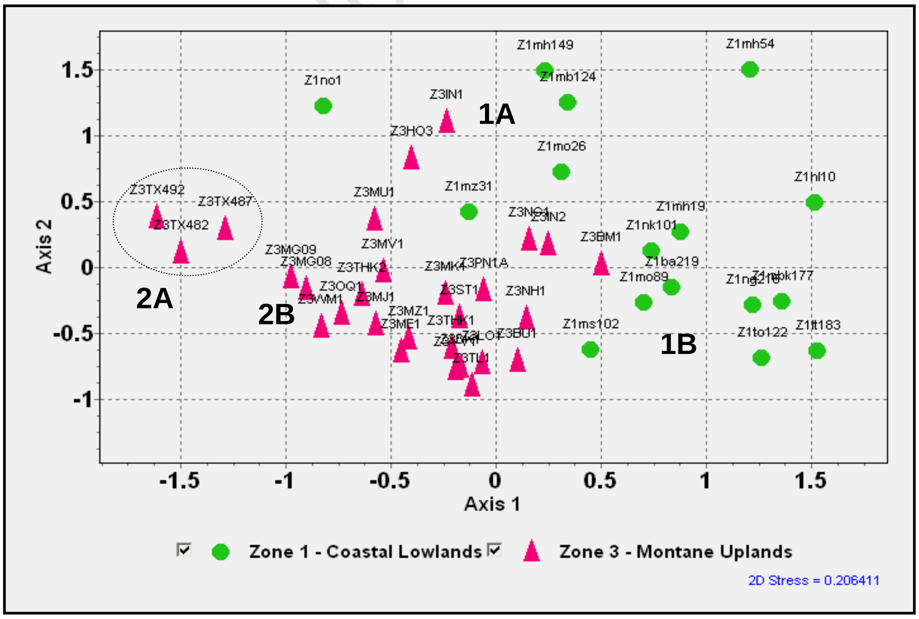


Figure 5.2.1 **NMDS ordination of diatom community data ($\log_{10}[x+1]$ transformed) showing the positioning of sites from headwaters of rivers originating in the Coastal Lowlands (Z1) and Montane Uplands (Z3)**.
[Present-day Resources A4, C3 (2006-2009)]

[SCENARIO B1] ASSESSMENT OF PRESENT- DAY SITES WITHIN SPATIAL ZONE 3 - LARGE RIVERS OF THE MONTANE UPLANDS

One **present-day** data set (A4), and three separate **historic** resources (A1, A2, A3)(**Table 1**) were examined to establish a pool of headwater sites from which candidate reference sites could be identified for the largest and most important Zone 3 Rivers as discerned from **Scenario B1 (present–day) and Scenario B2 (historic)**.

An Agglomerative Cluster Analysis dendrogram of **present-day** diatom data, extracted from rivers originating in the Montane Uplands (Zone 3), produced a main group of sites (Group1) which was separated into smaller scale discrete geographical headwater sub-regions within the large spatial zone of the Montane Uplands. The designated Zone 3 site groupings were drawn from headwaters of rivers originating in different sub-regions. Sites located in the headwaters of large rivers originating in the North-east of the province (Pongola PON1A, Black Mfolozi BMF1, Mkuze MKU1) were more closely affiliated to those in the sub-regional headwaters of the North-western arm of the Thukela (Subroup1A) (**Figure 1.2**).The headwaters of rivers originating in the South-western part of the study area (Subgroup1B) were grouped as a separate entity with the exception of Site WMF1 (White Mfolozi) which has its headwaters in the North-east. A set of high altitude headwater sites of the Thukela River (a component of Subgroup 1A) was also differentiated from other river sites in this spatial zone (**Figure 5.3**).

An NMDS ordination of the present-day Zone 3 sites produced a distinctive geographical pattern reflecting gradients of known but unmeasured geological differences. The high altitude sites (circled triad - pink triangle Subgroup1Aa) were separated from the rest of the sites at one end of the gradient (Axis1). Zone 3 sites located in the headwaters of the South-western sub-region (solid blue circles), were ordered along a gradient (Axis1) and separated from sites from Group 1A in rivers originating in headwaters of the Northwestern sub-region (**Figure 5.3.1**). The designated Zone 3 sites of the-North-western headwaters (solid orange circles) were essentially from the upper Buffalo River, itself the main tributary of the Thukela River (**Figure 1.2**).These sites showed a greater affinity with river sites originating in the eastern part of the study area with the exception of Site WMF1 (White Mfolozi), the diatom composition of which showed a greater affinity with the Zone 3 South-western group of sites. The sites located in headwaters of rivers in the North-eastern part of the province were distant from Group1A sites. Another small separate group of sites (Group 2), drawn from the western and southern headwaters of the study area (**Figure 1.2**), were separated on an unmeasured gradient (Axis2) from the bulk of the South-western grouping (Group1B).

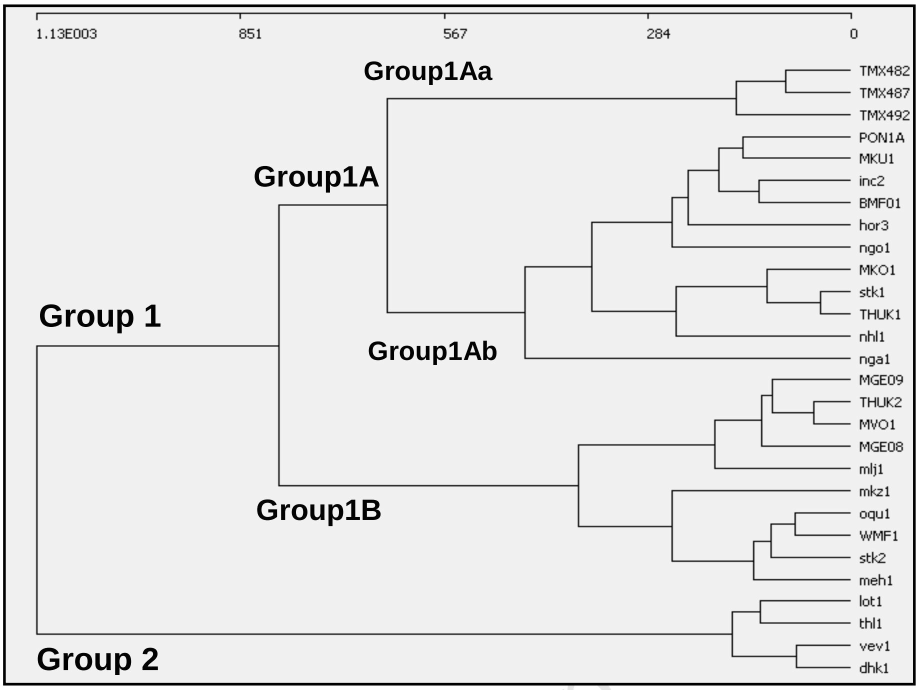


Figure 5.3 **Agglomerative Cluster Analysis dendrogram grouping of diatom communities from headwater sites of rivers originating in geographical sub-regions in spatial Zone 3 (Montane Uplands). Clustering using Ward's method and Bray Curtis similarity measure.**
[Present-day Resource A4 (2006-2009)]

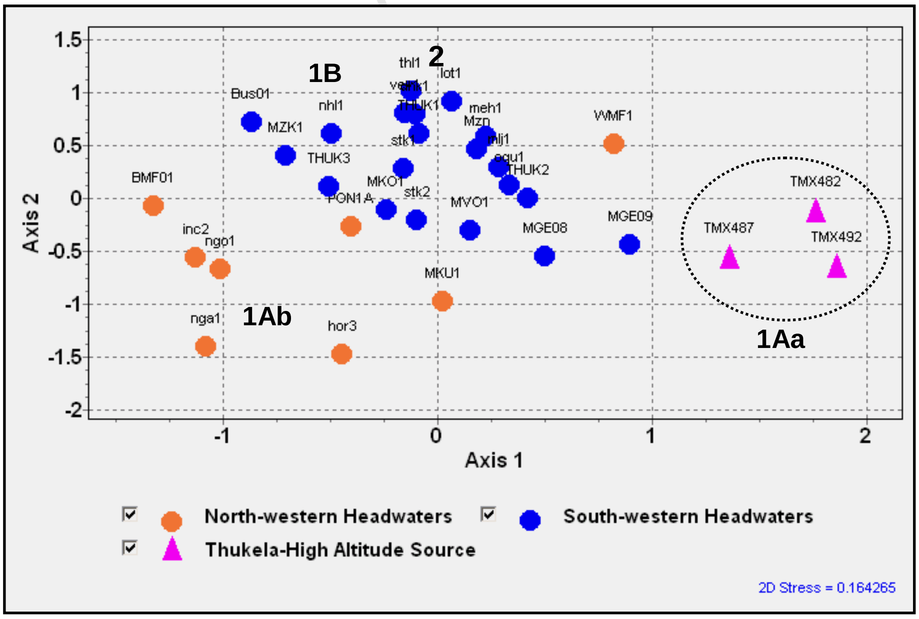


Figure 5.3.1 **NMDS ordination of diatom community data ($\log_{10}[x+1]$ transformed) showing the positioning of sites from headwaters of rivers originating in geographical sub-regions in spatial Zone 3 (Montane Uplands)**.
[Present-day Resources A4 (2006-2009)]

[SCENARIO B2] ASSESSMENT OF HISTORIC SITES WITHIN SPATIAL ZONE 3 – LARGE RIVERS OF THE MONTANE UPLANDS

A comparison was also made of **historic** Zone 3 sites drawn from the headwaters of different geographical sub-regions of the Thukela River and the adjacent Mkomazi headwaters (Historic Resources A1-A3, (Cholnoky 1956, 1957, 1960b) (**Figures 5.4, 5.4.1**).

Agglomerative Cluster Analysis produced two main groups of sites which were differentiated into Group1 representatives of headwaters of rivers originating in the Southern and Southwestern Montane Uplands. Group2 representatives were from the headwaters of the main Thukela River originating in the western headwaters, including the small group of high altitude sites (Subgroup2A) (**Figure 1.2**).

An NMDS ordination of the diatom data extracted from the headwater sites of rivers originating in Zone 3 indicated a condition gradient (Axis1) rendering individual groups of sites representing the sub-regional separation of the headwaters. The proximity of these sub-groupings indicates general similarities in the structure of diatom communities. A clearer gradient (Axis2) is portrayed by the separation of a group of similar high altitude sites (Subgroup 2A - pink triangle) from sites ordered on the main gradient (Axis1).

The similarity of many of the diatom samples, drawn from headwater tributaries of the upper western, southwestern and southern tributaries of the Thukela main channel (blue diamond, solid yellow circle, solid green circle) over two sampling periods (Cholnoky 1956, 1957) resulted in close ordination of these sites (Groups1A,1B and 2B). This group of sites, drawn from the headwaters of the adjacent Mkomazi and its tributaries, also showed close affinity with the larger grouping (Subgroup1B- solid orange circles).

Two disparate sites (GOL1 and TUG25) appear in separate subgroups of Group2B but both are distantly positioned together at one end of the condition gradient (Axis1) from the main cluster of sites drawn from the same sub-region.

[SCENARIO B2] ASSESSMENT OF HISTORIC SITES IN SPATIAL ZONE 3

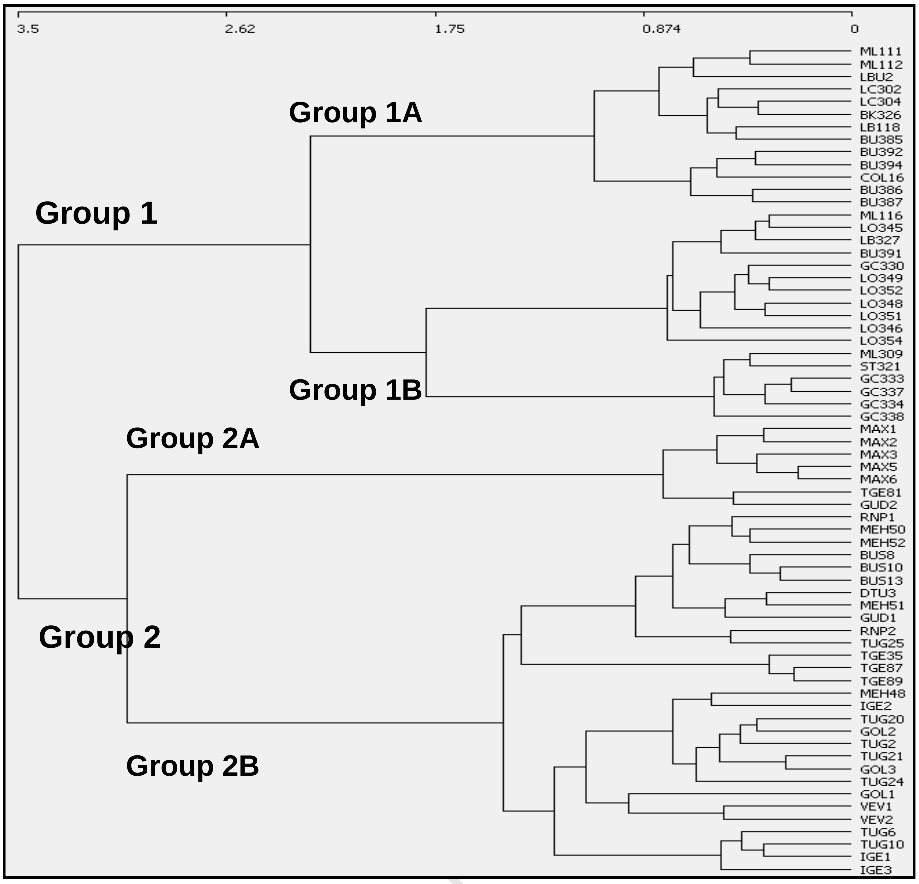


Figure 5.4 **Agglomerative Cluster Analysis dendrogram showing the groupings of historic sites from headwaters of rivers originating in Zone 3 (Montane Uplands).Clustering using Ward's method and Bray Curtis similarity measure** [Historic Resources A1-A3 (Cholnoky 1956-1957,1960b)]

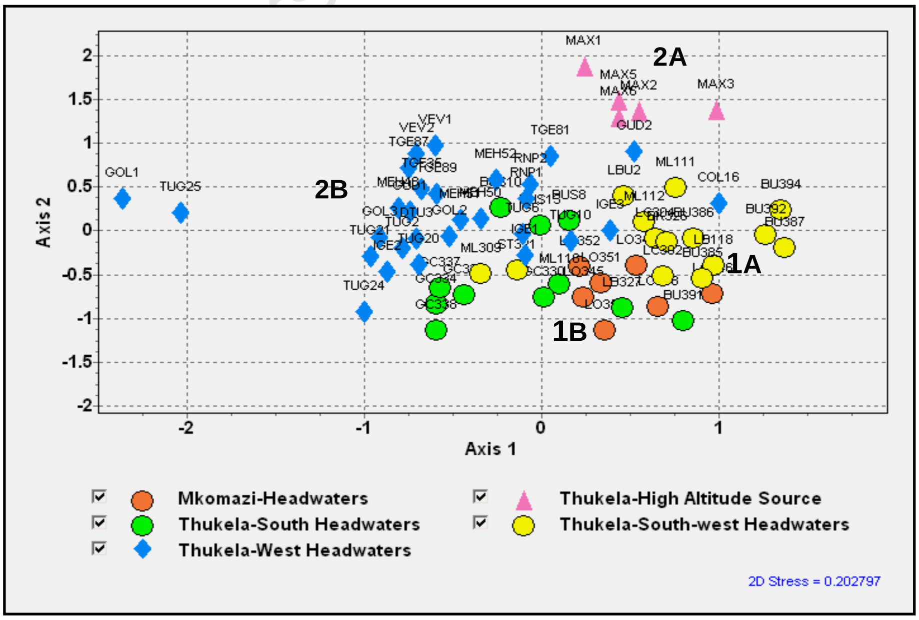


Figure 5.4.1 **NMDS ordination of diatom count data ($\log_{10}$ [x+1]) transformed) showing the positioning of sites from headwaters of rivers originating in Zone 3 (Montane Uplands)** [Historic Resources A1-A3 (Cholnoky 1956-1957,1960b)]

[SCENARIO B3] ASSESSMENT OF PRESENT-DAY AND HISTORIC DIATOM SITES OVER TIME WITHIN SPATIAL ZONE 3

***Present-Day versus Historic Zone 3 sites** - (**Resources: A4 versus A1-A3**)*

The possible effects of temporal changes needed to be tested in the quest to identify reference sites appropriate to present-day conditions. This was addressed by comparison of Zone 3 site data from both **present-day** and **historic** surveys of the large rivers of the study area (**Figures 5.5, 5.5.1**).

Two main, but different sized groups were produced by the Agglomerative Cluster Analysis. Group1 included a subgroup of high altitude sites from both recent and historic data (Subgroup1A) and these were discrete enough to remain as distinctive subgroups representing different time periods The historic group of sites of the second component (Group1B) was predominantly drawn from headwaters of rivers from the Montane Uplands (Upper case site codes). Two sites (MEH48 and VV101) were separated at a low level of variance but distinct from the rest of Subgroup1B. Group2 representatives were exclusively from present-day sites (lower case site codes). The ANOSIM statistic ('R'= 0.58 ; p = 0.001), which was used to compare the main groups, is relatively high in the range of +1 to -1 thus emphasizing that the most similar samples are retained separately within each of Group1 and Group2.

Since the 'Stress factor' (as demonstrated in **Figure 5.5.1**) is itself relatively low the ordination of the samples (sites) relative to one another is reliable.

Scenario	Comparison of Groups	ANOSIM Sample Statistic 'R'	p Value	Randomisation	No.of Samples	No.of Variables
B3	Z3 Historic vs Recent	0.58	0.001	1000	32	190

Seaby & Henderson (2006)

An NMDS ordination of the diatom data drawn from historic and recent samples indicated marked differences in the positioning of sites confined within the two main groups (Group1A and Group1B) and Group2 (solid orange circle) along an unmeasured condition gradient over time (Axis1). High Altitude sites (Subgroup1A) were ordered along a different gradient (Axis2) and were positioned as distinct triads of both the present-day recent (green triangle) and historic sites (pink triangle) quite distinct from Group1B (solid blue circle). Two historic sites (MEH48 and VV101- solid blue circles) were set apart from but closer to the main cluster of Subgroup1B at one end of a condition gradient (Axis1) with Group2 sites at the other end of the gradient.

[SCENARIO B3] PRESENT-DAY VERSUS HISTORIC ZONE 3 SITES

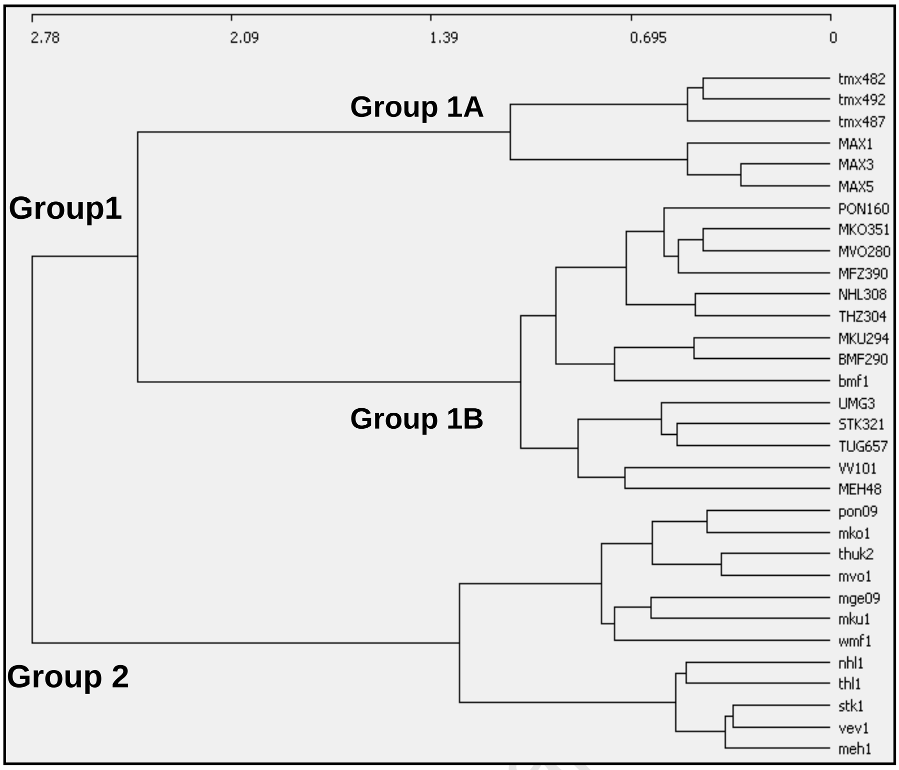


Figure 5.5 Agglomerative Cluster Analysis dendrogram of the groupings of present-day and historic sites from headwaters of rivers originating in Zone 3 (Montane Uplands). Clustering using Ward's method and Bray-Curtis similarity measure.

[Resources A1-A3 (Cholnoky 1956,1957,1960b) and Present-Day A4 (2006-2009)]

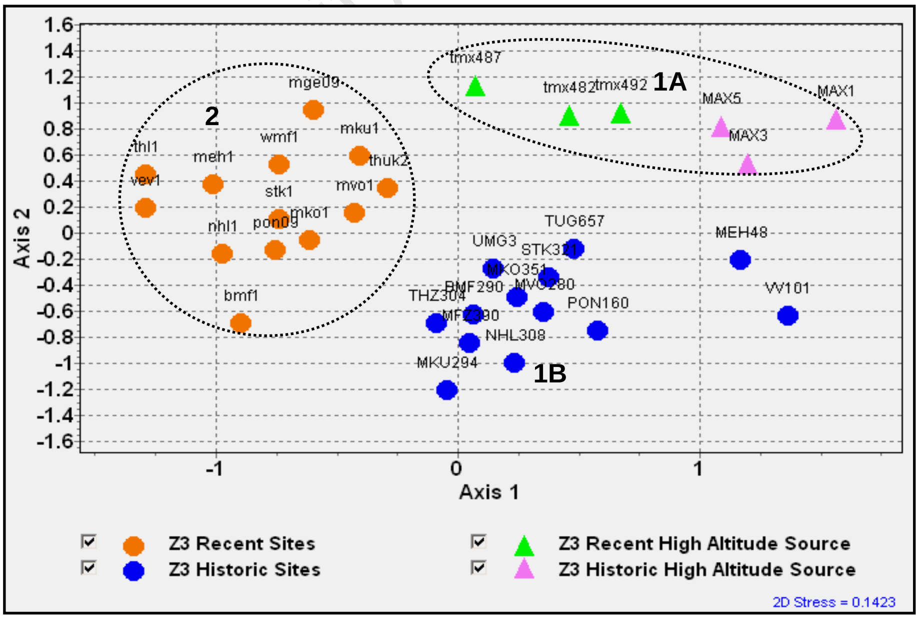


Figure 5.5.1 NMDS ordination of diatom count data ($\log_{10}$ [x+1] transformed) showing the positioning of recent and historic sites of headwaters of rivers originating in Zone 3 (Montane Uplands). Bray-Curtis similarity measure

[Resources A1-A3 (Cholnoky 1956,1957,1960b) and Present-Day A4 (2006-2009)]

[SCENARIO C1] ASSESSMENT OF PRESENT-DAY SITES IN SMALL RIVERS: SPATIAL ZONE 1 - COASTAL LOWLANDS

A suite of sites from the small rivers originating in the urbanized and rural areas of the Coastal Lowlands was assessed in the expectation that type specific candidate reference state sites could be identified from the headwaters for this category of river (**Figures 5.6, 5.6.1**). An Agglomerative Cluster Analysis dendrogram of the diatom count data from present-day sites differentiated the headwater sites (Group1) from impacted sites (Group2). The dissimilarity between impacted sites (mostly from the lower reaches) of the small rivers was indicated by the highest degree of variance (value ~2.78) between the two main groups. Disparate smaller subgroups within the respective larger groups were differentiated at a lower variance within the subgroups (< 0.696) (**Figure 5.6**).

The ANOSIM statistic (‘R’= 0.63; p = 0.001) which was used to compare the two main groups, is relatively high in the range of +1 to -1 thus emphasizing that the most similar samples are retained separately within each of the two main groupings. The ‘Stress factor’ (as displayed in **Figure 5.6.1**) is itself also relatively low implying that the ordination of the samples (sites) relative to one another is reliable.

Scenario	Comparison of Groups	ANOSIM Sample Statistic 'R'	*p* Value	Randomisation	No.of Samples	No.of Variables
C1	Z1 Contaminated Sites vs Source	0.63	0.001	1000	35	181

Seaby & Henderson (2006)

An NMDS ordination of the diatom data resulted in the positioning of sites along a condition gradient (Axis1) giving a clear separation between the main groups. The headwater sites (Group1) were, however, more widely distributed with some sites in positions distant from the main gradient. The contaminated group (Group2) incorporates a denser cluster of sites positioned closer together and confined to one end of the main gradient (Axis1) (**Figure 5.6.1).** Three headwater sites (MOL26, MHL149, MHL54) were positioned distantly from the main representatives of Group1 headwater sites at the opposite end of the gradient (Axis1). These are still however included in a subgroup within the Group1 headwater sites of Zone 1 Rivers.

[SCENARIO C1] PRESENT-DAY SITES DRAWN FROM SMALL RIVERS IN SPATIAL ZONE 1

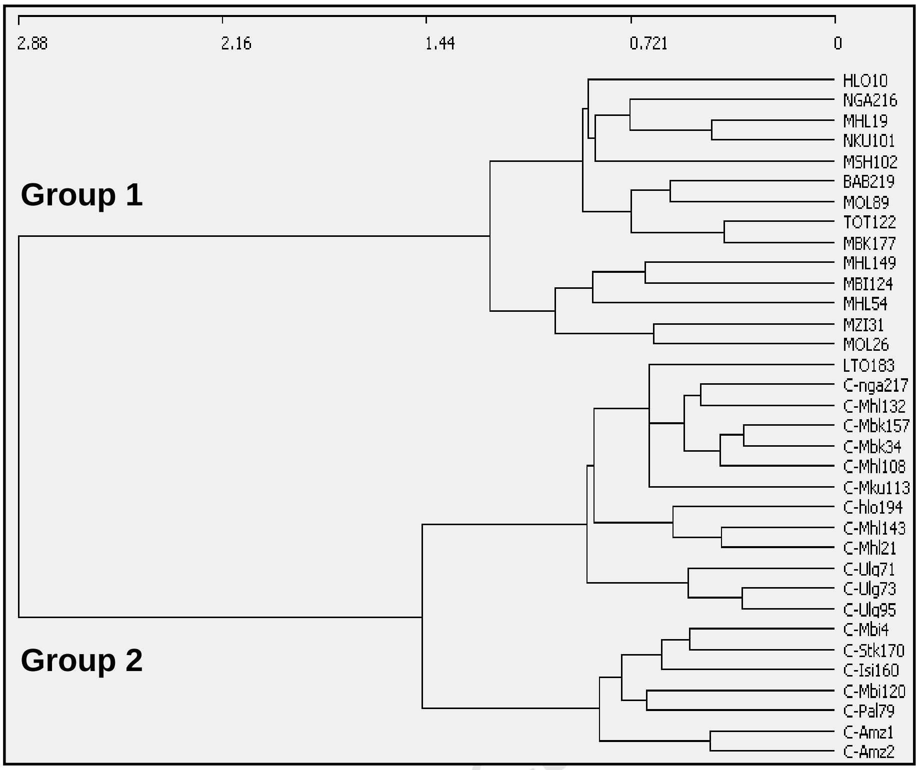


Figure 5.6 **Agglomerative Cluster Analysis dendrogram of diatom count data showing grouping of headwater sites and contaminated sites (c-) in rivers originating in Zone 1 (Coastal Lowlands).**

[Present-day Resources: C3, E1 (2006-2009)]

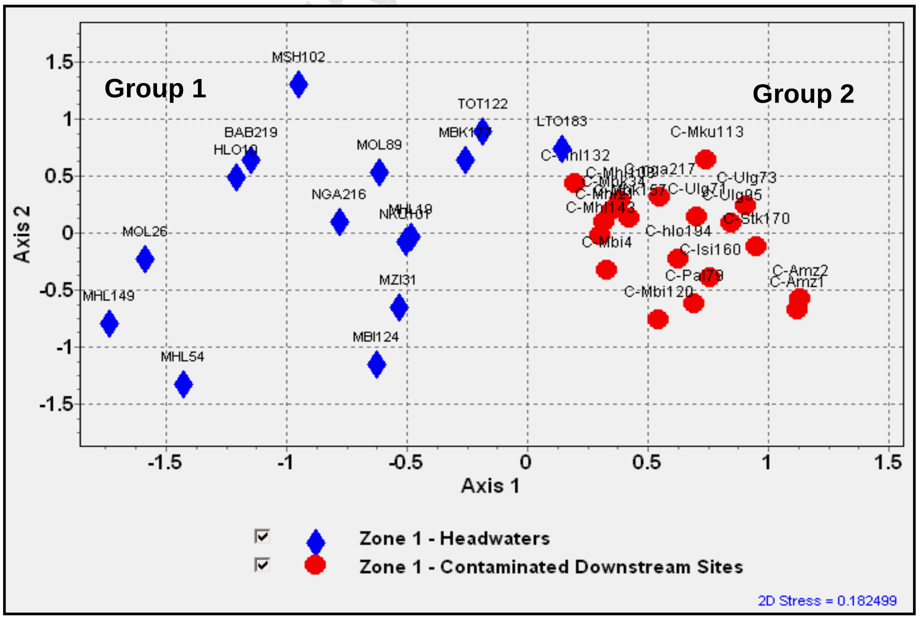


Figure 5.6.1 **An NMDS plot of diatom count data (log_{10}[x+1] transformed) showing the comparison in positioning of headwater sites with downstream impacted sites(C-) in rivers originating in Zone 1.**

[Present-day Resources: C3, E1 (2006-2009)]

[SCENARIO D1] ASSESSMENT OF HEADWATER SITES OF LARGE RIVERS OF SOUTH AFRICA

High ecological status sites would be expected in headwaters of large rivers originating in protected mountain areas. A simple comparison was made therefore of the diatom metrics of historic data from the headwaters of some large Zone 3 Rivers of the study area, and those of other large rivers of the eastern summer rainfall areas of South Africa **(Table 5.1).**

The data on the headwaters of several different large rivers was produced independently by three different leading diatomologists. This resource provided a good test of the application of an '*a posteriori'* interpretation of the position and / or separation of sites along an environmental gradient based solely on the structure of diatom communities using multivariate procedures.

An Agglomerative Cluster Analysis dendrogram of the diatom assemblage data was differentiated into two main groups of headwater sites (Group1 and Group2) (**Figure 5.7)**. Group 1 was distinguished by the headwater site of the Bilangil River, at the source of the Orange River (Site OD71), which lies on the western slopes of the Drakensberg Mountain divide, adjacent to the Group1 high altitude sites (TX1, TX3, TX5) at the source of the Thukela River which lies on the eastern side of the mountains. Similarly, the headwater site of the Sani River, also a source tributary of the Orange River (Site OC146), lies on the western slopes adjacent to Sites MK348, MK349, at the headwaters of the Mkomazi River on the high eastern slopes of the Drakensberg (Subgroup 2Bb). Both Orange River sites (OD71 and OC146) are situated in areas that share similar geological and hydrochemistry characteristics with large river sites located on the eastern slopes (De Villiers 2005).

An NMDS ordination also showed the positioning of Group2 sites along a condition gradient (Axis1) with a different gradient (Axis 2) separating the high altitude sites (Group1) **(Figure 5.7.1).** The main channel headwaters sites of the Thukela River (TM65, TM54) were positioned at one end of the gradient and all the sites from the Sundays River (Subgroup2Bb), which is known to be highly saline (Archibald 1981) occur at the opposite end of this gradient. By contrast, most of the southern headwater sites of the Vaal River (VL-sites) were grouped with the chemically dilute Mkomazi headwaters (Subgroup 2Ba) in an intermediate position.

The headwaters of the Vaal itself (VL204) and the Pongola River site (PON1 solid yellow circle), both geographically closer to each other and characterised by higher TDS waters, are positioned nearest to the discrete group of headwater sites of the more saline Sundays River (**Group 2Bb**) (solid brown circle) **(Figure 5.7.1)**.

[SCENARIO D1] HEADWATER SITES OF LARGE RIVERS OF THE EASTERN SUMMER RAINFALL REGION OF SOUTH AFRICA

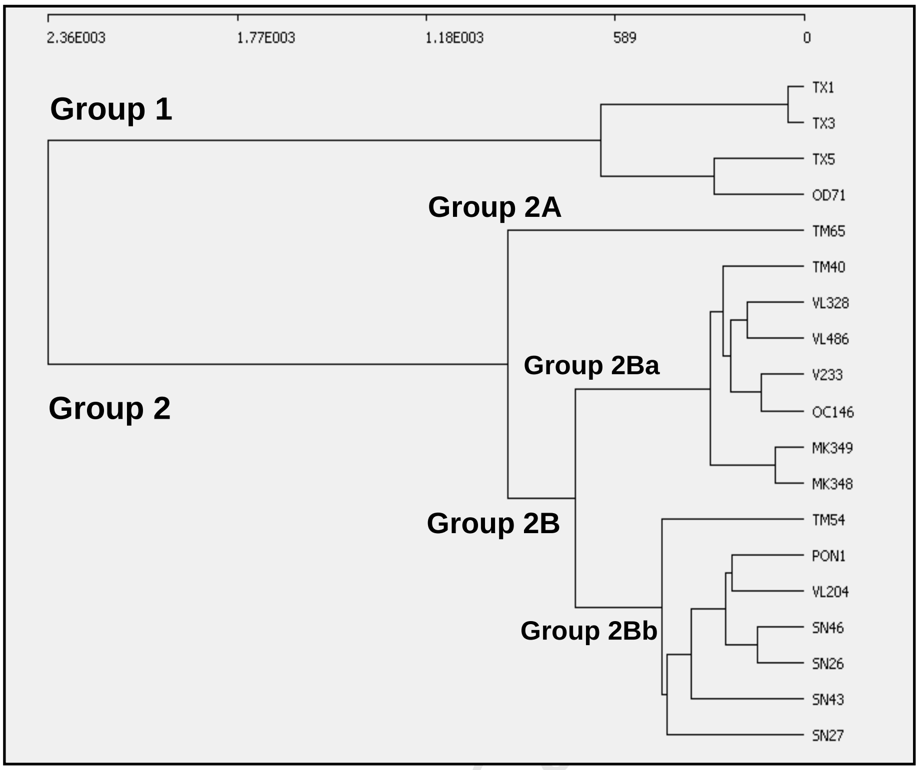


Figure 5.7 **Agglomerative Cluster Analysis of diatom count data from headwater sites of large South African Rivers originating in the Eastern Summer Rainfall region of South Africa.**
[Historic Sources (Cholnoky 1956, 1957; Schoeman 1971, Archibald 1968, 1981)]

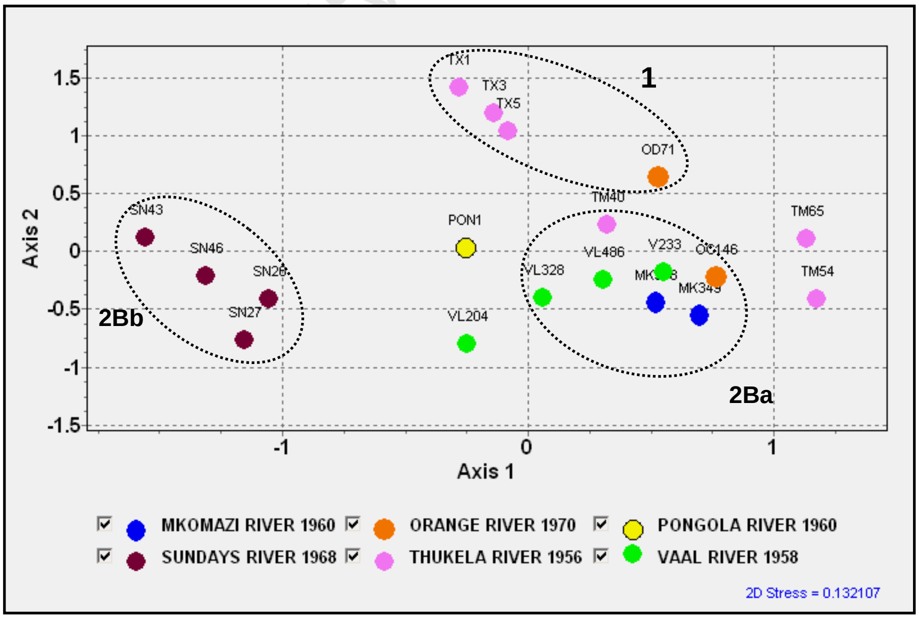


Figure 5.7.1 **An NMDS ordination plot of diatom count data (log_{10} [x+1] transformed) from headwater sites of large South African Rivers originating in the EasternSummer Rainfall region of South Africa.**
[Historic Sources (Cholnoky 1956, 1957; Schoeman 1971, Archibald 1968, 1981)]

Table 5.1 Summary statistics for various diatom water quality indices of the headwater sites of large rivers originating in the Eastern summer rainfall region of South Africa

Indices ▸ / Parameters ▾	IPS	BDI	GDI	EPI-D	TDI$	%PTV$
	General Water Quality Indices			Nutrient Enrichment Indices		
A. Headwaters of Large Zone 3 Rivers						
Maximum	19.8	20	18.8	18.1	69.9	51.1
75th Percentile	19.4	17.8	17.4	17.1	48.1	3.1
Median	18.8	16.5	16.6	15.8	34.9	0.9
25th Percentile	17.1	14.4	15.4	14.9	26.2	0.1
Minimum	9.9	9.2	10.1	7.0	18.0	0.1
Average	17.7	16.3	16.0	15.2	38.8	4.2
Standard Deviation	2.6	2.7	2.0	2.8	14.3	9.6
CV %	14.4	16.6	12.6	18.5	36.9	230.8
No.of Sites	39	39	39	39	39	39

[Present-Day Resource A4 (2006-2009)]

B. Headwater Sites from Other Large South African Rivers

Indices ▸ / Rivers ▾	IPS	BDI	GDI	EPI-D	TDI$	%PTV$
	General Water Quality Indices			Nutrient Enrichment Indices		
#Vaal 204	15.5	14.4	16.5	12.9	45.7	7.4
#Vaal 486	16.3	15.2	15.0	16.5	42.6	5.9
* Orange D71	18.0	20.0	17.7	17.3	26.5	5
* Orange C146	19.1	17.4	17.2	17.3	28.2	1.5
♦Sundays 27	16.1	8.5	13.7	14.6	34.2	10.4

[Historic Resources : Archibald # 1968, Schoeman*1971, Archibald♦1981)]

$ TDI Scale [(0-20) Low - (80-100) high nutrient contamination)]

$ %PTV Proportion of valves tolerant of organic pollution : Scale [(0-20) Low - (80-100) High]

IPS, BDI, GDI, & EPI-D Scale [0 (very poor status) - 20 (high ecological status)]

5.4.2 Ecological Status of Candidate Reference Sites

The ecological status of the candidate reference sites was determined to provide confirmatory evidence as to which sites exhibit a high ecological status, consistent with an expectation of river conditions free of human disturbance (**Tables 5.1-5.3**). Several diatom metrics that describe water quality conditions and ecological status were calculated from the diatom species level data using Omnidia (Lecointe *et al.* 1993) and SDR software (Seaby & Henderson 2006). There was an expectation that the candidate reference sites in these spatial zones would produce high IPS, BDI and GDI index values of general water quality (>15) if these were to be representative of a high ecological status. It was also expected that diatom water quality indices developed as measures of nutrient contamination, particularly phosphorus, would also reflect sufficiently low contamination in these same circumstances. The outputs from the Trophic Diatom Index (TDI) (Kelly 1998, Kelly *et al.* 2001) supplemented by % PTV values (% pollution tolerant valves) and the EPI-D index (Dell'Uomo 1996) were used as integrated measures of saprobic and trophic conditions as additional evidence to benchmark the ecological status of candidate reference sites.

The inclusion of GDI metrics allows for comparison with similar diatom indices because there is no other such published data for reference conditions in South Africa. Recent findings in South Africa have confirmed the similarities in values produced by a suite of diatom water quality indices for some other river conditions (Taylor 2004b, Taylor *et al.* 2007c, 2007d). Furthermore the consensus from these findings, adopted by the RHP, was that the IPS Water Quality Index is a metric that should be taken as the 'benchmark' to provide some standardisation in reporting diatom water quality index results in the absence of an accredited South African Diatom Water Quality Index (DWAF 2008). This is the first set of diatom metrics for KwaZulu-Natal Rivers of its kind (Appendix II). The data provides an early benchmark for the 21^{st} century for future research applications and comparison involving diatoms. Reference to other less well known indices produced by Omnidia software were infrequent in the literature across several continents (USA, Australia, Southern Africa and to a lesser extent Central - Eastern Europe**).** The applicability and transferability of these relatively unknown indices (possibly untested outside the country of origin) was therefore uncertain compared with the more established and frequently quoted suite of indices used in this thesis.

***Ecological status of Headwater sites* (*Rivers from all spatial zones*)**

'Box and Whisker' plots were developed separately for each zone to provide a comparison of the series of values of various Water Quality Indices extracted from diatom data of a pool of present-day and historic sites (**Figures 5.8, 5.8.1**). Similarly, tables were drawn up with the respective diatom metrics for headwater sites from each spatial zone (**Tables 5.2, 5.3**).

Comparison of these water quality index data provides key information of different aspects of the prevailing water quality condition and ecological status associated with the diatom assemblages that were commonly identified from the candidate reference sites within the various spatial zones.

High values (>15) for the three general water quality indices were recorded from historic data of most sites drawn from the headwaters of several rivers originating in Zone 3 and for some sites in Zone 2 and Zone 1 **(Table 5.2, Figure 5.8).** However the range of values of the three general water quality indices (IPS, BDI and GDI) differed between the spatial zones indicating that there were several disturbed sites in the Zone 2 and Zone 1 data sets. The results of the TDI index and the %PTV showed a greater range in values for Zone 1 rivers (81.8 - 8.9) than for conditions in Zone 3 rivers (55.2 - 6.1), as is evident also from the high coefficients of variation (CV %) for these measures (**Table 5.2**).

This trend is also presented graphically in the 'Box-and-Whisker' plots for historic data in each zone (**Figure 5.8**). The EPI-D index values of historic sites (Dell'Uomo 1996) show a similar pattern in results for Zone 3 sites (18.2 - 11.5) and Zone 1 sites (19.5 - 6.9) (**Table 5.2**). The output from these data showed that almost all the candidate sites in Zone 3 met the criteria for high ecological status. Those of Zone 2 and Zone 1 showed a reduction in the number of suitable sites which met the criteria for high ecological status.

Ecological status of present-day sites

Zone 3 –Montane Uplands Sites

A summary of **present-day** values for the diatom water quality indices of sites from headwaters of rivers originating in Zone 3 also showed high values for the three general water quality indices (medians > 16) (**Table 5.3)**. The EPI-D median value was also > 15 while the associated median TDI and %PTV values were 34.9 and 0.9 respectively indicating sites with sufficiently low nutrient contamination thus confirming their high ecological status.

Zone 1 – Coastal Lowland Sites

There was a marked increase in the range in index values for present-day sites when comparisons were made between Zone 3 data from sites which were uncontaminated (high ecological status) and those of Zone 1 sites. Nonetheless there were some headwaters in Zone 1 that were rated as high ecological status sites and these met the ecological status criteria of candidate reference state sites. There was a notable and expected increase in the variation in the TDI and %PTV values for sites from impacted reaches of Zone 1 indicating the change in response of the diatom assemblages to nutrient pollution in contaminated reaches of rivers originating in the Coastal Lowlands (**Table 5.3, Figure 5.8.1**).

Table 5.2 Range in values for various water quality indices from headwater sites of rivers originating in three different spatial zones (Historic Data 1954-1958)

Parameters	IPS	BDI	GDI	#TDI	#%PTV	EPI-D
	General Water Quality			Nutrient Status Indices		
[A] Zone 3 River Sites : Montane Uplands						
Maximum	19.8	20.0	19.5	55.2	30.9	18.2
75th Percentile	19.0	17.0	17.0	35.0	7.0	17.0
Median	17.3	16.6	15.9	31.1	3.0	16.7
25th Percentile	15.6	15.2	14.6	25.7	0.6	15.7
Minimum	13.3	12.5	10.5	6.1	0.1	11.5
Mean	17.1	16.3	15.6	30.3	5.2	16.2
Standard Deviation	1.7	1.8	1.8	10.0	6.4	1.6
CV %	9.9%	11.0%	11.5%	33.0%	123.1%	9.9%
No. of sites	56	56	56	56	56	56
[B] Zone 2 River Sites : Interior Midlands						
Maximum	18.9	17.2	18.0	78.0	57.1	18.2
75th Percentile	15.0	15.6	15.7	54.1	16.4	16.2
Median	14.4	14.3	13.8	41.2	11.2	15.1
25th Percentile	11.7	13.1	11.8	27.3	3.0	13.1
Minimum	10.3	10.2	8.0	3.2	0.6	7.9
Mean	14.1	14.3	13.5	38.9	13.7	14.4
Standard Deviation	2.5	1.8	2.9	21.0	14.5	2.7
CV %	17.7%	12.6%	21.5%	54.0%	105.8%	18.8%
No. of sites	24	24	24	24	24	24
[C] Zone 1 River Sites : Coastal Lowlands						
Maximum	20.0	18.7	17.1	81.8	82.4	19.5
75th Percentile	17.2	16.0	14.2	63.5	46.1	16.1
Median	13.3	14.4	12.6	52.0	16.8	13.7
25th Percentile	11.2	13.0	9.9	31.6	2.6	11.4
Minimum	7.3	6.2	6.3	8.9	0.1	6.9
Mean	13.6	14.2	12.1	48.0	24.9	13.5
Standard Deviation	3.5	2.6	3.1	22.6	25.1	3.5
CV %	25.7%	18.3%	25.6%	47.1%	100.8%	25.9%
No. of sites	25	25	25	25	25	25

(Header: Diatom Water Quality Indices)

[Resources : A1- A3, B1, B2,C1 (Cholnoky 1956-1960)]

IPS, BDI, GDI, EPI-D Scale : (0 Very poor) - (20 high quality) water

TDI Scale (0 Low) - (100 high) nutrient contamination

#%PTV Scale : % of valves tolerant of organic pollution (0-20) Low and (80-100) High organic contamination

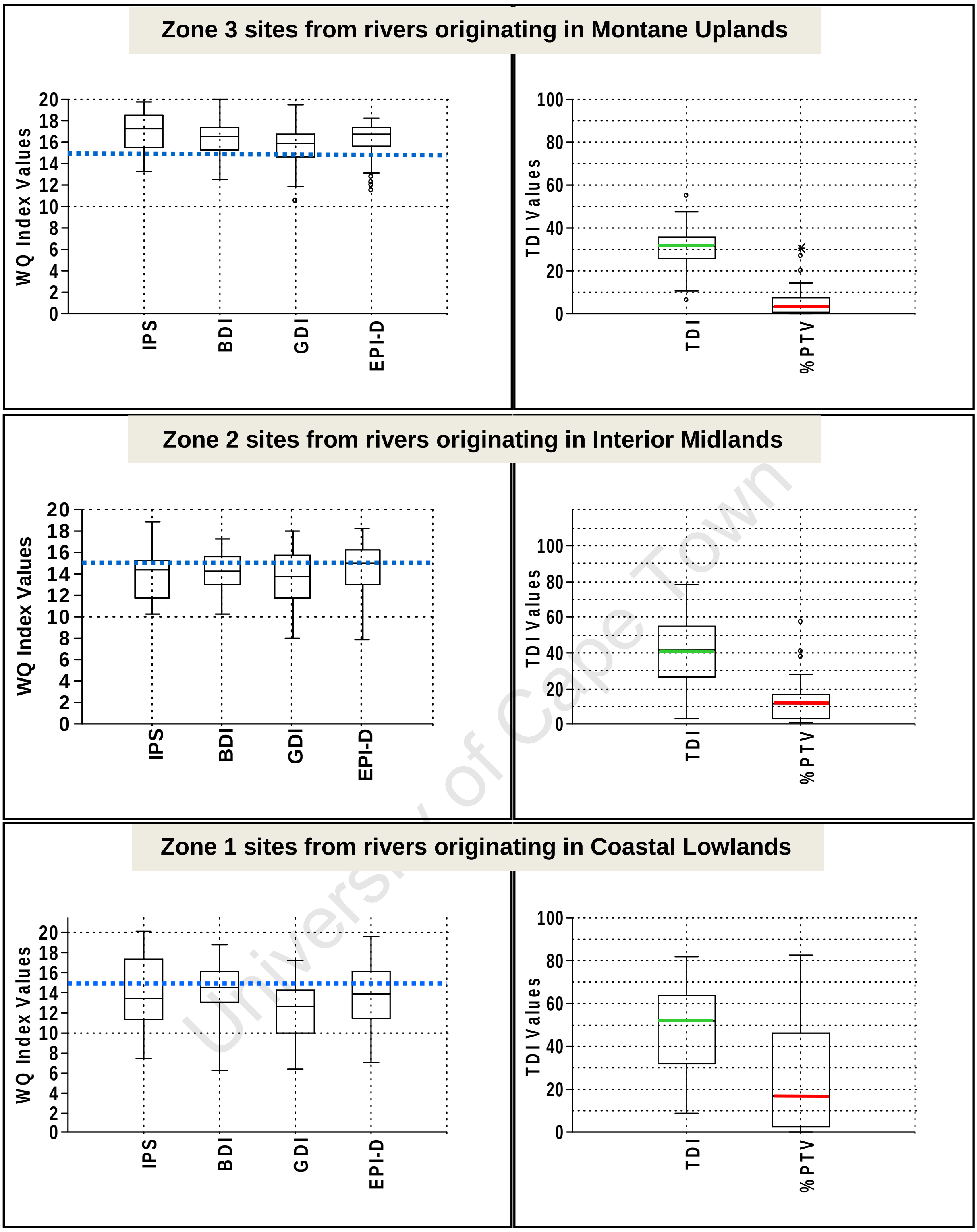


Figure 5.8 **'Box' plots showing the range in Water Quality Index values for historic sites from rivers originating in three spatial zones in KwaZulu-Natal**

[Resources A1-A3, B1, B2, C1 (Cholnoky 1956-1960b)]

Note: WQ Index values range from (**0-10** poor ecological status) to (**15-20** high ecological status). TDI and % PTV values range from (**0-20%** low nutrient contamination) to (**80-100%** high nutrient contamination). Vertical boxes represent the Inter Quartile Range (IQR) for each index and central bar represents the median value. Extreme points (Max or Min < 1.5 * IQR).

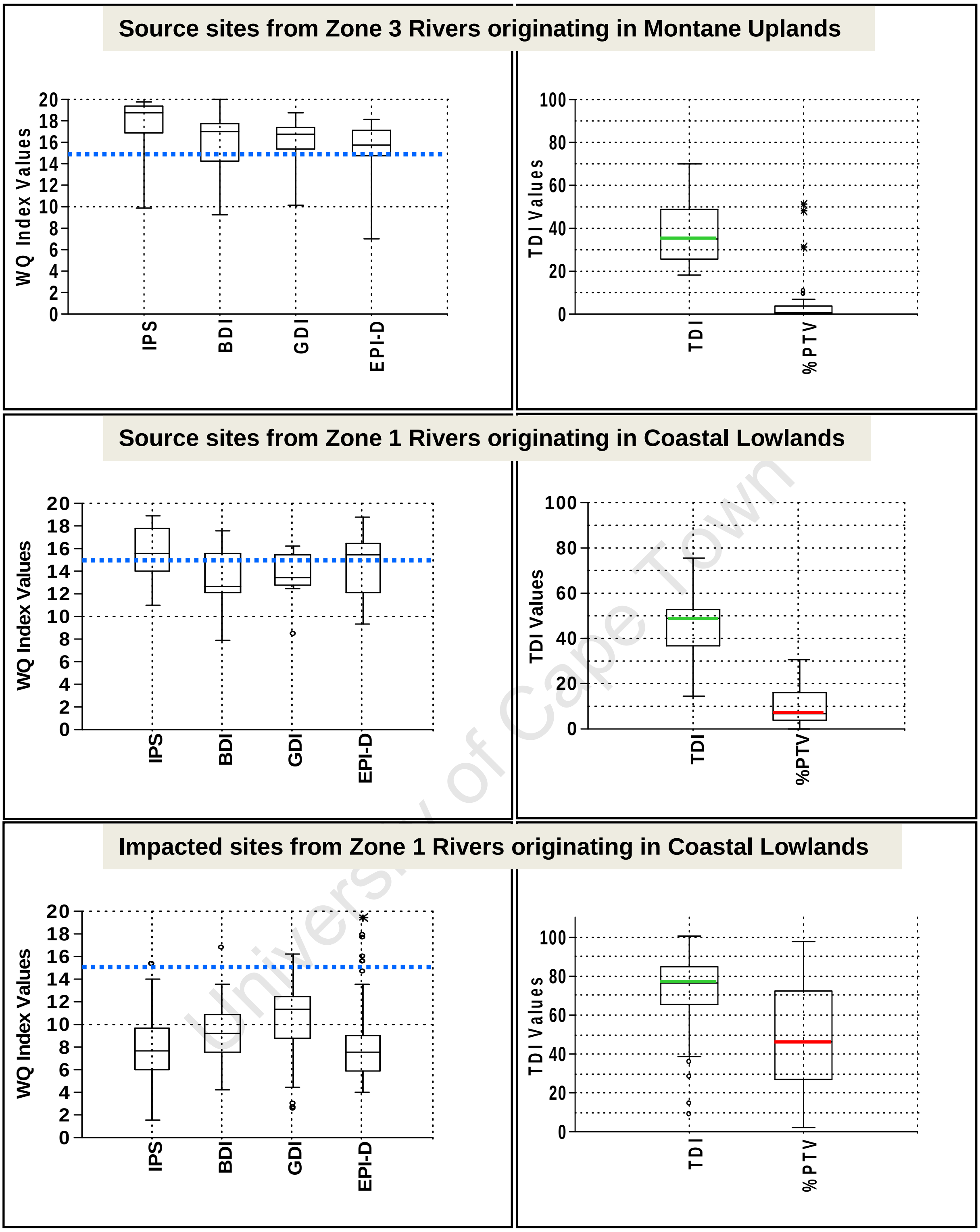


Figure 5.8.1 'Box Plots' showing the range of various Water Quality Index values for present-day sites in rivers originating in spatial Zone 1 and Zone 3

[Present-day Resources : A4, C1,C3 (2006-2009)]

Note : WQ Index values range from (**0-10** poor ecological status) to (**15-20** high ecological status). TDI and % PTV values range from (**0 - 40%** low nutrient contamination) to (**80 - 100%** high nutrient contamination). Vertical boxes represent the Inter Quartile Range (IQR) for each index and central bar represents the median value. Extreme points (Max or Min < 1.5 * IQR).

Table 5.3 Summary statistics for various diatom water quality indices from sites in rivers originating in spatial Zone 3 and Zone 1

Parameter	IPS	BDI	GDI	EPI-D	TDI	% PTV	Species Richness
	General Water Quality Index				Nutrient Index #	#	
Zone 3 Headwaters	**High Ecological Status Sites (WQI > 15) : TDI (0 - 20)**						
Maximum	19.8	18	18.9	17.1	48.9	9.6	30
75th Percentile	19.5	17.7	17.3	16.9	37.1	1.1	24
Median	19.3	17.2	17	16.5	29.9	0.3	16
25th Percentile	18.2	16.1	16.2	15.7	26.1	0.1	13
Minimum	16.9	15.1	14.3	14.7	19.6	0.1	9
Samples	14	14	14	14	14	14	14
Zone 1 Headwaters	**High Ecological Status Sites (WQI > 15) : TDI (0 - 20)**						
Maximum	18.9	17.5	16.2	18.8	52.8	18.1	39
75th Percentile	17.9	16.0	15.9	16.8	44.3	7.8	31
Median	16.9	14.3	14.9	16.3	38.1	5.2	25
25th Percentile	15.6	12.8	14.3	15.5	34.2	3.3	20
Minimum	15.3	12.4	13.3	13.0	14.2	0.1	11
Samples	11	11	11	11	11	11	11
Zone 1 Rivers	**Moderate Ecological Status Sites (WQI 10 - 15) : (TDI 40 - 60)**						
Maximum	14.9	16.8	16.2	19.4	83.1	52.1	40
75th Percentile	13.5	12.3	13.7	15.1	64.5	28.7	33
Median	12.2	11.4	12.8	11.8	55.7	16.4	28
25th Percentile	11.4	10.6	12	9.9	48.7	8.1	22
Minimum	10.3	7.4	8.4	6.6	9.1	2.2	10
Samples	32	32	32	32	32	32	32
Zone 1 Rivers	**Poor Ecological Status Sites (WQI < 10) : (TDI > 80)**						
Maximum	9.8	13.5	13.6	11.1	100	97.3	48
75th Percentile	8.4	10.2	11.9	8.0	90.4	77.4	31
Median	6.9	8.3	10.3	7.1	81.6	57.5	24
25th Percentile	5.5	7.0	8.0	5.6	72.6	39.8	19
Minimum	1.5	3.8	2.2	4.0	60.1	8.4	5
Samples	72	72	72	72	72	72	72

[Present-day Resources A4, C5 (2006-2009)]
IPS, BDI, GDI, EPI-D Scale : (0-5) Very poor - (15-20) high quality
TDI Nutrient contamination Scale (0-20) Low - (80-100 high)
#% PTV Scale : % of valves tolerant of organic pollution: (0-20) Low and (80-100) High contamination

5.5. Discussion

A diatom-based approach was applied in this part of the investigation to facilitate the assessment, identification and derivation of reference state sites and communities without recourse to environmental data. Various scenarios were tested as a means of identifying potential candidate reference state sites within the constraints of the availability of sites from specific resources. For example, there were no appropriate **historic sites** in the headwaters of Zone 1 Rivers with which to compare Zone 3 sites and therefore only the **present-day** resources were 'available' for analysis of Scenario A2.

[Scenario A1] Assessment of historic candidate sites in near-natural headwaters of three different spatial zones.

A pool of **historic sites** was originally part of a province-wide study of river diatoms with no formal sampling strategy to derive and identify reference state sites from the headwaters of any particular river system. However by using screening criteria (**Chapter 3**) a discrete group of historic sites within each of the three spatial zones was retained for analysis of the diatom assemblages to achieve the goals of this investigation (Appendix I). Near-natural undisturbed historical headwater sites of rivers sharing similar characteristics would be expected to sustain diatom assemblages of a high ecological status if these were in a near-natural reference state condition. Conversely, differences in the disturbance level among sites would be expected to produce different responses of diatom assemblages to contaminated conditions if these were impacted by human activities (Chapter 7).

The main finding from the output of the analysis of this historic data set (Scenario A1) revealed a large group of similar diatom communities from historic sites (Figure 5.1, Group2) of the Thukela River originating in several headwater tributaries and the main channel in spatial Zone 3 (Montane Uplands). These were distinct from a mixed group of sites from all three Zones (Group1). Analysis of the original indiscrete pool of sites drawn from across the study area produced this mixed group of diatom communities from sites registered as belonging to all three zones. It was evident that the screening process had not isolated the individual sites clearly enough to distinguish more discrete representation of near-natural headwaters from each of the three different zones. The objective screening procedure did not preempt or make presumptions as to the ecological status of any of the historic sites. It became obvious that most of the screened Zone 2 sites, although located in the appropriate spatial zone geographically, were drawn from headwaters of tributaries of larger Zone 3 Rivers. Similarly the historic Zone 1 sites were also not true headwaters of rivers originating in the Coastal Lowlands**.**

It was concluded that it was necessary to make further assessment of candidate reference state sites by restricting the analysis to sites 'within spatial zones' as discrete test groups.

[Scenario A2] Assessment of present-day sites from two spatial zones with different bedrock geology.

An assessment of the influence of bedrock geology on the water quality template and the consequent diatom responses was made by comparing representative assemblages of **present-day** sites drawn from two spatial zones. The outputs supported the initial interpretation from Scenario A1 that, with few exceptions, the most similar samples (sites) are separately retained within discrete spatial Zone 1 and Zone 3 groupings. These response patterns of present-day and historic diatom data showed differences in the diatom communities between spatial zones that justified further examination of patterns separately within each of the spatial zones.

Natural sub-regional differences in topography and geology, and therefore water quality were expected to influence the water quality of headwater streams in the absence of human disturbance, and affect the consequent collective response of diatom species (Biggs 1995, Leland & Porter 2000, Potapova & Charles 2003). The diatom assemblages of candidate reference sites within the three separate spatial zones would therefore also be expected to be different even without any confounding impacts from pollution. The diatom communities extracted from headwater river sites that fell within the same contour boundaries (i.e. contour elevation > 1200m in spatial Zone 3) or (contour elevation <800 m spatial zone 1) would be expected to be different unless the gradients of the geological and chemical templates were the same.

The main findings from the assessment of sites under Scenario A2 was that two distinct diatom communities were produced from two different geological templates and therefore it can be deduced that geological characteristics influence the different responses of the separate diatom assemblages.

It was concluded that there are scientific grounds therefore for deriving separate reference state communities representative of separate groups of sites retained within each of the headwaters of separate Zone 3 and Zone 1 spatial zones.

[Scenario B1] Assessment of <u>present-day sites</u> within spatial Zone 3 – Large Rivers of the Montane Uplands.

The assessment of these sites was first undertaken separately to determine if issues related to different time periods may confound interpretation of the status of these sites. The outputs from the present-day Zone 3 resource showed a clear separation of sub-regional headwaters. The diatom assemblages from sites in Group 1B included representatives of Zone 3 rivers located in the South-western sub-region. These sites retained similar diatom assemblages because the headwaters of these rivers (including the Mkomazi River) were geographically close to each other and shared similar geological and climatic characteristics. Likewise Group 1A sites were located in the North-western sub-region and included sites from the headwaters of tributaries of the Buffalo River (Basin 2) (**Figure 1.2**), the largest tributary of the Thukela River. The hydrochemistry of these headwaters is different from the chemically-dilute headwaters of the South-western sub-region but similar to the headwater sites located in the North-eastern sector of the province.

[Scenario B2] Assessment of historic sites within spatial zone 3 – Large Rivers of the Montane Uplands.

The ordination of diatom data from the historic sites also showed distinct sub-groupings associated with a sub-regional gradient of sites including the western headwaters of the main Thukela River. There was no historic diatom data for the headwaters of the North-western sub-region. The outcome from site assessments of both scenarios (B1 and B2) showed that there was a clear case for sub-regional differentiation of sites located in the headwaters of the Thukela and the adjacent catchment of the Mkomazi River for both present-day and historical data sets.

It was concluded that the historic and present-day headwater sites from large Zone 3 sites could justifiably be separated based on sub-regional differences. A separate set of candidate reference sites were therefore likely to be representative of the western sub-region of the province as distinct from the North-western and North-eastern sectors

[Scenario B3] Assessment of present-day and historic diatom sites over time with spatial Zone 3

It is inevitable that measurable physical attributes and chemical characteristics of a river reach will show variations over time and therefore a reference distribution (range of values) for any associated measure will best describe the prevailing conditions at a given point in time (Stoddard *et al*.2006). It follows that headwaters, free of human disturbance, will also engender a range of diatom responses conforming to the distributions alluded to previously. It is unrealistic and unscientific to expect a single absolute value to be representative of a reference state condition in a river. The clear clustering and separation in the ordination of the large groups of sites, representative of present-day and historic headwaters of rivers originating in spatial Zone 3, were described by a range of values associated with the diatom assemblages of the time. The range of diatom responses, captured by these distinct groupings over a time span of several decades, might be expected to be substantially different if there were cogent reasons for the separation of these groups. Examination of the range of diatom metrics associated with the present-day sites and historic sites showed that, despite the pattern of grouping and ordination, there are some similarities between the historic and present-day data sets. The water quality indices for both groups, for instance, reflect high ecological status in consort with sites free of human disturbance despite differences in diatom species compositional structure. It is apparent that the autecological characteristics of the dominant species fall into the same ecological categories with high ratings in each group and therefore water quality indices are expected to be similar.

The differences in species composition may, in part, be attributed to differences in sampling time, location of sites and differences in local microhabitats (Round 1991) rather than any substantive changes in gradients within the geological / water quality template given there is no record of major abnormal climatic or environmental interventions over the last 50 years. For example, *Achnanthidium minutissimum* is a dominant species which occurs frequently in samples in high numbers and is generally regarded as an indicator of good quality water. However, as an adnate species it has also been recorded in high numbers after storm events from rocky habitats to the exclusion of other species and its specific value as a reliable indicator therefore has been questioned (Acs *et al*. 2004). Sampling in periods prior to storm events or long after the first flush has passed may result in the collection from similar substrates of similar or different species (e.g. *Achnanthidium crassum*) but all these species may still retain equally high ecological ratings. A wide range in ecological tolerance has been demonstrated by the presence of various forms of *Achnanthidium minutissimum* from diverse aquatic environments (van Dam *et al.* 1994, Eloranta & Soininen 2002, Potapova & Hamilton 2007, Ector 2009, van de Vijver (2009), Wojtal *et al.* 2011).

Based on these findings it was concluded that candidate reference sites should be retained in each of these groupings to ensure that the information derived from each cluster vis-á-vis the main objective of establishing reference state communities for KwaZulu-Natal Rivers was not obscured or lost by simple amalgamation of the two groups.

[Scenario C1] Assessment of sites in small rivers of spatial Zone 1 – Coastal Lowlands

The assessment of candidate reference state sites for small rivers originating in the Coastal lowlands produced two very different groupings of diatom community responses to the prevailing condition gradients. The communities of headwater sites of the small rivers were clearly separated in the ordination of the entire data set in contrast to sites that retained diatom communities characterised by pollution-tolerant species in a contaminated urban environment (Newall & Walsh 2005). Several headwater sites of Zone 1 Rivers were, however, classed as being of high ecological status from information derived from the application of water quality indices **(Table 5.3**).

It was concluded from these findings that a group of headwater sites form rivers originating in Zone 1 could justifiably be used for the selection and identification of candidate reference sites peculiar to coastal waters with naturally higher ionic concentrations than the headwaters of the Montane Uplands.

[Scenario D1] Comparison of historic diatom data from headwater sites of other large Rivers in South Africa

The diatom community responses to headwaters of rivers in KwaZulu-Natal were compared with diatom communities conforming to headwaters of other large rivers of the eastern summer rainfall region. The data from most of the headwater sites of large rivers of the eastern part of South Africa including the study area produced high water quality ratings consistent with a high ecological status **(Table 5.1).** These large Zone 3 rivers of the study area drain eastwards and are all grouped together because their headwaters lie on the eastern slopes of the escarpment formed by the Drakensberg Mountain divide. The headwaters of the Orange River, by comparison, drain the opposite western slopes of the same divide but also originate from a similar geological template thus creating an expectation of similar diatom communities from the two adjacent areas. The ordination of these data confirmed the similarities in these communities. The exception to these findings was the condition observed in the more distant position of the Sundays River samples (sites) in the ordination determined by different diatom community structures **(Figure 5.7.1).** The BDI diatom index values for the Sundays River were however quite low (< 10) compared with the other large rivers, possibly because this index is more sensitive to large salinity ranges common to the upper reaches of this particular river (**Table 5.1).**

Summary

The primary purpose of biological assessment is to determine the effect of human impacts on rivers by comparison with reference conditions and therefore it is crucial to describe the near-natural biological status of river reaches in the absence of human disturbance. These reaches are nearly always located in the least disturbed headwaters of a catchment. Reference state conditions may therefore be defined using data derived from several reference sites that are located in the same spatial unit and /or from historical information derived from the same or very similar reaches. It has also been suggested that reference conditions do not necessarily have to equate *to "totally undisturbed, pristine conditions but rather that minimal disturbances may also include human pressures as long as these have very minor ecological effects"* (Wallin *et al.* 2003).

The aforementioned rationale was extended and applied to data from the pool of present-day sites which were obtained from a more distinct discrete present-day sampling strategy. This sampling strategy focused on the headwater sites of rivers in the study area as the prime target resource from which reference sites were expected to be identified.

***Differentiation of Sites within Spatial Zones*.**

The '*a priori*' pre-selection of a pool of sites in the upper headwater reaches of rivers also acted as a primary filter and dictated that these sites are more likely to be free of human disturbance. The additional filter of ensuring that the sampling was restricted to low flow periods when water quality was stable and to a single '*stones-in-current*' micro-habitat also reduced the potential effects of variability of the external environment and hence the response of diatom assemblages.

Threshold value of a water quality index associated with high ecological status

The selection of percentile values from a table of various abiotic criteria (e.g. a range of values for Water Quality indices) is the commonly used approach for setting an environmental benchmark for reference state sites (Wallin *et al.* 2003).The threshold value associated with the 25th Percentile of the water quality index values was therefore taken as a benchmark for interpreting the ecological status of the candidate reference sites located in **Zone 3**. This translated into candidate reference sites having IPS values >15.6, simultaneously corresponding with TDI and %PTV values < 25.7 and 0.6 respectively **(Table 5.2).** Sites with water quality index values greater than 15 and TDI values less than 25 were rated as high ecological status and sufficiently free of evidence of human disturbance pressures to meet target criteria representative of a reference state site. Abiotic water quality attributes of the near-natural headwaters were used to confirm implied differences in dilute water chemistries as a function of the lithologies of these headwaters.

A TDI value of 25.7 (a measure of trophic state) pertains to a water type of high ecological status which is chemically dilute and predominantly found in the undisturbed headwaters of Zone 3 rivers only (Table 5.2). It is directly associated with an IPS Water Quality index value of 15.6 derived from the 25th percentile (lower limit) of high ecological status in the same data set. However, the TDI value of 25.7 is considered an inappropriate reference value for comparison with lowland rivers. Data displayed in Table 5.2 indicates that there is some 'relaxation' of the trophic status with a higher TDI value of 31.6 pertaining to the diatom responses in coastal rivers.

The data from the historic river sites and those of the present-day sites of the **Zone 2** Rivers was found however to be inadequate and inappropriate vis-á-vis identifying discrete uncontaminated and disturbance-free headwaters of Zone 2 Rivers. The location of all the **present-day** headwater sites of the few Zone 2 Rivers was found to be confined within areas of private farmland or commercial forestry and as such these were usually transformed and disturbed land-uses e.g. headwaters converted into small domestic dams. These sites would therefore at best only qualify as '*minimally disturbed'* headwater conditions consistent with and described as '*the best available physical, chemical and biological habitat given the present-day state of the landscape'* (Stoddard *et al*. 2006).

The range of values of the Water Quality Indices (e.g. the inter-quartile range of the IPS metric), calculated for these streams, also indicated that the diatom communities were mostly representative of moderate ecological status and therefore cannot be classified or rated as suitable for reference state conditions in this spatial zone. The strategy therefore reverted to the principle of the '*best attainable condition'* for the remaining sites selected for Zone 2 Rivers This condition is described as '*places where the impact on the biota of inevitable land-use is minimal'* (Stoddard *et al*. 2006) but is also regarded as being too far removed from a near-natural condition expected of a true reference state site (Kelly *et al.* 2008).

The data from Zone 1 sites provided a small pool of candidate reference sites with a high ecological status while simultaneously retaining a distinctly different suite of diatom species from the Zone 3 communities (Output from Scenario A2). The ecological status of these headwater Zone 1 sites was also clearly differentiated from those of the impacted sites downstream (Outputs from Scenario C1)

This investigation has followed a specific diatom-based approach in the development of a protocol for comparison of test sites that qualify as candidate reference state site conditions (Grenier *et al.* 2006). The more general traditional approach has relied previously on abiotic characteristics described in terms of the constraints of an eco-region template (geographical and physical attributes) to facilitate the definition of reference state conditions. However the diatom-based approach has the advantage of making no *a priori* assumptions about the similarities / dissimilarities of diatom communities at different sites. In contrast, clustering analysis and ordination techniques of the response patterns of diatom assemblages differentiated and identified groups of candidate reference state sites from which reference state diatom communities were described **(Chapter 6)**.

This is not an entirely new approach in general bio-monitoring protocols (e.g. RIVPACS (Wright *et al* 1993), AusRivAS (Davies 2000), (Tison *et al* 2007, 2008) but it has not been applied previously for diatoms in any of the rivers of South Africa. The diatom-based approach was consistently and successfully followed in the identification of the diatom reference state conditions in KwaZulu-Natal river systems.

- The geomorphological structural features of the province provided a pragmatic solution to the classification of near-natural river headwaters. This classification was also underpinned by geological differences and natural water quality gradients between the spatial zones.
- Classification of diatom assemblages based solely on similarity of species composition effectively identified similarities and dissimilarities between groups.
- Ordinations of sites resulted in '*a posteriori'* logical interpretations of the apparent environmental gradients and consequential different clustering of candidate reference sites.

- The final interpretation resulted in the identification of reference state sites associated with reference conditions in the headwaters of specific river categories.
- The groups of diatom communities, positioned at the high quality end of a gradient (dilute chemistry) and with the highest ecological status, were designated as the most logical and scientifically appropriate representatives of a reference state.

The graphical outputs of the various multivariate assessment scenarios carried out on diatom assemblage data demonstrated that the positioning of sites along a water quality condition gradient was sufficiently reliable to differentiate between minimally disturbed sites and impacted sites. It is noteworthy that relatively recent investigations on diatom assemblages in mountain streams led to the observation and conclusion that "*there are two main classification methods employed in bio-assessment* (i) landscape classification (ii) "*biota-based stream classification. In regions with high environmental heterogeneity biota-based stream classification may delineate discrete stream types with more clear relationships between biota and environmental variables*" (Weilhoefer & Pan 2006). This approach has also been advocated for the "*definition of reference conditions for each river type according to diatom community composition*" (Tison *et al*. 2008).

CHAPTER 6

ATTRIBUTES OF REFERENCE STATE DIATOM COMMUNITIES FROM RIVERS IN KWAZULU-NATAL

6.1 Introduction

6.2 Aims

6.3 Methods

6.3.1 Definitions and principles

6.4 Results

6.4.1 Attributes of Reference State Diatom Communities

6.5 Discussion

ATTRIBUTES OF REFERENCE STATE DIATOM COMMUNITIES FROM RIVERS IN KWAZULU-NATAL

6.1 Introduction

The concept of using reference communities derived from a reference state condition, as the ecological quality benchmark in bio-monitoring programs, has been embraced in several countries in the implementation of national regulatory directives (USA Amended Clean Water Act of 1972; Australian Water Reform Framework (ANZECC & ARMCANZ 2000), the European Union Directive 2000/60/EC (European Union 2000). the South African National Aquatic Ecosystem Health Programme (DWA&F 2008). However, the difficulties in locating suitable reference conditions and sites has not been without challenges, particularly in intensively developed first world European countries (Soininen & Eloranta 2002, Acs *et al.* 2004, Yallop *et al*. 2004, Kelly *et al.* 2008).

The identification and description of diatom reference state communities was a primary goal of this investigation because there is a gap in our knowledge and an inadequacy in the description of these type-specific communities for KwaZulu-Natal Rivers. It is important to note therefore that in the context of this investigation, prior to 2006, when this investigation commenced, there had been no formal attempt at identifying, classifying and defining type specific **diatom reference state** communities as a basis for river health assessment. It was therefore meaningful and logical to describe the distinguishing features (traits) of the diatom communities encountered in present-day and historic (mid 20th century) reference sites if these are to be linked to valued ecological attributes such as biodiversity and biological integrity of a river system (Stevenson 2006).

The use of diatoms to describe a reference state community requires information of the structure of the diatom assemblage and the identification of near-natural sites together with corroborating evidence that these sites are free of human stress factors (Chapter 5 and Appendix III). Such a biological quality element can be defined as a **reference state community** when applied to an individual diatom assemblage associated with a **reference state condition** in a river. Information on the floral composition of a diatom reference state community is also premised on sufficiently robust and accurate identification of the diatoms to species level while being mindful of caveats around the concepts of '*consistency*' and '*correctness*' when making such determinations (Kociolek & Stoermer 2001). This investigation benefitted from previous South African taxonomic specialists who were able to identify endemic diatom species amongst the river flora while still retaining published concepts of the more familiar European species (Cholnoky 1956, 1957, 1960b, 1968a, Schoeman 1971, Schoeman & Archibald 1976, Archibald 1972, 1981). Many of these earlier taxonomic investigations of river diatoms were made before Cholnoky's death in 1972 but also prior to the appearance of the much quoted and commonly used 'standard' taxonomic literature (Krammer & Lange-Bertalot 1985 -1991). Diatom assemblages retain environmental 'signals' and therefore relevant information has been derived from several metrics associated with the assemblages encountered in type specific reference state conditions. A distinction had to be made for the diatom communities recorded from the sites of all rivers originating in the Interior Midlands spatial zone (Zone 2) in terms of these being inappropriate as reference state conditions for these rivers. It was determined previously that these sites were not truly representative of near-natural conditions for both the historic and present-day situation. However, a set of historic data was analysed and a species composition list was produced that, at best, may represent a condition described as minimally disturbed – "*places where the impact on the biota of inevitable land-use is minimal"* (Stoddard *et al*. 2006) but this may also be regarded as being too far removed from a near-natural condition expected of a true reference site (Kelly *et al.* 2008).

6.2 Aims

The primary aims of this part of the investigation were therefore:-

- Documentation of the species composition of diatom assemblages distinctive of reference state communities drawn from the headwaters of rivers originating in different sub-regional spatial zones.
- Comparison of the species composition of diatom assemblages of reference state communities recorded from present-day and historic conditions in the Montane Uplands spatial zone.
- Documentation of diatom communities from near-natural headwater sites and impacted sites in the Coastal Lowlands spatial zone.

The relative abundance scores of the dominant species were regarded as critical quantitative indicators of the ecological status of a river (Cholnoky 1968a, Lange-Bertalot 1979, Round 1991). The ubiquity and sub-cosmopolitan nature of diatoms implies that the autecology of these dominants might also be similar to those recorded from other parts of the world. These species may therefore also have relevance and applicability in bio-monitoring across continents, while still making allowances for endemism. The ecological preferences of the dominant species from uncontaminated and contaminated local river waters might also be expected to be similar to those found in other parts of the world.

6.3 Methods

Present-day data (2006 - 2009)

The present-day data set of diatom assemblages was generated from samples taken at headwater sites of several of the river resources used in this study area (**Resources A4,**

B3, C3, Table 1). This strategy automatically confined the distribution of the target population to the specific niche in the upper reaches of a river where there was no evidence of human activity. Equal emphasis was given to the data from historic and present-day surveys undertaken in the period 2006-2009 as an integral part of this investigation.

Historic data (pre 1960)

The use of historic data sets of diatom assemblages has been recommended for the establishment of reference sites where the data is considered appropriate and adequate (Wallin *et al*. 2003, Stoddard *et al*. 2006, Archibald *et al.* 2008). The matrix of historic data sets (**Resources A1-A3, B1, B2, C1)** was constituted from samples taken from several headwaters of rivers within the study area (**Table 1**). Historic data were assessed and compared with present-day data from similar sites in the same river reaches (**Appendix I**).

Only species with a relative abundance > 1% of the total and/or occurring at more than 10% of the sites formed part of the analysis. Biplots of the data drawn from both sets of species and sites were produced from Principal Components Analysis using CAP4 routines and outputs as an objective means of identifying the key species for a given scenario. All data was $\log_{10}$ transformed to reduce the effects of the range in variables scores (species counts) and the influence of the most common taxa.

6.3.1 Definitions and Principles

Several principles were used as a guideline to achieve the main goal of benchmarking these diatom reference state communities.

Principle # 1

A reference site is representative of a river water type normally selected within a spatial framework or longitudinal zone (Rowntree & Wadeson 1999). *Reference communities should be generated from data from several reference sites located in the same spatial framework* (DWA&F 2008).

Principle # 2

The ultimate objective of bio-monitoring is to use biota to reflect the degree of disturbance at a site. Reference conditions define what is expected to occur naturally at a site and hence provide a means of comparing observed biological conditions with expected conditions (DWA&F 2008).

It is logical that a biological component (e.g. diatom assemblage) of a reference state condition should be the target quality element if bio-monitoring is premised on assessing the biological integrity status of a living component downstream.

Principle #3

"Ecological quality status is based on the principle that biological responses represent an integration of the effect of the influential external variables (drivers) which manifest in an ecological endpoint. Diatom water quality indices are examples of numerical expressions of that ecological endpoint" (DWA&F 2008).

It is also useful to describe the thresholds for ecological classes derived from the diatom metrics for practical application of bio-monitoring using diatoms. Diatom attributes and metrics were generated from the data obtained from the group of reference state communities. For example, diatom water quality indices are integrated numerical expressions of diatom responses to prevailing water quality conditions and many of these indices have been developed in Europe. Some have been tested in South African rivers over the last several years and the outputs were found in most cases to be sufficiently robust and meaningful measures of general water quality (de la Rey *et al.* 2004; Harding *et al*. 2005; Taylor *et al.* 2005; Taylor 2006, Archibald & Taylor 2007, Taylor *et al*. 2007b; Taylor *et al*. 2007c). **The Index of Pollution Sensitivity** (**IPS**) (CEMAGREF 1982) was assessed as one of the most reliable and sensitive indices in local applications and is therefore now used to indicate general water quality conditions and ecological status of sites in the rivers of South Africa **in the absence of an improved locally-derived index**. The IPS diatom index has also been widely used and accepted in several European countries (Prygiel & Coste 1993, Kwandrans *et al*. 1998, Eloranta and Soininen 2002, Acs *et al* 2004, Rimet et al. 2004) and is part of the European inter-calibration process as a reference index (Tison *et al*. 2008). The IPS index was also used as a reference against which comparisons were made in Canadian rivers "*because it includes the highest number of taxa*" and the "*results showed that the sensitivity values used to calculate the IPS generally followed the pollution gradient found in Quebec*" (Lavoie *et al.* 2009) despite earlier reservations of its transferability to Canadian rivers (Lavoie *et al.* 2006). However, water quality index values provide no indication of the structure of diatom assemblages or the autecological values of species contributing to such an index.

A comparison was made therefore of the distinctive species composition of different diatom assemblages which characterised the suite of reference state communities drawn from sites in the various sub-regional spatial zones. The autecological data of diatom species used for ecological evaluation of these river sites was obtained from previously published works on the subject (Lowe 1974, van Dam *et al*. 1994, Lange-Bertalot 1979, De Nicola 2000a, Porter *et al*. 2008, Porter 2008).

6.4 Results

6.4.1 Attributes of Reference State Diatom Communities

Reference State Communities from the Montane Uplands (Zone 3 Rivers)

The aggregate values obtained of the relative abundance data for each species was assessed within a diatom community matrix from headwaters of rivers within a spatial zone. Reference taxa are those species expected to be at a site which is free of human disturbance and for which the environmental regime and water quality characteristics are regarded as evidently near-natural conditions. The criteria set for inclusion in the assessment restricted diatom counts to more than 1% for individual species scores and the occurrence of a species at more than 10% of the sites (**Tables 6.1, 6.2**). The initial analysis of diatom assemblage responses from a suite of historic sites from rivers originating in the headwaters of Zone 3 Rivers showed that the top ten most prolific species constituted 70% of the total count and each of these species contributed more than 2% of the total count. The inclusion of species which contributed 1% or more of the total count increased the number of species to 17, giving a total contribution of 76.3% of the count (**Table 6.1**).

The small ubiquitous diatom*, Achnanthidium minutissimum Kg.,* produced the highest proportion of the total count in the matrix and it was also recorded at every site in the Montane Uplands although it did not necessarily always attain the highest count per sample. The relative abundance scores for this species at a site ranged from 91.5% (Total dominance of the species composition) to a minimum contribution of 3.1% of the count. Counts of *Tabellaria flocculosa (Roth) Kg.* reached a maximum of 89.4% of the count in a high altitude sample in which it was the dominant diatom. This species was only recorded from 15% of the total sites incorporated within spatial Zone 3 Rivers. *Fragilaria ulna* (Nitzsch) Lange-Bertalot and *Gomphonema longiceps* v.*subclavatum* Grun. constituted 4.3% and 4.1% of the total count respectively. The distribution of the former was more widespread, occurring in 76% of the sites whereas the latter only occurred at 43.7% of the sites. *Encyonema ventricosum* (Agardh) Grunow and *Cymbella turgidula* Grunow contributed 3.8% and 3.0% respectively to the total count and also exhibited wide ranging distributions across the sampling grid (71.8% and 46.5% of site occurrences respectively) (**Table 6.1**).

A Principal Components Analysis Covariance plot of the historic diatom data shows the eight largest eigenvectors associated with the most influential species in the ordination of sites, giving a separation of the Western, Southern and South-western group of headwater sites along a gradient on Axis 1. *Gomphonema clevei* (GCLE) and *Fragilaria capucina var*.*vaucheriae* (SVAU) were positioned at the opposite end of a gradient from that of *Cymbella turgidula* (CTGL) and *Encyonema ventricosum* (CVEN) which were dominant high ecological status indicators.

Table 6.1 Species composition of reference state diatom assemblages from a matrix of historic headwater sites of Zone 3 Rivers originating in the Montane Uplands

Omnidia Database Autecology Rating s'	v'	Code	(A) BY PERCENTAGE OF TOTAL COUNT Species	% Total Count	(B) BY FREQUENCY OF OCCURENCE Species	% Occurrence	No. of sites
5	1	**AMIN**	***Achnanthidium minutissimum*** (Kützing)Czarnecki	**33.9**	***Achnanthidium minutissimum*** (Kützing)Czarnecki	**100.0**	**71**
5	1	**TFLO**	***Tabellaria flocculosa*** (Roth) Kg.	**10.4**	**Fragilaria ulna** (Nitzsch) Lange-Bertalot	**76.1**	**54**
3	1	**SULN**	***Fragilaria ulna*** (Nitzsch) Lange-Bertalot	**4.3**	*Navicula crytptocephala* Kg.	73.2	52
5	2	**GLSU**	***Gomphonema longiceps* var.*subclavatum*** Grun.	**4.1**	**Encyonema ventricosum (Agardh) Grunow**	**71.8**	**51**
4.8	2	**CVEN**	***Encyonema ventricosum*** (Agardh) Grunow	**3.8**	*Nitzschia linearis* (Ag.)W.Sm.	69.0	49
4	2	**CTGL**	***Cymbella turgidula*** Grunow	**3.0**	*Gomphonema clevei* Fricke	62.0	44
4	2	CMIC	*Encyonemopsis microcephala* (Grunow) Krammer	2.9	*Gomphonema lagenula* Kutzing	59.2	42
5	3	GCLE	*Gomphonema clevei* Fricke	2.8	*Nitzschia kuetzingiana* Hilse	54.9	39
3	1	SVAU	*Fragilaria capucina var.vaucheriae* (Kützing) Lange-Bertalot	2.7	*Encyonemopsis microcephala* (Grunow) Krammer	49.3	35
3	2	NLIN	*Nitzschia linearis (*Ag.)W.Sm.	2.1	*Nitzschia palea* (Kg.)W.Sm.	49.3	35
2	3	GLGN	*Gomphonema lagenula* Kützing	1.6	***Cymbella turgidula* Grunow**	**46.5**	**33**
3	3	NITR	*Nitzschia interrupta* (Reicheldt) Hustedt	1.2	*Navicula gregaria* Donkin	46.5	33
3.5	2	NCRY	*Navicula crytptocephala* Kg.	1.1	***Gomphonema longiceps* var. *subclavatum* Grun.**	**43.7**	**31**
5	2	AMIC	*Achnanthidium microcephalum* Kg.	1.0	*Gomphonema parvulum* (Kg.) Grun.	43.7	31
2	3	NKUT	*Nitzschia kuetzingiana* Hilse	1.0	*Nitzschia perminuta* Grun.	43.7	31
4	3	FJAV	*Frustulia javanica* L.Rabenhorst	1.0	*Cocconeis placentula* Ehrenberg	42.3	30
5	2	ANEX	*Anomoeoneis exilis* (Kg)Cl	1.0	*Nitzschia interrupta* (Reicheldt)Hustedt	35.2	25
3.4	1	NGRE	*Navicula gregaria* Donkin	0.9	Fragilaria capucina var.rumpens(Kützing)Lange-Bertalot	35.2	25
4	1	CPLA	*Cocconeis placentula* Ehrenberg	0.8	*Fragilaria ungeriana* Grunow	35.2	25
2	1	GPAR	*Gomphonema parvulum* (Kg.) Grun.	0.8	*Navicula miniscula* Grunow.	33.8	24
1	3	NPAL	*Nitzschia palea* (Kg.)W.Sm.	0.8	*Fragilaria capucina var.vaucheriae(* Kützing) Lange-Bertalot	26.8	19
4	1	SRUM	*Fragilaria capucina var.rumpens* (Kützing) Lange-Bertalot	0.7	*Fragilaria intermedia* (Kg.) Grun.	26.8	19
5	2	CAPH	*Cymbopleura amphicephala* (Naegeli) Krammer	0.7	*Amphipleura pellucida* Kützing	23.9	17
5	1	NIPM	*Nitzschia perminuta* Grunow	0.6	*Anomoeoneis exilis* (Kg)Cl	19.7	14
3	1	NMIS	*Navicula miniscula* Grunow	0.6	*Cymbopleura amphicephala* (Naegeli) Krammer	19.7	14
0	0	FUNG	*Fragilaria ungeriana* Grunow	0.5	*Frustulia vulgaris* (Thwaites) De Toni	19.7	14
4	3	FVUL	*Frustulia vulgaris* (Thwaites) De Toni	0.5	*Planothidium rostratum* (Oestrup) Round & Bukhityarova	18.3	13
4.6	1	ALAN	*Planothidium rostratum* (Oestrup) Round & Bukhityarova	0.5	***Tabellaria flocculosa* (Roth) Kg.**	**15.5**	**11**

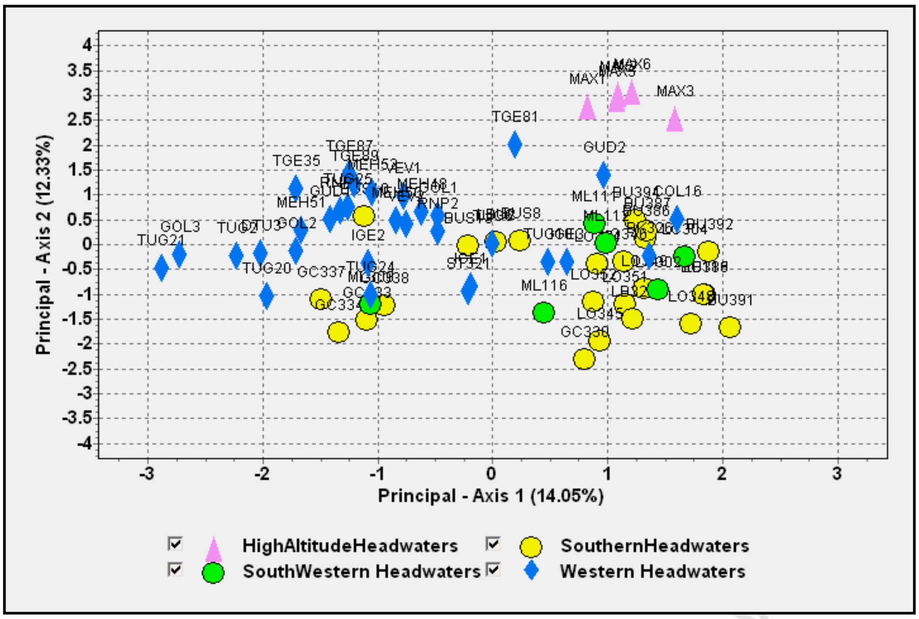
Bold indicates (%of total count and %site occurrence) related to dominant and subdominant species contributing >5% and 3% of the total count respectively.

[Historic Resources A1-A3 (Cholnoky 1956-1960)] Only species with Relative Abundance values >1% of the total count were included in the assessment

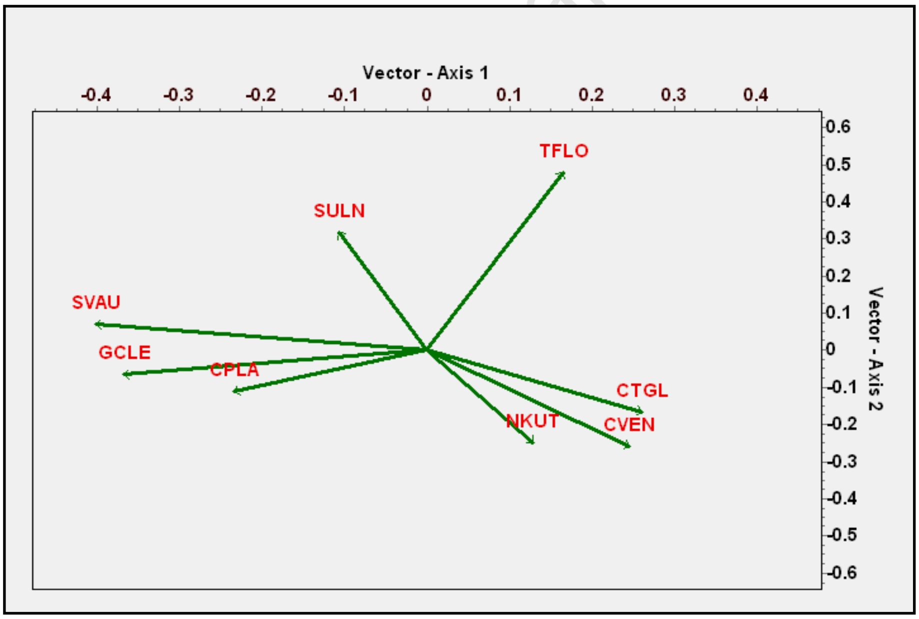
Table 6.2 The ecological status of dominant diatom species from historic headwater sites in Zone 3 Rivers originating in the Montane Uplands

OMNIDIA CODE	Species Composition	Site Occurrences	Percentage of Site Count Max %	Min %	Range %	Tolerance Rating Sensitivity s'	Reliability v'
HIGH ECOLOGICAL STATUS							
AMIN	***Achnanthidium minutissimum***	**45**	**91.5**	**3.1**	**88.4**	**5**	**1**
TFLO	***Tabellaria flocculosa***	**6**	**89.4**	**22.1**	**67.3**	**5**	**1**
GLSU	***Gomphonema longiceps v subclavatum***	**7**	**69.2**	**3.8**	**65.4**	**5**	**2**
FJAV	*Frustulia javanica*	3	66.8	23.6	43.2	4.8	3
RGIB	*Rhopalodia gibba*	1	54.8		0	5	3
ANEX	*Anomoeoneis exilis*	3	54.5	4.4	50.1	5	2
CVEN	***Encyonema ventricosum***	**9**	**48.6**	**3.2**	**45.4**	**4.8**	**2**
CTGL	***Cymbella turgidula***	**8**	**34.1**	**3.2**	**30.9**	**4**	**2**
GCLE	*Gomphonema clevei*	13	32.8	3.3	29.5	5	3
CTUR	*Cymbella turgida*	1	28.1	28.1	0	5	3
FCAP	*Fragilaria capucina*	3	27.7	3.5	24.2	4.5	1
CMIC	*Encyonopsis microcephala*	9	21.9	4.0	17.9	4	2
SPHO	*Stauroneis phoenicenteron*	1	21.6		0	5	3
NDIS	*Nitzschia dissipata*	1	20.9		0	4.5	3
ETUR	*Epithemia turgida*	1	20.6	20.6	0	5	2
ESOR	*Epithemia sorex*	3	19.7	3.4	16.3	4	2
APEL	*Amphipluera pellucida*	4	17.2	3.2	14.0	5	3
CPLA	*Cocconeis placentula*	4	13.8	3.8	10.0	4	1
NVUL	*Navicula vulpina*	1	13.3	13.3	0	5	3
PAPP	*Pinnularia appendiculata*	1	12.6		0	5	3
CHER	*Cymbella hercynica*	1	12.1	12.1	0	5	2
NIPM	*Nitzschia perminuta*	4	11.7	3.9	7.8	5	1
CAPH	*Cymbopleura amphicephala*	1	11.4	11.4	0	5	2
AMIC	*Achnanthidium microcephalum*	2	11.1	4.0	7.1	5	2
EZEB	*Epithemia zebra*	2	8.9	4.6	4.3	4	3
NSHM	*Sellaphora subhamulata*	1	8.3	8.3	0	5	2
ALAN	*Planothidium rostratum*	1	8.3	8.3	0	4.6	1
GIPU	*Gomphonema intricatum v pumila*	2	8.1	4.7	3.4	5	1
NMED	*Navicula mediocris*	1	7.9	7.9	0	4	2
NRTE	*Navicula radiosa var.tenella*	2	7.5	4.3	3.2	4	1
SRUM	*Fragilaria capucina var. rumpens*	3	6.3	3.2	3.1	4	1
FVUL	*Frustulia vulgaris*	1	5.6	5.6	0	4	3
NRHY	*Navicula rhyncocephala*	1	4.9	4.9	0	4	3
MEDIUM ECOLOGICAL STATUS							
SVAU	*Fragilaria capucina var.vaucheriae*	7	51.0	4.5	46.5	3	1
SULN	***Fragilaria ulna***	**17**	**47.2**	**3.0**	**44.2**	**3**	**1**
NLIN	*Nitzschia linearis*	3	27.8	4.3	23.5	3	2
NCRY	*Navicula cryptocephala*	5	21.4	3.4	18	3.5	2
NGRE	*Navicula gregaria*	4	8.3	3.9	4.4	3.4	1
NMIS	*Navicula miniscula*	3	7.3	4.6	2.7	3	1
FINT	*Fragilaria intermedia*	1	5.4	5.4	5.4	3	1
LOW ECOLOGICAL STATUS							
SPUP	*Sellaphora pupula*	1	6.9	6.9	0	2.6	2
GPAR	*Gomphonema parvulum*	5	6.8	3.5	3.3	2	1
NPAE	*Nitzschia paleaecea*	2	5.5	3.7	1.8	2.5	1
UNCLASSIFIED STATUS							
#EOLI	*Encyonema oliffii*	3	23.8	8.1	15.7	0#	0#
#ETSC	*Eunotia tschirchiana*	2	16.2	3	13.2	0#	0#
#FUNG	*Fragilaria ungeriana*	6	15.6	3.5	12.1	0#	0#
#RPAR	*Rhopalodia parallela*	1	11.7		0	0#	0#
#GSCH	*Gomphonema shweickerdtii*	3	11.4	4.4	7	0#	0#
#NINT	*Nitzschia intermissa*	1	10.9		0	0#	0#
TOTAL No OF SITES		**48**					

Note: Zero values 0# = No classification and / or Endemic species * : **Bold indicates data asociated with the dominants**
Ecological sensitivity ratings [(S 5 = Very pollution sensitive; 1 = Pollution tolerant) : (V 3 = High reliability ;1= Low reliability]



[a]



[b]

Figure 6.1 **[a] PCA Variance-Covariance plot of diatom count data ($\log_{10}[x+1]$ transformed) showing [b] the main species eigenvectors associated with sub-regional groupings of historic sites of Zone 3 headwaters originating in the Montane Uplands.**

[Note : Acronyms of species are found in the corresponding series of Tables 6.1– 6.4]

Some overlap in the positioning of sites occurs because the sub-regions lie adjacent to each other and the diatom responses at the intermediate sites reflect similarities in the lithology of these headwaters. *Tabellaria flocculosa* (TFLO) was the most influential species closely associated with the high altitude headwaters in the arrangement of sites along Axis 2 which can be considered a response to an altitude gradient (**Figure 6.1**).

The species list was also rearranged to account for reported autecological tolerance ratings or sensitivity to pollution of key species (Lecointe *et al. 1*993, van Dam *et al.*1994). A total of 18 species had a maximum sensitivity rating of 5 (high ecological quality rating) with 7 of these having the highest reliability rating of 3 **(Table 6.2.1)** *Sellaphora pupula* (Kutzing) Mereschkowsky*, Gomphonema parvulum* (Kg.) Grunow, and *Nitzschia paleacea* Grunow were the only three species rated as having a low ecological status and all had a low level of occurrence (< 5 sites) and only rarely contributed slightly more than 5% of the population count at a site. Six species have no rating value because these are not registered in the Omnidia database. All occurred infrequently across the spatial zone in relatively low numbers **(Table 6.2.1).**

A similar analysis of the **present-day** species data matrix was undertaken for sites in Zone 3 Rivers originating in the Montane Uplands spatial zone. Seventeen genera were represented in the upper portion of the count which was constituted by approximately 291 species that were recorded initially from this matrix of sites (**Table 6.3).** The 5 dominant species collectively contributed 66.2% of the total count, and with the addition of a further 9 species (all contributing more than 1% of the total count) the collective aggregate increased to 80.3% of the total count. The individual dominance of *A. minutissimum* was reduced from 33.9% of the total count in the historic data set to 23.0% of the total count in the present-day samples (**Tables 6.1, 6.3**) but this particular species again had the highest frequency of occurrence, being present at 100% of the present-day sites. The co-dominant species of *Achnanthidium crassum* Hustedt (19.4%), C*occoneis. placentula* Ehrenberg (11.3%), *T. flocculosa (6.7%)* and *Reimeria sinuata (*Gregory) Kociolek & Stoermer (5.9%) all exceeded 5% of the total count and all had high ecological tolerance ratings (s >4) **(Table 6.3**).

Twenty-eight species were rated as high ecological status indicators and these collectively constituted 80.3% of the total count. Just over 50% of the species carried high ecological ratings (s >5) and less than 8% were recorded as rare or possibly endemic and therefore do not have a recorded ecological rating in the Omnidia database. The latter made up less than 3% of the total count and therefore were not considered to be sufficiently influential in defining the diatom responses unique to local rivers **(Table 6.3.1)**.

Table 6.2.1 Ecological status ranking of diatom species from a matrix of historic sites in rivers originating in Zone 3 (Montane Uplands) (Rearranged data ex Table 6.2)

OMNIDIA CODE	Species Composition	Site Occurrences	Percentage of Count Max. %	Min. %	Range %	Tolerance Rating Sensitivity s'	Reliability v'
HIGH ECOLOGICAL STATUS							
RGIB	*Rhopalodia gibba*	1	54.8	-	0	5	3
GCLE	*Gomphonema clevei*	13	32.8	3.3	29.5	5	3
CTUR	*Cymbella turgida*	1	28.1	28.1	0	5	3
SPHO	*Stauroneis phoenicenteron*	1	21.6	-	0	5	3
APEL	*Amphipluera pellucida*	4	17.2	3.2	14.0	5	3
NVUL	*Navicula vulpina*	1	13.3	13.3	0	5	3
PAPP	*Pinnularia appendiculata*	1	12.6	-	0	5	3
GLSU	***Gomphonema longiceps var. subclavatum***	**7**	**69.2**	**3.8**	**65.4**	**5**	**2**
ANEX	*Anomoeoneis exilis*	3	54.5	4.4	50.1	5	2
ETUR	*Epithemia turgida*	1	20.6	20.6	0	5	2
CHER	*Cymbella hercynica*	1	12.1	12.1	0	5	2
CAPH	*Cymbopleura amphicephala*	1	11.4	11.4	0	5	2
AMIC	*Achnanthidium microcephalum*	2	11.1	4.0	7.1	5	2
NSHM	*Sellaphora subhamulata*	1	8.3	8.3	0	5	2
AMIN	***Achnanthidium minutissimum***	**45**	**91.5**	**3.1**	**88.4**	**5**	**1**
TFLO	***Tabellaria flocculosa***	**6**	**89.4**	**22.1**	**67.3**	**5**	**1**
NIPM	*Nitzschia perminuta Grun.*	4	11.7	3.9	7.8	5	1
GIPU	*Gomphonema intricatum var. pumila*	2	8.1	4.7	3.4	5	1
FJAV	*Frustulia javanica*	3	66.8	23.6	43.2	4.8	3
CVEN	***Encyonema ventricosum***	**9**	**48.6**	**3.2**	**45.4**	**4.8**	**2**
ALAN	*Planothidium rostratum*	1	8.3	8.3	0	4.6	1
FCAP	*Fragilaria capucina*	3	27.7	3.5	24.2	4.5	1
NDIS	*Nitzschia dissipata*	1	20.9	-	0	4.5	3
EZEB	*Epithemia zebra*	2	8.9	4.6	4.3	4	3
FVUL	*Frustulia vulgaris*	1	5.6	5.6	0	4	3
NRHY	*Navicula rhyncocephala*	1	4.9	4.9	0	4	3
CTGL	***Cymbella turgidula***	**8**	**34.1**	**3.2**	**30.9**	**4**	2
CMIC	*Encyonopsis microcephala*	9	21.9	4.0	17.9	4	2
ESOR	*Epithemia sorex*	3	19.7	3.4	16.3	4	2
NMED	*Navicula mediocris*	1	7.9	7.9	0	4	2
CPLA	*Cocconeis placentula*	4	13.8	3.8	10.0	4	1
NRTE	*Navicula radiosa var.tenella*	2	7.5	4.3	3.2	4	1
SRUM	*Fragilaria capucina var. rumpens*	3	6.3	3.2	3.1	4	1
MEDIUM ECOLOGICAL STATUS							
NCRY	*Navicula cryptocephala*	5	21.4	3.4	18	3.5	2
NGRE	*Navicula gregaria Donkin*	4	8.3	3.9	4.4	3.4	1
NLIN	*Nitzschia linearis*	3	27.8	4.3	23.5	3	2
SVAU	*Fragilaria vaucheriae*	7	51.0	4.5	46.5	3	1
SULN	*Fragilaria ulna*	17	47.2	3.0	44.2	3	1
NMIS	*Navicula miniscula*	3	7.3	4.6	2.7	3	1
FINT	*Fragilaria intermedia*	1	5.4	5.4	5.4	3	1
LOW ECOLOGICAL STATUS							
SPUP	*Sellaphora pupula*	1	6.9	6.9	0	2.6	2
NPAE	*Nitzschia paleaecea*	2	5.5	3.7	1.8	2.5	1
GPAR	*Gomphonema parvulum*	5	6.8	3.5	3.3	2	1
UNCLASSIFIED STATUS							
#EOLI	*Encyonema olifii*	3	23.8	8.1	15.7	0#	0#
#ETSC	*Eunotia tschirchiana*	2	16.2	3	13.2	0#	0#
#FUNG	*Fragilaria ungeriana*	6	15.6	3.5	12.1	0#	0#
#RPAR	*Rhopalodia parallela*	1	11.7	-	0	0#	0#
#GSCH	*Gomphonema shweickerdtii*	3	11.4	4.4	7	0#	0#
#NINT	*Nitzschia intermissa Hustedt*	1	10.9	-	0	0#	0#
Total No. of Sites		**48**					

Historic Resources A1-A3 (Cholnoky 1956, 1957, 1960) : Note: Zero values 0# = No classification and / or Endemic species *
Ecological sensitivity ratings [(S 5 = Very pollution sensitive; S1 = Pollution tolerant) : (V 3 = High reliability ; V1 low reliability]

Table 6.3 Species composition of Reference state diatom assemblages from a matrix of present-day headwater sites from large Zone 3 Rivers originating in the Montane Uplands.

(A) BY PERCENTAGE OF TOTAL COUNT

Acronym Code	Species Composition	Total Count %	% Sites	s'	v'
AMIN	***Achnanthidium minutissimum***	**23.0**	**100.0**	**5**	**1**
ACRA	***Achnanthidium crassum***	**19.4**	**87.2**	**5**	**2**
CPLA	***Cocconeis placentula***	**11.3**	**61.5**	**4**	**1**
TFLO	***Tabellaria flocculosa***	**6.7**	**23.1**	**5**	**1**
RSIN	***Reimeria sinuata***	**5.9**	**56.4**	**4.8**	**1**
CTGL	*Cymbella turgidula*	2.4	43.6	4	2
FCAP	*Fragilaria capucina*	2.4	43.6	4.5	1
NLIN	*Ntzschia linearis*	1.9	30.8	3	2
NGRE	*Navicula gregaria*	1.7	41.0	3.4	1
NCRY	*Navicula cryptocephala*	1.4	46.2	3.5	2
GPRI	*Gomphonema pumilum var. rigidum*	1.2	25.6	5	1
GVEN	*Gomphonema venusta*	1.1	38.5	0	0
CSLE	*Encyonema silesiacum*	1.1	33.3	5	2
GLSU	*Gomphonema subclavatum*	1.0	33.3	5	2
NVEN	*Navicula veneta*	0.9	28.2	1	2
AAFF	Achnanthes affinis	0.9	25.6	5	1
ENLE	*Encyonopsis leei var.sinensis*	0.9	25.6	5	2
GIPU	*Gomphonema intricatum var. pumila*	0.9	2.6	4	2
CMIN	*Encyonema minutum*	0.8	41.0	4.8	2
PLFR	*Planothidium frequentissimum*	0.8	33.3	3.4	1
CDEL	*Delicatula delicatulum*	0.7	7.7	5	2
ALIN	*Achnanthidium linearum*	0.6	2.6	5	3
SULN	*Fragilaria ulna*	0.6	59.0	3	1
BVIT	*Brachysira vitrea*	0.5	15.4	5	2
CMIC	*Encyonopsis microcephala*	0.5	17.9	4	2
GPUM	*Gomphonema pumilum*	0.5	5.1	5	1
ADMA	*Achnanthidium macrocephalum*	0.5	5.1	5	1
NNOT	*Navicula notha*	0.5	23.1	4.8	1
NFON	*Nitzschia fonticola*	0.4	17.9	3.5	1
ECKR	*Encyonopsis krammeri*	0.3	10.3	0	0
EADN	*Epithemia adnata*	0.3	10.3	4	3
GANT	*Gomphonema angustum*	0.3	12.8	5	1
GLGN	*Gomphonema lagenula*	0.3	35.9	2	3
GDEC	*Geissleria decussis*	0.2	12.8	5	2
NROS	*Navicula rostellata*	0.2	17.9	3	3
GMIN	*Gomphonema minutum*	0.2	15.4	4	1
CMLF	*Craticula molestiformis*	0.2	15.4	2	1
NHMD	*Navicula heimansioides*	0.2	17.9	5	2
NTRV	*Navicula trivialis*	0.2	5.1	2	3
NPAL	*Nitzschia palea*	0.2	23.1	1	3
AEXG	*Achnanthidium exiguum*	0.2	10.3	3	2
CPLI	*Cocconeis placentula var. Lineata*	0.2	7.7	4	1
GCLA	*Gomphonema clavatum*	0.2	7.7	5	2
AROK	*Achnanthes rosenstockii*	0.2	5.1	4	1
ALAN	*Planothidium rostratum*	0.2	7.7	4.6	1

(B) BY FREQUENCY OF SITE OCCURRENCE

Code		Total Count %	Sites	%Sites
AMIN	***AchnanthIdium minutissimum***	**23.0**	**39**	**100.0**
ACRA	***Achnanthidium crassum***	**19.4**	**34**	**87.2**
CPLA	***Cocconeis placentula***	**11.3**	**24**	**61.5**
SULN	*Fragilaria ulna*	0.6	23	59.0
RSIN	***Reimeria sinuata***	**5.9**	**22**	**56.4**
NCRY	*Navicula cryptocephala*	1.4	18	46.2
CTGL	*Cymbella turgidula (kappii)*	2.4	17	43.6
FCAP	*Fragilaria capucina*	2.4	17	43.6
NGRE	*Navicula gregaria*	1.7	16	41.0
CMIN	*Encyonema minutum*	0.8	16	41.0
GVEN	*Gomphonema venusta*	1.1	15	38.5
GLGN	*Gomphonema lagenula*	0.3	14	35.9
CSLE	*Encyonema silesiacum*	1.1	13	33.3
GLSU	*Gomphonema longiceps var. subclavatum*	1.0	13	33.3
PLFR	*Planothidium frequentissimum*	0.8	13	33.3
NLIN	*Ntzschia linearis*	1.9	12	30.8
NVEN	*Navicula veneta*	0.9	11	28.2
GPRI	*Gomphonema pumilum var. rigidum*	1.2	10	25.6
AAFF	Achnanthes affinis	0.9	10	25.6
ENLE	*Encyonopsis leei var.sinensis*	0.9	10	25.6
SPUP	*Sellaphora pupula*	0.1	10	25.6
TFLO	***Tabellaria flocculosa***	**6.7**	**9**	**23.1**
NNOT	*Navicula notha*	0.5	9	23.1
NPAL	*Nitzschia palea*	0.2	9	23.1
CMIC	*Encyonopsis microcephala*	0.5	7	17.9
NFON	*Nitzschia fonticola*	0.4	7	17.9
NROS	*Navicula rostellata*	0.2	7	17.9
NHMD	*Navicula heimansioides*	0.2	7	17.9
NDME	*Nitzschia dissipata var. Media*	0.2	7	17.9
GPAR	*Gomphonema parvulum*	0.2	7	17.9
CTUM	*Cymbella tumida*	0.1	7	17.9
NHAN	*Nitzschia hantzschiana*	0.1	7	17.9
CASP	*Cymbella aspera*	0.1	7	17.9
NSHR	*Navicula schroeteri*	0.1	7	17.9
BVIT	*Brachysira vitrea*	0.5	6	15.4
GMIN	*Gomphonema minutum*	0.2	6	15.4
CMLF	*Craticula molestiformis*	0.2	6	15.4
HCAP	*Hippodonta capitata*	0.1	6	15.4
GANT	*Gomphonema angustum*	0.3	5	12.8
GDEC	*Geissleria decussis*	0.2	5	12.8
PLEN	*Planothidium engelbrechti*	0.1	5	12.8
NBRY	*Adlafia bryophila*	0.1	5	12.8
APEL	*Amphipleura pellucida*	0.1	5	12.8
FVUL	*Frustulia vulgaris*	0.1	5	12.8
SANG	*Surirella angusta*	0.1	5	12.8

Resource : A4 (2006-2009**) : Bold data** represents information pertaining to the dominant species contributing >5% of total count

Table 6.3.1 Ecological status ranking of diatom species from a matrix of present day sites in rivers originating in Zone 3 (Montane Uplands) (Data rearranged ex Table 6.3)

Omnidia Acronym	Species Composition	Total Count %	Total Sites	Sites %	Tolerance Rating Sensitivity s'	Reliability v'
HIGH ECOLOGICAL STATUS						
APEL	*Amphipleura pellucida*	0.1	5	12.8	5	3
ACRA	***Achnanthidium crassum***	**19.4**	**34**	**87.2**	**5**	**2**
GLSU	*Gomphonema longiceps var. subclavatum*	1.0	13	33.3	5	2
CSLE	*Encyonema silesiacum*	1.1	13	33.3	5	2
NHAN	*Nitzschia hantzschiana*	0.1	7	17.9	5	2
NHMD	*Navicula heimansioides*	0.2	7	17.9	5	2
NBRY	*Adlafia bryophila*	0.1	5	12.8	5	2
BVIT	*Brachysira vitrea*	0.5	6	15.4	5	2
GDEC	*Geissleria decussis*	0.2	5	12.8	5	2
ENLE	*Encyonopsis leei var.sinensis*	0.9	10	25.6	5	2
AMIN	***Achnanthidium minutissimum***	**23.0**	**39**	**100**	**5**	**1**
TFLO	***Tabellaria flocculosa***	**6.7**	**9**	**23.1**	**5**	**1**
GPRI	*Gomphonema pumilum var. rigidum*	1.2	10	25.6	5	1
GANT	*Gomphonema angustum*	0.3	5	12.8	5	1
AAFF	*Achnanthidium affine*	0.9	10	25.6	5	1
CMIN	*Encyonema minutum*	0.8	16	41	4.8	2
RSIN	***Reimeria sinuata***	**5.9**	**22**	**56.4**	**4.8**	**1**
NNOT	*Navicula notha*	0.5	9	23.1	4.8	1
NDME	*Nitzschia dissipata var. media*	0.2	7	17.9	4.5	3
FCAP	*Fragilaria capucina*	2.4	17	43.6	4.5	1
FVUL	*Frustulia vulgaris*	0.1	5	12.8	4	3
CASP	*Cymbella aspera*	0.1	7	17.9	4	3
CMIC	*Encyonopsis microcephala*	0.5	7	17.9	4	2
CTGL	*Cymbella turgidula*	2.4	17	43.6	4	2
CPLA	***Cocconeis placentula***	**11.3**	**24**	**61.5**	**4**	**1**
SANG	*Surirella angusta*	0.1	5	12.8	4	1
HCAP	*Hippodonta capitata*	0.1	6	15.4	4	1
GMIN	*Gomphonema minutum*	0.2	6	15.4	4	1
MODERATE ECOLOGICAL STATUS						
NCRY	*Navicula cryptocephala*	1.4	18	46.2	3.5	2
NFON	*Nitzschia fonticola*	0.4	7	17.9	3.5	1
PLFR	*Planothidium frequentissimum*	0.8	13	33.3	3.4	1
NGRE	*Navicula gregaria*	1.7	16	41	3.4	1
SULN	*Fragilaria ulna*	0.6	23	59	3	1
NLIN	*Ntzschia linearis*	1.9	12	30.8	3	2
NROS	*Navicula rostellata*	0.2	7	17.9	3	3
CTUM	*Cymbella tumida*	0.1	7	17.9	3	3
LOW ECOLOGICAL STATUS						
PLEN	*Planothidium engelbrechti*	0.1	5	12.8	2.9	2
NSHR	*Navicula schroeteri*	0.1	7	17.9	2.8	3
SPUP	*Sellaphora pupula*	0.1	10	25.6	2.6	2
GLGN	*Gomphonema lagenula*	0.3	14	35.9	2	3
GPAR	*Gomphonema parvulum*	0.2	7	17.9	2	1
CMLF	*Craticula molestiformis*	0.2	6	15.4	2	1
NPAL	*Nitzschia palea*	0.2	9	23.1	1	3
NVEN	*Navicula veneta*	0.9	11	28.2	1	2
UNCLASSIFIED STATUS						
GVEN	*Gomphonema venusta*	1.1	15	38.5	0	0

Present-day Resource A4 (2006-2009) : **Bold** indicates data associated with dominant species

Table 6.4 The present-day and historic dominant diatom species from high altitude Thukela River Mont-aux-Sources sites.

Omnidia Acronym	Species composition	(2009* survey) Count %	Sites	Occurrence %	Mont-aux-Sources Sites (1954) % Count #MAX1	#MAX3	#MAX5	Tolerance Rating Sensitivity 's'	Reliability 'v'
A : SPECIES COMPOSITION ANALYSIS									
NHAN	*Nitzschia hantzshiana*	0.4	3	42.9			1.2	5	3
BVIT	*Brachysira vitrea*	2.5	5	71.4				5	2
GCLA	*Gomphonema clavatum*	1.1	5	71.4				5	2
ACRA	*Achnanthidium crassum*	0.7	4	57.1				5	2
AMIC	*Achnanthidium microcephalum*	0.5	1	14.3	0.6	2.9	4	5	2
NBRY	*Adlafia bryophila*	0.3	1	14.3				5	2
EVEN	*Encyonopsis ventricosum*	0.4	1	14.3				4.8	2
AMIN	***Achnanthidium minutissimum***	**40.8**	**7**	**100.0**	**0.3**	**1.7**	**3.4**	**5**	**1**
TFLO	***Tabellaria flocculosa***	**38.8**	**7**	**100.0**	**89.4**	**81.8**	**77.9**	**5**	**1**
NNOT	*Navicula notha*	0.4	4	57.1				4.8	1
FCAP	***Fragilaria capucina***	**10.5**	**6**	**85.7**				**4**	**1**
SULN	*Fragilaria ulna*	1.0	6	85.2	6.7	5.5	7.2	3	1
GLGN	*Gomphonema lagenula*	0.4	4	57.1	0.1		0.2	2	3
ENKR	*Encyonopsis krammeri*	0.7	2	28.6				0	0
PART B : SPECIES ECOLOGICAL STATUS RATING									
HIGH ECOLOGICAL STATUS									
NHAN	*Nitzschia hantzshiana*	0.4	3	42.9				5	3
BVIT	*Brachysira vitrea*	2.5	5	71.4				5	2
GCLA	*Gomphonema clavatum*	1.1	5	71.43				5	2
ACRA	*Achnanthidium crassum*	0.7	4	57.1				5	2
AMIC	*Achnanthidium microcephalum*	0.5	1	14.3	0.6	2.9	4	5	2
NBRY	*Adlafia bryophila*	0.3	1	14.3				5	2
AMIN	***Achnanthidium minutissimum***	**40.8**	**7**	**100**	**0.3**	**1.7**	**3.4**	**5**	**1**
TFLO	***Tabellaria flocculosa***	**38.8**	**7**	**100**	**89.4**	**81.8**	**77.9**	**5**	**1**
EVEN	*Encyonopsis ventricosum*	0.4	1	14.3				4.8	2
NNOT	*Navicula notha*	0.4	4	57.1				4.8	1
FCAP	***Fragilaria capucina***	**10.5**	**6**	**85.7**				**4**	**1**
MODERATE ECOLOGICAL STATUS									
SULN	*Fragilaria ulna*	1.0	6	85.2	6.7	5.5	7.2	3	1
LOW ECOLOGICAL STATUS									
GLGN	*Gomphonema lagenula*	0.4	4	57.1	0.1		0.2	2	3
UNCLASSIFIED STATUS									
ENKR	*Encyonopsis krammeri*	0.7	2	28.6				0	0

Resources : A4 * (Archibald 2009) #A1 (Cholnoky (1956) : **Bold** indicates data associated with the dominant species

#MAX 1,3, 5 : Acronyms for high altitude Mont-aux-Sources sites of the Thukela River

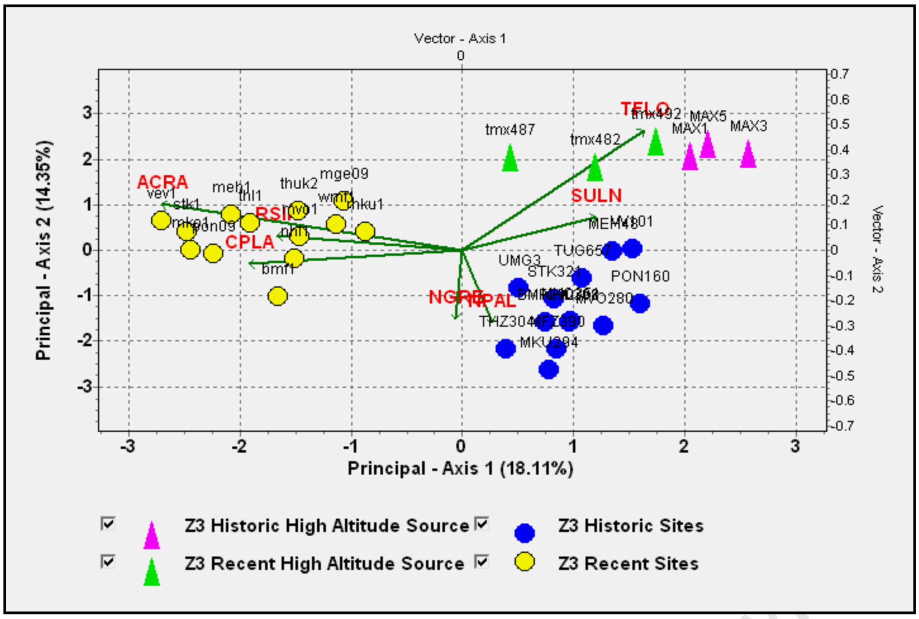


Figure 6.2 **PCA Variance-Covariance plot of diatom count data ($log_{10}[x+1]$ transformed) showing the main species eigenvectors associated with historic and recent sites originating from Zone 3 Rivers**
[Resources A1-A3 (Cholnoky 1956-1960) & C3 (2006-2009)]

Analysis of the species composition of the high altitude sites near the source of the Thukela River, at the summit of the Amphitheatre, warranted separate attention because of the restricted niche. The analysis of both present-day and historic data revealed the dominance of *Achnanthes minutissimum* and *Tabellaria flocculosa,* both in terms of the proportion of the count and in the 100% occurrence of these species in the small group of high altitude sites. All the species that were recorded from this habitat had high ecological status ratings with the exception of *Fragilaria ulna* and *Gomphonema lagenula* Kutzing both of which made up a low proportion of the total count (**Table 6.4)**.

A Principal Components Analysis Covariance plot of the historic and present-day diatom data diatom data shows the largest eigenvectors associated with the most influential species in the ordination of sites over a time gradient (Axis 1) (**Figure 6.2**). The response of the species was reflected in distinct differences in the historic and present-day groupings. *Achnanthidium minutissimum* (AMIN*)* was numerically dominant at most sites in both time periods (**Tables 6.1, 6.3**) and therefore was less influential in separating the groupings. *Achnanthidium crassum* (ACRA), *Cocconeis placentula* (CPLA) and *Reimeria sinuata* (RSIN) were the three most influential species with a wide distribution and high occurrence amongst the present-day sites. *Fragilaria ulna* (SULN*)* was the most influential diagnostic species in the historic matrix of Zone 3 River sites while *Tabellaria flocculosa* (TFLO) was the most influential species associated with the high altitude headwaters.

The latter species dominated the counts in high altitude historical and present-day diatom data versus small counts from lower altitude sites. This consistent response reflected an altitude gradient (Axis 2) for both time periods (**Figure 6.2**).

Diatom Communities within the Interior Midlands Spatial Zone (Zone 2 Rivers)

These communities were not considered to be at a reference state condition for reasons listed previously. Nonetheless, the species composition at the selected historic sites was dominated by only three species, namely, *A.minutissimum* (35.1%), *Nitzschia linearis* Agardh W. Smith (8.6%) and *Cymbella turgidula* Grunow (5.1%) in terms of the proportion of the total count from the matrix of Zone 2 sites. The highest counts per site in Zone 2 Rivers (in excess of 60%) were attributed to *Anomoeoneis exilis* (Grunow) Cleve, *Achnanthidium microcephalum* (Kutzing) Grunow and *A*. *minutissimum* with the former two species only occurring in 3 out of 39 sites. Fifteen species were rated within the high ecological status band and a further fifteen were in the moderate to lower status categories **(Table 6.5)**.

Reference State Diatom Communities within the Coastal Lowlands Spatial Zone (Zone 1 Rivers)

Analysis of the reference state diatom communities from a matrix of headwater sites of rivers originating in Zone 1 (Coastal Lowlands) showed that four diatoms dominated the total count. viz. *C. placentula* (19.6%), *Psammothidium oblongellum* Oestrup (17.4%), *A.minutissimum (12.0%)* and *Gomphonema pumilum v rigidum* Reichardt & Lange-Bertalot (6.3%).These 4 dominant species contributed 55.3% of the total count and with an additional 13 species (each contributing more than 1% of the total count) the total contribution increased to 78.6% of the total count (**Table 6.6)**. All four dominant species contained in the Zone 1 River matrix were rated in the high ecological status category. A further 10 species were also rated in the high ecological status making a total of 14 out of 25 species in the count. This condition can be contrasted with the low ecological status ratings carried by all the species which contributed to the low individual percentage of the total count **(Table 6.6.1).** The species composition of diatom assemblages drawn from the contaminated lower reaches of urban rivers showed that 5 species (viz. Sellaphora *seminulum* (Grunow)DG Mann*, Nitzschia frustulum (Kützing)Grunow, C. placentula, Mayamaea atomus var. permitis* (Hustedt) Lange-Bertalot*, Nitzschia palea* (Kützing)W.Smith dominated the total count, obtaining the highest individual percentage counts at several sites. There was however a greater evenness in the contributions (5.2-10.4%) made by each species in this matrix of low ecological status indicators from the contaminated sites **(Table 6.6.2)**.

TABLE 6.5 Matrix of dominant species from the upper most sites of rivers originating in Zone 2 (Interior Midlands)

Species Acronym Code	Species Composition	Site Occurences	Dominance in species composition Maximum %	Minimum %	%Count	Tolerance Rating Sensitivity *'s'	Reliability **'v'
HIGH ECOLOGICAL STATUS							
CTUR	*Cymbella turgida* (Gregory) Cleve	1	28.1			5	3
GCLE	*Gomphonema clevei* Fricke	4	6.4	3.9	2.0	5	3
ANEX	*Anomoeoneis exilis (* Kg.)Cl	3	81.4	0.2	1.0	5	2
AMIC	*Achnanthidium microcephalum Kg.*	3	73.5	0.2		5	2
CAPH	*Cymbopleura amphicephala* (Naegeli)Krammer	2	39.0	0.2		5	2
CCHA	*Caloneis chasei* Cholnoky	1	18.4			5	2
NIPM	*Nitzschia perminuta* Grun.	4	14.5	4		5	1
AMIN	*Achnanthidium minutissimum* Kg.	25	68.9	2.6	35.1	5	1
CVEN	*Encyonema ventricosum* Kg.	11	27.5	0.2	2.2	4.8	2
NDIS	*Nitzschia dissipata* (Kg.) Grun.	3	3.6	0.8		4.5	3
CCIS	*Cymbella neocistula Krammer*	1	15.7			4	3
CMIC	*Encyonopsis microcephala* (Grunow) Krammer	7	54.1	0.2	1.9	4	2
CTGL	*Cymbella turgidula* Grunow	6	28.2	0.2	5.1	4	2
SRUM	*Fragilaria capucina var.rumpens* Kützing)Lange-Bertalot	4	10.4	1.8		4	1
NRTE	*Navicula radiosa* var.*tenella* (Breb.)Grun.	3	7.5	4.3		4	1
MEDIUM ECOLOGICAL STATUS							
NCRY	*Navicula crytptocephala* Kg.	14	26.3	0.2		3.5	3
NFON	*Nitzschia fonticola* Grun.	2	7.8	0.6		3.5	1
NGRE	*Navicula gregaria* Donkin	10	23.3	0.2		3.4	3
NROS	*Navicula rostellata* Kg.	6	34.4	1.8		3	2
NITR	*Nitzschia interrupta (* Reicheldt)Hustedt	7	20.1	3.1		3	2
NLIN	*Nitzschia linearis* (Ag.)W.Sm.	12	32.4	0.2	8.6	3	1
FINT	*Fragilaria intermedia* (Kg.) Grun.	5	27.9	0.3		3	1
SULN	*Fragilaria ulna* (Nitzsch) Lange-Bertalot	7	10.1	0.2		3	1
NMIS	*Navicula miniscula* Grun.	2	6.6	3.3		3	1
LOW ECOLOGICAL STATUS							
NPSH	*Craticula buderi (* Hustedt) Lange-Bertalot	5	15.3	5.1		2	3
GLGN	*Gomphonema lagenula* (Grun.)Hust.	10	11.5	0.6		2	3
NSHR	*Navicula schroeteri* Meister	1	8.3			2	3
NKUT	*Nitzschia kuetzingiana* Hilse	7	6.2	0.5		2	3
NSMO	*Navicula submolesta* Hustedt	3	4.9	0.9		2	2
GPAR	*Gomphonema parvulum* (Kg.) Grun.	2	35.6	4.8		2	1
NMIC	*Nitzschia microcephala* Grun.	1	9.1			1	3
NPAL	*Nitzschia palea (* Kg.)W.Sm.	8	8.1	0.6		1	3
UNCLASSIFIED STATUS							
FUNG	# *Fragilaria ungeriana* Grunow	2	15.1	7.7		0#	0#
EOLF	# *Encyonema olifii* (Cholnoky)Krammer	2	12.5	5.3		0#	0#
NIMS	# *Nitzschia intermissa* Hustedt	5	4.5	0.3		0#	0#
	No of sites	**39**					

Note : Zero ratings # 0 = Unclassified tolerance of endemic species
Pollution Tolerance Range Ratings [* S5 = High Sensitivty; S1= Low sensitivity to pollution] [** Reliability of rating V3 = high; V1= low]

Table 6.6 Species composition of Reference state diatom assemblages from a matrix of present-day headwater sites from rivers originating in Zone 1 (Coastal Lowlands)

(A) BY COUNT : RELATIVE ABUNDANCE

Omnidia Code	Ecological Tolerance s'	v'	Species	Relative Abundance % count	Sites % Occurence
CPLA	**4**	**1**	***Cocconeis placentula***	**19.6%**	**74**
AOBG	**4.5**	**1**	***Psammothidium oblongellum***	**17.4%**	**59**
AMIN	**5**	**1**	***Achnanthidium minutissimum***	**12.0%**	**79**
GPRI	**5**	**1**	***Gomphonema pumilum var. rigidum***	**6.3%**	**47**
GPXS	5	1	*Gomphonema exillisimum*	2.8%	15
PLFR	3.4	1	*Planothidium frequentissimum*	2.5%	47
GPUM	5	1	*Gomphonema pumilum*	2.4%	24
FBID	5	1	*Fragillaria bidens*	2.3%	21
CDIR	0	0	*Cocconeis dirupta*	2.0%	32
NSHR	2.8	3	*Navicula schroeteri*	1.5%	76
NSEM	1.5	2	*Sellaphora seminulum*	1.5%	50
PLEN	2.9	2	*Planothidium engelbrechti*	1.4%	35
ENLE	5	2	*Encyonopsis leei var.sinensis*	1.3%	26
GPAR	2	1	*Gomphonema parvulum*	1.2%	50
ACRA	5	2	*Achnanthidium crassum*	1.2%	35
NIFR	2	1	*Nitzschia frustulum*	1.1%	53
NGRE	3.4	1	*Navicula gregaria*	1.1%	59
GAUR	5	1	*Gomphonema auritum*	1.0%	21
CTGL	4	2	*Cymbella turgidula*	0.9%	26
PRST	4.4	1	*Planothidium rostratum*	0.7%	18
GPSA	3	1	*Gomphonema pseudoaugur*	0.7%	12
GVEN	0	0	*Gomphonema venusta*	0.7%	35
DSUB	2	2	*Denticula subtilis*	0.6%	3
NCON	4	1	*Diadesmis contenta*	0.6%	29
Total			**24 species**		

(B) BY SITE : FREQUENCY OF OCCURENCE

Omnidia Code	Species	Occurrence No.of Sites	Occurrence %	Total count %
AMIN	***Achnanthidium minutissimum***	**27**	**79**	**12.0%**
NSHR	*Navicula schroeteri*	26	76	1.5%
CPLA	***Cocconeis placentula***	**25**	**74**	**19.6%**
AOBG	***Psammothidium oblongella***	**20**	**59**	**17.4%**
NGRE	*Navicula gregaria*	20	59	1.1%
NIFR	*Nitzschia frustulum*	18	53	1.1%
NSEM	*Sellaphora seminulum*	17	50	1.5%
GPAR	*Gomphonema parvulum*	17	50	1.2%
GPRI	***Gomphonema pumilum var. rigidum***	**16**	**47**	**6.3%**
PLFR	*Planothidium frequentissimum*	16	47	2.5%
PLEN	*Planothidium engelbrechti*	12	35	1.4%
ACRA	*Achnanthidium crassum*	**12**	35	1.2%
GVEN	*Gomphonema venusta*	12	35	0.7%
CDIR	*Cocconeis dirupta*	11	32	2.0%
NCON	*Diadesmis contenta*	10	29	0.6%
ENLE	*Encyonopsis leei var.sinensis*	9	26	1.3%
CTGL	*Cymbella turgidula*	9	26	0.9%
GPUM	*Gomphonema pumilum*	8	24	2.4%
FBID	*Fragillaria bidens*	7	21	2.3%
GAUR	*Gomphonema auritum*	7	21	1.0%
PRST	*Planothidium rostratum*	6	18	0.7%
GPXS	*Gomphonema exillisimum*	5	15	2.8%
GPSA	*Gomphonema pseudoaugur*	4	12	0.7%
DSUB	*Denticula subtilis*	1	3	0.6%

[Resource C3 (2006-2009)] : **Bold** indicates data relating to dominant species

Table 6.6.1 Matrix of dominant species from Zone 1 river sites of the Coastal Lowlands listed according to ecological status categories and by ecological ratings

Omnidia Data Base Acronym	Species Composition	Relative Abundance % count	Sites % Occurence	Tolerance Rating Sensitivity s'	Tolerance Rating Reliability v'
HIGH ECOLOGICAL STATUS					
ENLE	*Encyonopsis leei var.sinensis*	1.3%	26	5	2
ACRA	*Achnanthidium crassum*	1.2%	35	5	2
AMIN	***Achnanthidium minutissimum***	**12.0%**	**79**	**5**	**1**
GPRI	***Gomphonema pumilum var. rigidum***	**6.3%**	**47**	**5**	**1**
GPXS	*Gomphonema exillisimum*	2.8%	15	5	1
GPUM	*Gomphonema pumilum var.rigidum*	2.4%	24	5	1
FBID	*Fragillaria bidens*	2.3%	21	5	1
GAUR	*Gomphonema auritum*	1.0%	21	5	1
AOBG	***Psammothidium oblongellum***	**17.4%**	**59**	**4.5**	**1**
PRST	*Planothidium rostratum*	0.7%	18	4.4	1
CTGL	*Cymbella turgidula*	0.9%	26	4	2
CPLA	***Cocconeis placentula***	**19.6%**	**74**	**4**	**1**
NCON	*Diadesmis contenta Grunow*	0.6%	29	4	1
MODERATE ECOLOGICAL STATUS					
PLFR	*Planothidium frequentissimum*	2.5%	47	3.4	1
NGRE	*Navicula gregaria*	1.1%	59	3.4	1
GPSA	*Gomphonema pseudoaugur*	0.7%	12	3	1
LOW ECOLOGICAL STATUS					
PLEN	*Planothidium engelbrechti*	1.4%	35	2.9	2
NSHR	*Navicula schroeteri*	1.5%	76	2.8	3
DSUB	*Denticula subtilis*	0.6%	3	2	2
GPAR	*Gomphonema parvulum*	1.2%	50	2	1
NIFR	*Nitzschia frustulum*	1.1%	53	2	1
NSEM	*Sellaphora seminulum*	1.5%	50	1.5	2
CDIR	#*Cocconeis dirupta*	2.0%	32	#0	#0
GVEN	#*Gomphonema venusta*	0.7%	35	#0	#0

\# 0 Indicates endemic species or no rating in the Omnidia Database

Bold indicates data associated with dominant species

Table 6.6.2 Species composition and ecological status rating from a matrix of urban sites in Zone 1 Rivers impacted by sewage and industry waste

OMNIDIA CODE	Species Composition	% count	Sites	Ecological Ratings S'	V'
NSEM	***Sellaphora seminulum***	**10.4**	**58**	**1.5**	**2**
CPLA	***Cocconeis placentula***	**9.8**	**52**	**4**	**1**
NIFR	***Nitzschia frustulum***	**9.8**	**53**	**2**	**1**
NAPE	***Mayamaea atomus var. permitis***	**5.5**	**48**	**2.3**	**1**
NPAL	***Nitzschia palea***	**5.2**	**54**	**1**	**3**
NSHR	*Navicula schroeteri*	4.8	50	2.8	3
NSBM	*Eolimna subminuscula*	4.6	52	2	1
GPAR	*Gomphonema parvulum*	4.6	58	2	1
AEXG	*Achnanthidium exiguum*	4.4	51	3	2
SPUP	*Sellaphora pupula*	3.9	49	2.6	2
NAMP	*Nitzschia amphibia*	3.0	40	2	2
NGRE	*Navicula gregaria*	2.9	39	3.4	1
NVEN	*Navicula veneta*	2.2	56	1	2
NPAD	*Nitzschia palea debilis*	1.5	3	1	3
PLEN	*Planothidium engelbrechti*	1.5	25	2.9	2
NMIN	*Eolimna minima*	1.4	39	2.2	1
ADSA	*Achnanthidium saprophilum*	1.2	9	3	1
NCLA	*Nitzschia clausii*	1.2	13	2.8	3
AOBG	*Psammothidium oblongellum*	1.1	13	4.5	1
DCOF	*Diadesmis confervacea*	1.0	13	1	3
PLFR	*Planothidium frequentissimum*	1.0	28	3.4	1
GPSA	*Gomphonema pseudoaugur*	0.9	22	3	1
NUMB	*Nitzschia umbonata*	0.8	12	1	3
AMIN	*Achnanthidium minutissimum*	0.8	9	5	1
NMEN	*Navicula antonii*	0.7	17	4	1
NINT	*Nitzschia intermedia*	0.5	15	1	3
NLIN	*Ntzschia linearis*	0.5	19	3	2
NVGE	*Navicula viridula var. germainii*	0.5	14	3	2
LOW ECOLOGICAL STATUS					
NPAL	***Nitzschia palea***	**5.2**	**54**	**1**	**3**
NVEN	*Navicula veneta*	2.2	56	1	2
NPAD	*Nitzschia palea debilis*	1.5	3	1	3
DCOF	*Diadesmis confervacea*	1.0	13	1	3
NUMB	*Nitzschia umbonata*	0.8	12	1	3
NINT	*Nitzschia intermedia*	0.5	15	1	3
NSEM	***Sellaphora seminulum***	**10.4**	**58**	**1.5**	**2**
NIFR	***Nitzschia frustulum***	**9.8**	**53**	**2**	**1**
NSBM	*Eolimna subminuscula*	4.6	52	2	1
GPAR	*Gomphonema parvulum*	4.6	58	2	1
NAMP	*Nitzschia amphibia*	3.0	40	2	2
NMIN	*Eolimna minima*	1.4	39	2.2	1
NAPE	***Mayamaea atomus var. permitis***	**5.5**	**48**	**2.3**	**1**
SPUP	*Sellaphora pupula*	3.9	49	2.6	2
NSHR	*Navicula schroeteri*	4.8	50	2.8	3
NCLA	*Nitzschia clausii*	1.2	13	2.8	3
PLEN	*Planothidium engelbrechti*	1.5	25	2.9	2
MEDIUM ECOLOGICAL STATUS					
AEXG	*Achnanthidium exiguum*	4.4	51	3	2
ADSA	*Achnanthidium saprophilum*	1.2	9	3	1
GPSA	*Gomphonema pseudoaugur*	0.9	22	3	1
NLIN	*Ntzschia linearis*	0.5	19	3	2
NVGE	*Navicula viridula var. germainii*	0.5	14	3	2
NGRE	*Navicula gregaria*	2.9	39	3.4	1
PLFR	*Planothidium frequentissimum*	1.0	28	3.4	1
HIGH ECOLOGICAL STATUS					
CPLA	***Cocconeis placentula***	**9.8**	**52**	**4**	**1**
NMEN	*Navicula menisculus*	0.7	17	4	1
AOBG	*Psammothidium oblongellum*	1.1	13	4.5	1
AMIN	*Achnanthidium minutissimum*	0.8	9	5	1

Resource (2006-2009) : **Bold** indicates data associated with dominant species

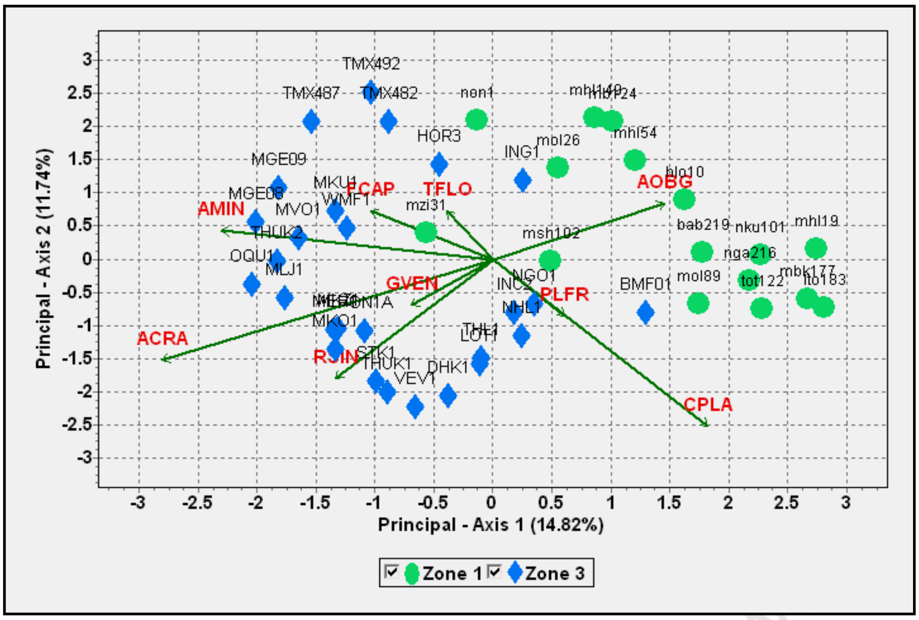


Figure 6.3 **PCA Variance-Covariance plot of diatom count data ($log_{10}[x+1]$ transformed) showing the comparison between the main species vectors associated with headwater sites originating in Zone 1 and Zone 3 Rivers**

[Resources A4, C3 (2006-2009)]

[Note : Acronyms of species are explained in the corresponding series of Tables 6.1 - 6.6.]

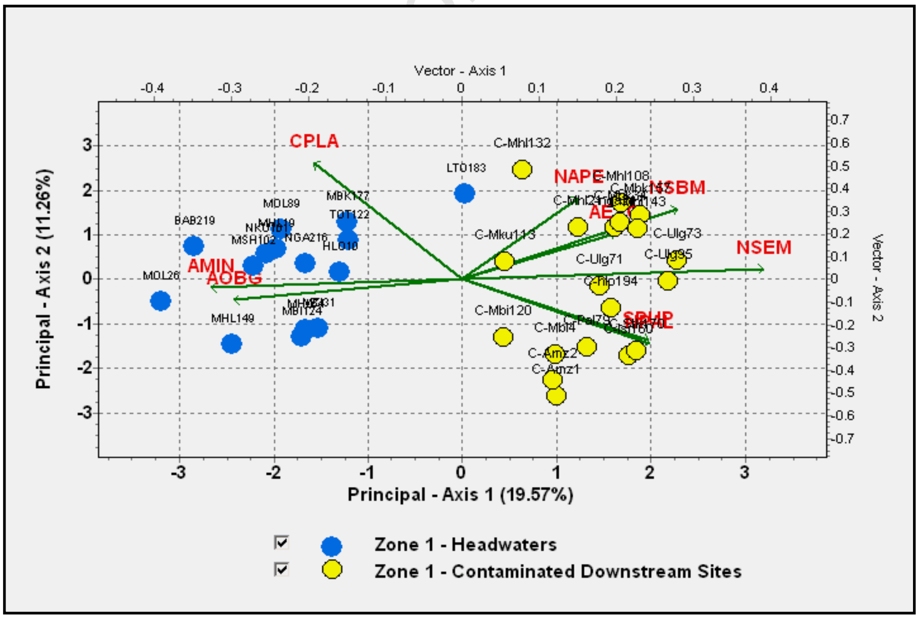


Figure 6.4 **PCA Variance-Covariance plot of diatom count ($log_{10}[x+1]$ transformed) showing the comparison between the main species vectors associated with headwater sites versus contaminated sites originating from Zone 1 Rivers** [Resource C3 (2006-2009)]

A Principal Components Analysis Covariance plot of the present-day diatom data from Zone 1 headwaters and diatom data of the headwaters of Zone 3 Rivers shows the largest eigenvectors associated with the most influential species in the ordination of sites along an environmental gradient. There was a clear separation of the groups of Zone 1 and Zone 3 sites along this gradient (Axis 1). *Psammothidium oblongellum* (AOBG) and *Cocconeis placentula* (CPLA) were the two influential species associated with Zone 1 sites, while *Achnanthidium crassum* (ACRA) and *Achnanthidium minutissimum* (AMIN) were the species most closely associated with headwaters of Zone 3 rivers to the left of the ordination (**Figure 6.3**).

A Principal Components Analysis Covariance plot of all the present-day Zone 1 River diatom data (near-natural headwaters versus downstream contaminated sites) shows the largest eigenvectors associated with species that influenced the ordination of sites.
A distinct separation exists along a pollution gradient (Axis 1) between near-natural headwaters and a group of contaminated sites of these small coastal rivers. *Psammothidium oblongellum* (AOBG) and *Achnanthidium minutissimum* (AMIN) were the most abundant and influential species in the near-natural headwaters, while *Sellaphora seminulum* (NSEM) was the most abundant species influencing the ordination of the contaminated sites (Axis 1) (**Figure 6.4).**

6.5 Discussion

The narrative contained in the European Directive documentation that describes the proposed characteristics for reference conditions allows for a wide ranging interpretation and understanding of the physical criteria to fulfil a reference state condition (Eloranta & Soininen 2002, Wallin *et al*. 2003, Kelly *et al*. 2008). The key issue, however, remains the identification of the suite of diatoms, which characteristically conform to the naturalness of type-specific reference state conditions.

There are two fundamentally different approaches for classifying reference sites and generating information on reference state biota, namely *the Regional Approach and the Multivariate Approach.* The former classifies reference sites *a priori* based on geographic and physical attributes whereas the latter approach (used in this investigation) seeks to classify reference sites from an '*a posteriori'* interpretation of the ordination of sites based on the responses of diatoms. Diatom data were used to group sites that have similar taxonomic composition, thus providing an objective way of grouping high ecological status reference sites according to the structure of diatom assemblages. Reference taxa are those expected to be at a site which is free of human disturbance and for which environmental and water quality characteristics are evidently at near-natural concentrations.

Previous other studies on lotic systems have also relied on diatoms as a basis for establishing reference state communities by using multivariate methods to group sites of similar species composition (Foerster *et al*. 2004, Descy *et al*. 2005, Tison *et al*. 2005, 2007; Grenier *et al.* 2006). These more recent initiatives are regarded as a paradigm shift in the way river assessments are conducted today with emphasis given to ecological status and Ecological Quality Ratios (EQR) based on the responses of the biota (diatoms) as "*an expression of the quality of the structure and functioning of aquatic life*" (Kelly *et al*. 2008).

Diatom counts at present-day and historic reference state sites were dominated in local rivers by a few species (**Table 6.1- 6.6**). *Achnanthidium minutissimum* was the most common taxon in the headwaters of most local rivers. *Tabellaria flocculosa* was abundant and dominant in a restricted high altitude niche and *Psammothidium oblongellum* was characteristic of the type specific headwaters of small coastal Zone 1 Rivers. These data were also compared with diatom counts obtained from literature surveys which, however, did not reveal comprehensive lists of reference state taxa for type specific river conditions but rather gave records of species that were common or dominant in the headwaters of various rivers. Comparison with local reference taxa revealed that *A. minutissimum, T. flocculosa and P. oblongellum* were three of the species that were recorded most frequently in headwaters of rivers elsewhere in the world **(Table 6.7)**.

Comparisons of the diatom reference communities within a matrix of headwater reference sites were also made together with their associated ecological status ratings within spatial zones. It was necessary to work at the species level for this investigation because differentiation of the ecological status of an assemblage at a site is based on the collective 'scores' of the species constituting a diatom assemblage. Analysis by frequency of site occurrence (dominant species names in bold) indicated that the most abundant species were not always well represented at a large number of sites (**Tables 6 - 6.6.2**). The dominance of *T. flocculosa,* for example*,* in the high altitude source zone of the Thukela River in both historic and present-day samples was an example of a restricted but characteristic distribution.

This species was only recorded from 15% of the total sites incorporated in a group of Zone 3 Rivers. The ubiquitous diatom, *Achnanthidium minutissimum,* on the other hand, produced the highest proportion of the total count and also occurred at every site in the Montane Upland rivers, although it did not necessarily obtain the highest count in every sample. The high range in percentage relative abundance and wide spread occurrence of *A. minutissimum* in near-natural waters makes it less useful as a diagnostic species.

Table 6.7 Examples of diatom taxa recorded at 'Reference State' in headwaters of rivers from other parts of the world

Spatial Zone	South Africa KwaZulu-Natal Rivers Present-day (2006-2009)	South Africa KwaZulu-Natal Rivers Historic (Cholnoky 1956-1960)	Canada St Maurice River (Grenier *et al*. 2006)	United States Oregon Coast Range Streams (Weilhoefer & Pan 2006)	United Kingdom Diverse Rivers (Kelly *et al.* 2008)	Hungary Kemence River (Acs *et al.* 2004)	Australia Kiewa River (Newall *et al*. 2006)	Australia West Australian Rivers (John 1998)
Montane Uplands	*Achanthidium minutissimum*	*Achnanthidium minutissimum*	*Achnanthidium minutissimum*	*Achnanthidium minutissimum*	*Achnanthidium minutissimum*	*Achnanthidium minutissimum*	*Tabellaria flocculosa*	*Tabellaria flocculosa*
	Tabellaria flocculosa	*Tabellaria flocculosa*	*Tabellaria flocculosa*	*Achnanthes pyrenacium*	*Tabellaria flocculosa*	*Gomphonema micropus*	*Actinella eunotioides*	*Eunotia curvata*
	Achnanthidium crassum	*Fragilaria ulna*	*Brachysira microcephala*	*Nitzschia inconspicua*	*Psammothidium oblongellum*	*Gomphonema pumilum*	*Fragilariaforma virescens*	*Eunotia flexuosa*
	Reimeria sinuata	*Encyonema ventricosum*	*Eunotia pectinalis*	*Rhoicosphaenia abbreviata*	*Fragilaria capucina*	*Diatoma mesodon*		*Navicula cryptocephala*
	Cocconeis placentula	*Gomphonema longiceps*	*Fragilaria capucina*	*Cocconeis placentula*	*Cocconeis placentula*			*Fragilaria ulna*
			Gomphonema sphaerophorum		*Nitzschia dissipata*			
					Hannaea arcus			
Coastal Lowlands	*Achanthidium minutissimum*						*Navicula rhyncocephala*	*Amphora coffeaeformis*
	Psammothidium oblongellum						*Reimeria sinuata*	*Cocconeis placentula*
	Cocconeis placentula						*Navicula cryptocephala*	*Amphora holsatica*
	Gomphonema pumilum v rigidum						*Navicula gregaria*	*Rhopalodia musculus*
							Nitzschia palea	
							Placoneis elginensis	
							Geissleria decussis	

Its usefulness and ecological indicator rating has also been questioned because of difficulties in separating it from very similar small *Achnanthidium* species which have different ecological ratings, and because of its ecological response pattern as an early coloniser after heavy rain events (Acs *et.al.* 2004, Kelly *et al*. 2008). It carries a high ecostatus rating but has also been reported from contaminated reaches of rivers (Soininen & Eloranta 2002, Acs *et al.* 2004) which has led to an ongoing debate about the autecology of various forms of this taxon (Ector *et al.* 2009, Wojtal *et al*. 2011)

Some species, such as *Navicula cryptocephala, Navicula gregaria and Fragilaria ulna* occurred relatively frequently in the Zone 3 headwaters (>40% of the sites) but generally in low numbers which is sensible because these are uncontaminated waters and these species carry a medium ecological status rating of just over 3 (**Table 6.2**). Low ecological status indicators (*Nitzschia palea, Craticula molestiformis, Sellaphora pupula)*, as would be expected, occur less frequently and in relatively low numbers in chemically dilute waters.

All of the dominant reference species recorded in the headwaters of Zone 1 Rivers carried high ecological ratings (> 4) and yet only *A. minutissimum* and *P. oblongellum* are reported to be indicative of clean, well oxygenated electrolyte poor waters (Taylor *et al.* 2007) (**Table 6.6.1**).The structure of these assemblages is distinctly different from those of the headwaters from the Montane Uplands. *Cocconeis placentula, a species with an* enigmatic response pattern, was also recorded in high numbers in some different localities.

Sellaphora seminulum Grunow, *Nitzschia frustulum (Kützing)Grunow, C. placentula, Mayamaea atomus var. permitis* (Hustedt)Lange-Bertalot, *Nitzschia palea* (Kützing)W.Smith are all species listed as indicators of pollution and were therefore common in more than 50% of the contaminated sites that were sampled (**Tables 6.6.1, 6.6.2**). Several other species, such as *Navicula schroeteri, Eolimna subminuscula, Gomphonema parvulum, Achnanthidium exiguum, Sellaphora pupula and Nitzschia amphibia* have also been reported as commonly occurring in organically and nutrient enriched environments in relatively high numbers. All these species, as expected, carry a low ecological status indicator value consistent with contaminated waters and were seldom if ever recorded from any near-natural headwater sites.

A prime objective of defining the attributes of diatom assemblages at a reference site is to establish the characteristics and variations in these reference communities at sites free of human disturbance (Wallin *et al.* 2003) because this is the basis on which impacted sites can be assessed (Stoddard et al 2006). The diatom component is a quality element that has been promoted as a general proxy for assessment of the biological integrity of the phytobenthos (Round 1991, Kelly *et al.* 2008).

This is because diatom assemblages are considered to have both distinct technical attributes over other algal groups and practical advantages for bio-assessment (Stevenson & Smol 2003, John 2004, Chessman *et al*. 2007).

Temporal and spatial variation of reference communities

A reference condition and its associated biological attributes is best described by a distribution of metrics rather than by a single set of values and this range may be attributed to variations in sampling practices as well as to natural temporal and spatial variations (*Stoddard et al*. 2006). Variations will occur naturally in any inherent measure of communities at the same site over time even without the confounding influences of human interventions. Yet, the goal is to describe the attributes of reference communities associated with a high degree of naturalness.

Natural temporal variations in the reference state communities were recorded from present-day and historic sites of the same undisturbed headwaters originating in Zone 3 Rivers in the Montane Uplands. The two reference communities were separated by a time interval yet the index scores from a suite of water quality indices showed a **high ecological quality** for both communities, thus observing the basic criterion of naturalness of a reference state condition for each time period. It has been established previously that diatom community structure in a headwater reference state is effectively influenced by natural sub-regional geology and broad climatic factors, leading to an expectation of similar reference communities in the same spatial zone (Biggs 1995, Stevenson 1997). However, the differences in these two diatom community responses (in terms of species composition) may also be attributed to other variable causes such as slightly different growing periods and sampling times of the winter season, natural variations in different flow regimes and / or sampling of different substrates in the microhabitat (Round 1991, Lenoir & Coste 1996, Kelly *et al.* 1995, Kwandrans et al. 1998, Kelly *et al.* 2008).

The naturally high variability of water quality variables and the wide fluctuation of spot measurements of the physico-chemical properties of river water justified the shift in emphasis to the integrative attributes retained by diatom communities. Diatoms are at the direct interface with water quality constituents and therefore are the biological component best positioned to bridge these spatial variations. The measurement of the distribution of attributes of diatom reference assemblages from undisturbed reference conditions gives complementary and non-redundant information on ecosystem status. Bio-indication is therefore key information used in the '*a posteriori*' identification of reference sites derived directly from 'signals' retained in the diatom community structure (Pan *et al.* 2000, Grenier *et al.* 2006). It is precisely this latter approach, which circumvents inherent spatial variability between sites, and which provides the integrative responses contained in the biotic assemblages in near-natural present-day and historic conditions. This approach facilitates and enables objective identifications of reference state communities.

A counter argument has been advanced for not using biotic assemblage data which is generated from a reference site because it was held that one cannot know '*a priori'* how much variation is inherent in any typical given assemblage (Stoddard *et al.* 2006). It was therefore considered to be inadvisable to use the structure of an assemblage because preconceived expectations of such a structure may lead to a circular argument in the process of selecting reference sites (Stoddard *et al.* 2006). This argument may be more applicable if an '*a priori'* approach is followed but contradicts the merits and essence of an '*a posteriori*' chemistry-free approach which relies directly on information from biotic assemblages which conform to and correspond with an extant, naturally stable geological template. Such an approach makes no prior assumptions about the similarity of diatom assemblages at different sites or of the environmental factors that may explain the distribution and variation in the diatom assemblages. Rather, diatom assemblage data are used to group sites that have similar taxonomic composition, thus providing an objective protocol for identifying potential candidate reference sites from the headwaters within each sub-region or spatial zone.

Reference State Conditions and Diatom Assemblages

The structure of diatom assemblages has often been shown to be dominated by a few species, favoured by conditions at a site, in association with a limited number of rare resident species, termed '*casual*' or '*alien'* to the site (Round 1991). The dominants and subdominants are the most influential species of an assemblage in the deduction and assignment of the ecological status of a river condition based on numerical attributes (absolute counts or relative abundance) and their autecological ratings. Diatom communities from **undisturbed near-natural reference sites** are therefore held to be the fundamental basis for attainment and assignment of a high ecological quality status consistent with a river reference condition (Eloranta & Soininen 2002). Natural variability in diatom community responses must encompass a range in values of physical and chemical attributes of a reference condition within a given spatial zone (Stoddard *et al.* 2006).The geomorphological characteristics of the province of KwaZulu-Natal predetermined that the headwaters of local river systems would be the most logical physical spatial zones for fulfilling these requirements. The fundamental conditions for fulfilling a reference state condition in these headwaters that provide a good correspondence with a representative reference state community were based on the following criteria :-

- headwaters free of human disturbance
- headwaters with an undisturbed, natural flow regime
- Median values of water quality parameters that have not deviated from the natural range in concentrations expected from the catchment geology and lithology of each sub-region
- Predominance of diatom species indicators which are consistent with high ecological quality ratings

These communities would be aligned to a high degree of *naturalness at the high end of the biological condition gradient* (Davies & Jackson 2006).

The spatial and temporal variation of reference communities was addressed in this part of the study by examining the differences between the dominants and sub-dominants recorded in (a) different sub-regions within the same spatial zone (**Figure 6.1, Tables 6.1, 6.2, 6.2.1**) (b) in historic and present-day communities drawn from the same spatial zone (**Figure 6.2, Tables 6.3, 6.3.1**) and (c) in different spatial zones (**Figures 6.3, 6.4**, **Tables 6.5, 6.6, 6.6.1, 6.6.2**).

Additional measurement of the distributions of the metrics of these reference communities will provide firmer criteria for a more refined classification of reference conditions. Restrospective analysis of historic diatom reference data was found to provide valuable information of the pre-existing river conditions captured and retained by the diatom assemblages. This enabled valid conclusions about the existence of historic high ecological status reference communities commensurate with a reference state in Zone 3 Rivers.

The headwaters of selected rivers of the Montane Uplands (Zone 3 Rivers) and the Coastal Lowlands (Zone 1 Rivers) in KwaZulu-Natal fulfilled the criteria for diatom reference conditions and their associated reference communities. The metrics associated with these diatom reference communities can be taken as benchmarks at the high end of a water quality condition gradient (see also Appendix II).

CHAPTER 7

MEASURES OF DIATOM RESPONSES TO HUMAN DISTURBANCE OF RIVER WATER QUALITY

7.1 Introduction

7.2 Aims

7.3 Methods

7.4 Results Measures of Diatom Responses

7.4.1 Diatom Species Composition Changes

7.4.2 Diatom Assemblage Metrics

7.5 Discussion

MEASURES OF DIATOM RESPONSES TO HUMAN DISTURBANCE OF RIVER WATER QUALITY

7.1 Introduction

A corollary to the use of diatoms to define reference state conditions in near-natural river conditions is the inference that diatom responses to human-induced environmental stresses will provide reliable measures of such disturbances. The ultimate objective of biological surveillance of rivers is to use the attributes and measures of the resident biota (e.g. diatom assemblages) to measure and characterise such human disturbances at an impacted site. Diatom metrics have been developed out of attempts to quantify diatom responses and compare 'signals' of environmental stress emanating from human impacts on river water quality (Round 1991, Sabater 2000, Fore & Grafe 2002, Stevenson 2006, Potapova & Charles 2007, Porter 2008, Porter *et al.* 2008, Urrea-Clos & Sabater 2009).

The attributes of diatom assemblages coupled with the sensitivity of various diatom species to particular pollution stresses have made diatoms reliable and informative bio-indicators for assessing such impacts (Cholnoky 1968a, Cairns *et al.* 1972, Patrick 1973, Lowe 1974, Descy 1979, van Dam *et al*. 1994, Hill *et al*. 2001, Hamsher *et al.* 2004, Porter 2008). The use of diatoms in the assessment of river condition has therefore become more widely accepted judging by the wealth of recent literature on many ecological aspects (Kutka & Richards 1996, Kelly 1998, John 1998, Acs *et al*. 2004, Potapova *et al.* 2004, Bellinger *et al*. 2006, Salomoni 2006, Taylor 2007c, 2007d, Raunio & Soininen 2007, Beyene *et al*. 2009).

However, there are still uncertainties relating to the choice and interpretation of diatom metrics for the assessment of water quality changes despite the almost universal application of these measures (Round 1991, Besse-Lototskaya *et al*. 2006). The appropriateness of using some diversity indices and diatom metrics has also been questioned by other findings (van Dam 1974, 1982, Round 1991). Diatom metrics may also be less appropriate when applied in regions other than where species responses and relationships with environmental factors were originally developed (Potapova & Charles 2007). A decrease in species richness and a decrease in evenness and diversity have 'traditionally' been taken as implying a corresponding increase in dominance by a few species capable of tolerating pollution and vice versa. However, the change in diversity indices as a measure of deteriorating water quality is apparently not a simple reliable relationship (Clarke & Warwick 1997) as shown also by specific studies on this aspect (Archibald 1972). Nonetheless, the knowledge base on diatom responses to human–induced impacts has increased and improved considerably in terms of the development of tools for assessment of these impacts (Fore & Grafe 2002, Chessman *et al*. 2007, Porter 2008, Porter *et al.* 2008,).There has been an advance from the expert systems containing descriptions of the autecology of diatom species (e.g. Lowe, 1974, van Dam *et al*. 1994) to more quantitative computerised methods to construct diatom assemblage metrics (Fore & Grafe 2002, Porter 2008). Water quality indices incorporated in diatom databases (Lecointe *et al*. 1993) and procedures to calculate a range of diatom metrics (Seaby & Henderson 2006) are now easily available. These metrics have also been shown to be positively correlated with resource or stressor gradients such as nutrient concentrations, organic enrichment and salinity (Sabater 2000, Porter *et al.* 2008).

Response measures of diatom community structures can reveal that such impacts have occurred (van Dam *et al*. 1994, Blinn & Bailey 2001, Weilhoefer & Pan 2006, Porter 2008). Autecological attributes and the physiological tolerance or sensitivity of several diatom species have been well documented over the last 30 years and this information lends itself to the use of additional diatom-derived metrics, at the species level, for the detection of pollution stresses in rivers (Palmer 1962, Lowe 1974, Lange-Bertalot 1979, Krammer & Lange-Bertalot 1986-1991, van Dam *et al*.1994, Fore & Grafe 2002, Porter *et al*. 2008). The rapid and predictable response of diatom communities to changes in stream condition has also been promoted as an advantageous attribute for the assessment of pollution impacts (Lange-Bertalot 1979, Hill *et al.* 2001, Potapova & Charles 2003, Weilhoefer & Pan 2006, Salomoni *et al.* 2006, Potapova & Charles 2007, Bray *et al.* 2008).

Studies focused on the chemical aspects of pollutants alone may unintentionally overlook the detrimental impacts on the biological integrity of a stream or river system (Bray 2007, Sgro *et al*. 2007, Zalack *et al.* 2010). The use of diatom community structure to indicate river quality changes therefore has distinct advantages over chemical and physical measures of the state of a river (John 2004, Stevenson 2006). Diatoms have also been used successfully as biological measures of human disturbances in streams contaminated by mining activities (De Nicola 2000a, 2000b, DeNicola & Stapleton 2008, Bray *et al*. 2008); by sewage and industry wastes (Kelly & Whitton 1998, Pan *et al*. 1996, Kelly 1998, Kelly 2004, Potapova & Charles 2007) and by agricultural pollutants (Leland & Porter 2000, Smucker & Vis 2009). It was therefore meaningful in the context of this investigation to assess diatom species composition changes from case studies of human disturbance of local rivers. **Such specific responses and diatom metrics could also potentially be considered examples of local diatom indicators of specific pollution impacts**.

The influence of eutrophication-related environmental stressors on diatoms in some rivers of South Africa has also been documented previously in various studies (Cholnoky

1960b, Schoeman 1976, 1979a; van der Molen *et al*.1998, van der Molen 2000**,** de la Rey *et al*. 2004, Taylor 2004a, 2004b, Archibald &Taylor 2007, Taylor *et al.* 2007c, 2007d).

Independent ad hoc studies were made of pollution stresses on diatom communities of rivers in the study area prior to the more recent research on the derivation of diatom reference state conditions. The data from these ad hoc surveys was therefore re-visited and is presented here as pertinent examples of differences in measures of diatom responses to five different types of pollution.

Acid Mine Drainage in the Tshoba River

Documented examples of extreme acidic stream conditions are commonly associated with coal mining activities (Kemp 1962, Archibald & Taylor 2007, Bray 2007, Bray *et al.* 2008, Zalack *et al.* 2010). Diatom assemblages have been used to assess the impacts of such acid mine drainage and organic pollution elsewhere in the world (Bahls 1993,Verb & Vis 2000, Hamsher *et al*. 2004, Bray 2007, Novis & Harding (2007), Sgro *et al.* 2007, Bray *et al*. 2008).

Coal mining has long been practiced in the north-western sector of the province of KwaZulu-Natal, especially in the Newcastle–Vryheid-Dundee industrial triangle where the geological conditions are favourable for recovery of coal (**Figure 1.1**). The small Tshoba River is located in this area and lies downstream of a decommissioned and abandoned coal mine dump near Hlobane (Latitude -27^{o}.738S, Longitude 30^{o}.970E) (Archibald &Taylor 2007) (**Figure 7.1**). The earliest studies benchmarking the impact of acid drainage from coal mines focused on water quality changes and response measures of river invertebrates in the upper reaches of the Thukela River (Kemp 1962, 1967; Oliff 1960). Deterioration of the water quality was already evident in some localities and this was ascribed to organic pollution and mineralisation from drainage out of coal mines in the headwaters of the Mkuze and Mfolozi Rivers (Archibald *et al.*1969). Both these rivers drain into Lake St Lucia, a unique World Heritage Site (**Figure 1.1**).

Sugar Mill Effluent in the Nonoti River

Sugar cane processing, by comparison with other industries, requires relatively little water because the water content of sugar cane may be as high as 70% of the processed biomass (Archibald *et al.* 1971). Nevertheless, the primary pollutant in this waste water is entrained sugar, a carbonaceous pollutant which causes a high oxygen demand in the downstream receiving waters. A multi-disciplinary study of the river impacts in 1967 involved the collection of diatom data and water quality data from small Nonoti River, from above and below the point of effluent discharge, during the winter low flow period. (Archibald 1971, Archibald *et al*. 1971) (**Figure 1.1**).

Pulp and Paper Effluent in the Lower Thukela River

Pulp and paper milling operations, in contrast to other industries, consume large quantities of fresh water in the processing of paper and related products (Lange-Bertalot 1979)**.** Hence many of these operations are situated in the coastal zone of the lower reaches of large rivers. The location at these points in the river basin carries with it an expectation of sufficient volumes of intake water from upstream for the milling process and sufficient assimilative capacity of the downstream reaches before the river discharges into the marine environment (**Figure 1.1**).The pulp and paper industry has also been identified as a serious polluter of the aquatic environments in the past, not only because of the large volumes of waste discharged but also because of the refractory nature of the solid waste material (Lange-Bertalot 1979). The impacts of pulp and paper waste are therefore complex because of several interactions between different constituents involving toxicity, oxygen demand substances, and suspended matter (Sudhakar *et al*.1994).

Sewage and Industry Waste in the Mbilo and Amanzimnyama Rivers

Diatom response measures of nutrient enrichment have also been shown to be reliable indicators of eutrophication-related water quality changes in disturbed urbanised environments (Kelly *et al.* 1995, 2003; Kelly & Whitton 1998, Bellinger *et al.* 2006). This condition is probably the most common of all human-induced pollution impacts and a wide range of diatom studies have been conducted elsewhere in the world over several decades (Bahls 1973, Lange-Bertalot 1979, Round 1991, Lowe & Pan 1996, Kelly & Whitton 1995, Fore *et al*. 2002, Soininen 2002, Potapova *et al*. 2004, Newall & Walsh 2005) The Mbilo River and the Amanzimnyama River are examples of two small nutrient enriched and chemically polluted urban rivers flowing into Durban Bay, a valuable ecological resource within the confines of the local harbour (**Figure 1.1**). Both rivers drain a dense, mixed residential area with light industry and the headwaters are not entirely devoid of irregular mild pollution from diffuse urban runoff. There are numerous wastewater treatment plants discharging treated sewage and other industry waste into rivers of the Coastal Lowlands. The impact of eutrophication, using responses of diatoms, has been measured in several rivers in South Africa (Cholnoky 1968a, Schoeman 1976, 1979a; van der Molen 2000, de la Rey *et al*. 2004, Taylor *et al*. 2005b).

7.2 Aims

A key purpose of this part of the investigation was to document the changes in the structure of diatom assemblages and their associated metrics, as measures of the diatom response to water quality changes in rivers that have been impacted by various types of human disturbance. The expectations in undertaking such an analysis were based on:-

- Structural changes in the diatom assemblages reflecting the different response measures of **sensitive** species compared with response measures of species **tolerant** of specific human disturbance pressures.
- A change in the value of specific diatom metrics derived from the diatom assemblages would be expected to provide discernible measures (biological signals) of the impacts (e.g. a decrease in species richness and/or water quality index values) (Patrick 1973, Cairns 1974, Stevenson 1984, Biggs 1989, Blinn & Bailey 2001).
- An analysis of these measures, in terms of relative proportions of the diatom assemblages tolerant of <u>or</u> sensitive to human disturbance could potentially be a useful suite of pollution indicators, distinct from those associated with near-natural conditions (Raunio & Soininen 2007).

7.3 Methods

Several structural diatom community metrics were derived to describe the measures of diatom responses to human disturbances including (i) changes in species composition of assemblages (ii) changes in species richness, species diversity and evenness (iii) changes in values of diatom water quality indices (iv) changes in the relative proportions of acidobiontic, eutraphentic, and saprobic groups of diatoms using ecological profiling of an impacted site (van Dam *et al.* 1994).

Measures of species richness, species diversity and evenness were derived in each of the different pollution impact scenarios. However, the absence of replicate data for diatom metrics such as the diversity indices for each pollution scenario made reliable statistical comparisons impossible. More significance is therefore attached to the major structural changes of the species composition as the key measure of the collective response by diatoms to pollution impacts. The assessment of pollution impacts using biological elements should take into account the response of the whole community (Descy 1979). To this end, the relative proportions of the diatom assemblages occurring in six ecological classes provides a basis for interpretation of the prevailing ecological condition of a river (van Dam *et al*. 1994). The quantitative dominance of species (in terms of changes in the relative abundance) revealed aspects of the quality of the water from this type of analysis. The environmental stressors (pH, salinity, nitrogen metabolism, nutrients, and oxygen regime) were subdivided into value ranges to indicate the prescribed degree of tolerance exhibited by a particular diatom species (van Dam *et al.* 1994). All these metrics were derived from the analysis of the species composition of each diatom assemblage at each site above and below the point of human disturbance.

The diatom species composition data for all the case studies of impacted rivers was processed with the Omnidia and SDR software and the results of selected diatom water quality indices were calculated together with other pertinent metrics. A rating scale for water quality condition divided into five classes ranging from 1 (low quality) to 20 (high quality) is given for interpretation of the numerical values of the Diatom Water Quality Indices. This is based on an original concept of water quality classes which ranged from 1 (heavy pollution) to 4.5 (no pollution, best biological quality) (Descy 1979) and incorporated in Omnidia (Lecointe *et al.*1993) and subsequently modified to the present 1-20 scale (Eloranta & Soininen 2002). However, it is more pragmatic and practical for water quality management applications to retain only three classes, namely 15-20 (high quality conditions) 10-15 (intermediate conditions) and <10 (polluted conditions) for all indices except the Trophic Diatom Index (TDI).

The TDI nutrient-specific index operates on a different scale of 0-100 and a different interpretation with the lowest values (0-20) representing higher quality conditions. This index has also to be interpreted in conjunction with the % PTV value (0-20% represents low organic content) - a measure of the pollution tolerant valves in the sample as confirmatory evidence of the presence / absence of organic pollution (Kelly 1998, Kelly *et al.* 2001)

Modus Operandi

A *control site* upstream in the 'generating environment' was sampled in each case as the only available and appropriate comparison of upstream conditions with changes downstream, a circumstance referred to as the "*best available condition*" (Stoddard *et al*. 2006).

The receiving environment was subdivided into two segments, namely:-

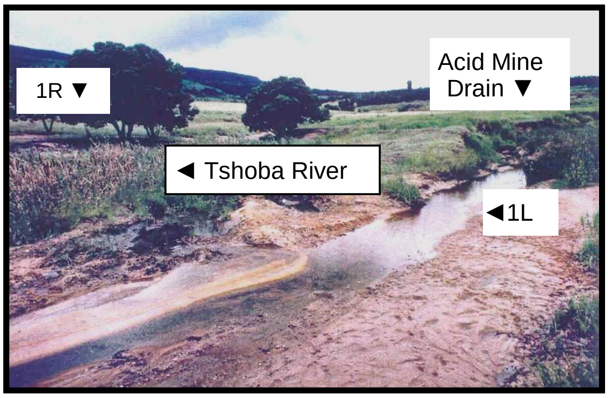
[i] An ***impacted zone*** which was sampled where the first mixing took place at a point downstream of the entry of the effluent.

[ii] A ***recovery zone*** which was sampled further downstream to assess the changes in the diatom response measures as an indication of the efficacy of self-purification capacity of the system.

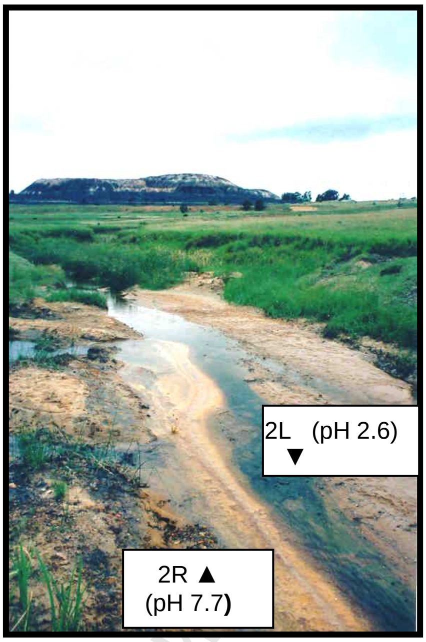
[Note The data from the original diatom material collected from the Nonoti River (Archibald 1971) was published in a pre-laptop computer age (Cholnoky 1970a). This data has now been updated and subjected to additional analysis using recent Omnidia software (Lecointe *et al.* 1993)].

7.4 Results: Measures of Diatom Responses

The results obtained from the independent ad hoc river surveys have been collated as examples of response measures of diatom assemblages to human disturbances (**Tables 7.1 - 7.4.2)**. The philosophy of this thesis is premised on the attributes and metrics of diatom assemblages being quite precise and accurate response measures of the quality of water in a river, including that related to varied human disturbance pressures.



[a] Tshoba River (Tsh1) at confluence (1R) with Acid Mine Drain (1L)



[b] Tshoba River (Tsh2) downstream of confluence showing integrity of twin inflows (2L and 2R) maintained with a distinct gradient in water quality and diatom assemblages across the channel.

(Images : CGM Archibald 16/02/1998)

Figure 7.1 Upper reaches of the Tshoba River showing [a] the confluence and [b] sites downstream of a disused and abandoned coal mine dump.

7.4.1 Diatom Species Composition Changes

The Impact of Acid Mine Drainage on the Tshoba River

The coal mine drainage water at Site Tsh1 (Sample 1L) was extremely acid (pH 2.6) and highly contaminated with metals, cations and anions giving elevated concentrations of total dissolved solids and high conductivity values. The water quality characteristics of the natural inflow at Site Tsh1 (Sample 1R) represented a 'target' condition of the upper Tshoba River system even though elevated concentrations of some constituents were measured in this water (**Table 7.1**).

There was no obvious mixing of these two inflows in the combined channel at Site Tsh2 downstream of the confluence. An intermediate condition at Site Tsh2 was measured in midstream (Sample 2C) indicating the presence of a marked cross-stream water quality gradient (**Table 7.1, Figure 7.1**).The quality of water of Sample 3, taken at Site Tsh3, some 15 km downstream showed a marked improvement in terms of reduced concentrations of chemical pollutants derived from the mine dump Alkaline conditions were restored at Site Tsh3 and concentrations of chemical constituents were much reduced (**Table 7.1**).

Table 7.1 Physico-chemical characteristics of water from the Tshoba River impacted by acid mine drainage (AMD) (16/02/1998)

Water Quality Variables ▼	Site ► / Sample ► / Units ▼	Tsh1 1L	Tsh1 1R	Tsh2 2L	Tsh2 2C	Tsh2 2R	Tsh3 3
		AMD	Natural	Main channel downstream of confluence			Mixed
		Upstream		Impacted Reach 0.1 km			Recovery 10 km
Temperature	℃	25.2	21.3	21.7	21.7	21.7	22.3
pH value		2.6	7.7	2.6	4.3	7.0	8.1
Alkalinity	mg $CaCO_3 ℓ^{-1}$		62			56	110
Conductivity	mSm^{-1}	459	277	468	289	281	53
Total dissolved solids	mg $TDSℓ^{-1}$	5020	2360	5100	2600	2370	352
Suspended solids	mg $SSℓ^{-1}$	27	15	21	41	16	26
Turbidity	JTU	1.1	0.4	0.3	56	0.2	24
COD	mg $Oℓ^{-1}$	5	16	12	16	8.1	16
Dissolved oxygen	$mgOℓ^{-1}$	6.7	8.7		8.1		7.4
Dissolved oxygen	% saturation	82.9	98.1		94.6		86.4
NH_4-N	µgN $ℓ^{-1}$	12	40	30	1000	110	40
NO_3-N	µgN $ℓ^{-1}$	70	2300	670	2200	2300	670
Kjeldahl-N	µgN $ℓ^{-1}$		1200	1100	1000	1110	510
TSP (soluble fraction)	µgP $ℓ^{-1}$	30	10	30	10	10	10
Calcium	mg Ca $ℓ^{-1}$	363	198	368	222	208	31
Magnesium	mg Mg $ℓ^{-1}$	198	85	200	92	83	22
Sodium	mg Na $ℓ^{-1}$	115	350	100	338	360	45
Potassium	mgK $ℓ^{-1}$	1.8	5.8	1.5	5.7	6.0	1.9
Cation Ratio M:D		0.21	1.26	0.18	1.09	1.26	0.88
Sulphate	mg SO_4 $ℓ^{-1}$	3100	354	3720	1650	1530	158
Chloride	mgCl $ℓ^{-1}$	31	8.7	31	11	9.2	13
Iron	mgFe $ℓ^{-1}$	151	0.03	133	0.24	0.01	0.14

Cation Ratio M : D - Calculated Ratio of Monovalent to Divalent Cations
COD - Chemical Oxygen Demand

Diatom Community Responses to Acid Mine Waters

The structure of the diatom community in the Tshoba River was markedly altered downstream of the impact from the acid mine drainage (**Table 7.1.1**). The relative abundance of species, characteristic of alkaline waters, was reduced in the reaches contaminated by the acid drain. The five dominant species in the control reach were *Brachysira vitrea* (Grun.) Ross (25.2%), *Achnanthidium minutissimum* (Kützing) Czarnecki (19.2%), *Encyonopsis cesatii* (Rabenhorst) Krammer (17.7%), *Nitzschia nana* Grunow (9.4%) and *Craticula buderi* (Hustedt) (9.4%) and constituted a total of 80.9% of the community at the control site. Seven dominants out of 20 species were recorded on the right bank of the impacted channel and these constituted 73.9% of the species count. The diatom community structure was quite consistent with that recorded from alkaline conditions at Site 1R upstream of the confluence with the acid drain.

Table 7.1.1 Changes in the diatom species composition (% Relative Abundance) at sites on the Tshoba River impacted by Acid Mine Drainage (AMD) (16/02/1998)

Sites ▶	Tsh1		Tsh2			Tsh3
Samples ▶	1L	1R	2L	2C	2R	3
Species composition ▼	AMD	Natural	Acid	Central	Alkaline	10 km
	Twin streams		Left bank	Mid-stream	Right bank	Downstream
Achnanthidium minutissimum		**19.2**		**7.0**	**14.6**	**56.2**
Brachysira vitrea		**25.2**		2.0	**18.5**	2.4
Caloneis bacillum						1.5
Caloneis molaris		0.4			0.6	2.3
Craticula buderi		**9.4**			2.1	
Cyclotella meneghiniana		0.8			0.6	
Cymbella affinis		1.2				**5.1**
Encyonema neogracile						0.8
Encyonema silesiacum						0.8
Encyonopsis cesatii		**17.7**			4.8	
Encyonopsis microcephala		1.7			2.1	
Fragilaria fasciculata						2.4
Fragilaria nanana		4.1		1.0	2.6	
Fragilaria ulna		2.8		**5.0**	1.2	3.2
Gomphonema gracile				2.0		
Gomphonema parvulum		2.8			2.8	**10.5**
Navicula heimansii					0.3	
Navicula schroeteri		0.7			0.3	1.5
Navicula tenelloides					0.3	
Navicula vandamii						0.8
Navicymbula pusilla						1.5
Nitzschia denticula						1.5
Nitzschia linearis						1.5
Nitzschia microcephala						0.8
Nitzschia nana		**9.4**		**6.8**	**16.8**	
Nitzschia palea		2.4			**6.8**	1.5
Nitzschia palea var. debilis		0.4			3.1	3.0
Nitzschia paleaeformis	**85.5**		**88.0**	**40.0**	4.1	
Nitzschia reversa		0.8			**6.3**	0.8
Nitzschia tropica		0.9		**6.0**	**5.2**	
Rhopalodia operculata						0.8
Sellaphora pupula						0.8
Stauroneis kriegerii	**14.4**		**11.6**	**30.0**	**5.7**	
Diatom Metrics						
Total count	532	499	517	515	571	334
Species Richness	**2**	**17**	**2**	**9**	**20**	**21**
No. of dominants (**> 5%** of count)	**2**	**5**	**2**	**6**	**7**	**3**
No.of subdominants	0	1	0	0	3	2
Aggregate % of dominants	**99.9**	**80.9**	**99.6**	**94.8**	**73.9**	**72.6**

[Note: *A dominant species in an assemblage is defined as a species with a relative abundance greater than 5% of the total count. A sub-dominant is a species which has a relative abundance count between 3-5% of the total count (Cholnoky 1968a, Schoeman 1971). It is commonly found that the aggregate % of the dominants and sub-dominants of a sample reaches 80% or more of the total count. It is unusual to find more than 5 dominants and sub-dominants in an assemblage*].

Two diatoms, *N. paleaeformis* (88.0%) *and S kriegerii* (11.6%)*,* dominated the impacted site (2L) in waters on the left bank and made up 99.6% of the population count of this composition which was near identical to that found in the upstream acidic feeder stream (1L).(**Table 7.1.1**).

There was a marked reduction in the species tolerant of acidic conditions in mid-stream at Site 2C and in the alkaline component on the right bank (2R) where *N***.** *paleaeformis* was reduced to 4.1% and *S.kriegerii* to 5.7%***.*** *B.vitrea (*18.5%)*, N.nana (*16.8%) *and A. minutissimum (*14.6%) were the dominant species together with *Nitzschia reversa* W.Smith and *Nitzschia tropica* Hustedt making up 6.3% and 5.2% of the total count respectively at Site 2R. The latter two species showed an increase in relative abundance over Site 1R, and have been reported from freshwaters with high electrolyte content (Krammer & Lange-Bertalot 1988, Taylor *et al*. 2007a).

The dominance of the acidophilous species *N. paleaformis* (40.0%*)* was reduced in the mid-stream sample (2C) by the appearance of several other diatoms. Six dominants out of 9 species made up 95% of the population count in this midstream sample. There was a measurable shift towards a greater percentage of *S. kriegerii* (30.0%) which is favoured by circum-neutral waters (van Dam *et al.* 1994). *A.minutissimum* (56.4%), *Gomphonema parvulum Kützing* (10.8%) and *Cymbella affinis (Kützing)(5.1%)* were the three dominants out of 21 species making up 72.6% of the total count in the alkaline recovery zone at Site Tsh3 (**Table 7.1.1**).

The Impact of Sugar Mill Effluent on the Nonoti River

The river water upstream of the sugar mill at Site Non1 was well oxygenated, alkaline with relatively low conductivity (Total Dissolved Solids), and a low biochemical oxygen demand (BOD). A normal winter temperature range (15-20℃) prevailed upstream of the mill. The river water immediately below the mill (Site Non3), however, registered abnormal winter temperature increases of up to 9℃ higher than ambi ent in-stream temperatures because of the hot effluent discharged from the sugar mill. High concentrates of sugars which are essentially dissolved carbonaceous material (BOD > 15 mg Oℓ^{-1}), were entrained in the effluent and markedly reduced the downstream oxygen content of the Nonoti River (**Table 7.2**). The oxygen 'sag' phenomenon resulted from bacterial degradation of the excessive organic matter in the impacted reach during winter months (**Table 7.2**). A polluted man-made stream (Site Non2) carried dunder water into the Nonoti River via a drain. '*Dunder water'* is a term encompassing wet-waste containing chemicals of various kinds such as sewage, oil spills and process waste from the mill operations. This wastewater had a high organic nitrogen and BOD content from entrained sugar in the effluent (**Table 7.2)**.

Table 7.2 Physico-chemical characteristics of water from the Nonoti River impacted by carbonaceous waste from a sugar mill (10/05/1967)

Water Quality Variables ▼	Sites ▶ Units ▼	Non1 Distance upstream 1.6 km	Non2 Mill Dunder water drain	Non3 Distance downstream 1.2 km	Non4 8.3 km
Temperature	°C	20.3	29.2	28.2	21.3
pH		7.2	6.5	6.8	7.4
Alkalinity	$mgCaCO_3\ell^{-1}$	17.4	26.6	30.7	25.6
Conductivity	mSm^{-1}	11.1	11.8	13.7	16.0
Total Dissolved Solids#	$mg\ \ell^{-1}$	73.3	77.9	90.4	94.9
Biochemical Oxygen Demand	$mgO\ell^{-1}$	0.6	14.3	15.4	2.0
Dissolved oxygen	$mgO\ell^{-1}$	8.2	6.5	3.6	8.6
Dissolved oxygen	%saturation	92	83	46	96
Kjeldahl-N	$\mu gN\ \ell^{-1}$	300	1200	900	900
NO_3-N	$\mu gN\ \ell^{-1}$	420	240	90	300
TSP (soluble fraction)	$\mu gP\ \ell^{-1}$	10	200	100	200
Calcium	$mgCa\ell^{-1}$	5.1	8.5	8.5	6.3
Magnesium	$mgMg\ell^{-1}$	3.0	1.5	5.6	5.0
Sodium	$mgNa\ell^{-1}$	14.8	3.5	17.0	19.0
Potassium	$mgK\ell^{-1}$	0.9	15.6	1.6	1.6
M:D Cation ratio		1.94	1.91	1.32	1.82
Chloride	$mgCl\ell^{-1}$	24.3	24.3	25.8	30.4
Sulphate	$mgSO_4\ell^{-1}$	4.5	8.0	8.0	9.5

Reference : (Archibald 1969) (**M:D** Cation Ratio Calculated Ratio of **M**onovalent cations **:** **D**ivalent cations)

Calculated TDS($mg\ \ell^{-1}$) = Conductivity value (mSm^{-1}) x 6.6 (Dallas & Day 1993)

Diatom Responses to Carbonaceous Sugar Mill Waste

The diatom community at the upper control Site Non1 was dominated by three species out of the complement of 19 recorded in the count. The count was made up of *Achnanthidium minutissimum (Kűtzing)* Czarnecki (28.1%), *Nitzschia gracilis* Hantsch *(18.1%) and Fragilaria intermedia* Grun. (12.9%) totaling 59.1% of the total count (**Table 7.2.1)**.

Marked changes in the diatom community structure were recorded downstream of the discharge from the sugar mill waste at Site Non3 in the river itself. The relative abundance of pollution-sensitive dominant species from the upstream control sites were all substantially reduced at this river site (**Table 7.2.1)**. The response of diatoms tolerant of or favoured by constituents of sugar mill waste was measured by the increased relative abundance of four dominant species at Site Non3, namely *Navicula schroeteri* Patrick (46.1%), *Nitzschia clausii* Hantsch (28.2%), *N. palea* (8.4%) and *Navicula tenella* Brebisson (*N. cryptotenella*) Lange-Bertalot (5.9%).

Table 7.2.1 Changes in the diatom species composition (% Relative Abundance) at sites on the Nonoti River impacted by carbonaceous sugar mill waste (10/05/1967)

Sampling sites ▶ / Species composition ▼	Non1 Upstream of mill 1.6 km	Non2 Mill Dunder water	Non3 Distance downstream of mill 1.2 km	Non4 4.7 km
Achnathidium microcephalum	2.2			
Achnanthidium minutissimum	**28.1**		1.6	2.7
Encyonema ventricosum	2.9			
Fragilaria capucina var. rumpens	4.6		0.4	
Fragilaria intermedia	**12.9**		1.8	
Fragilaria ulna	2.2		0.2	
Gomphonema parvulum	4.1		2.2	4.2
Luticola mutica	1.0	0.4		
Diadesmis contenta	4.6			
Navicula cryptocephala	3.6	0.4	0.2	1.2
Navicula gregaria			0.6	3.9
Navicula longicephala	1.4		0.2	
Navicula rostellata	1.4	0.6		3.0
Navicula schroeteri			**46.1**	**12.1**
Navicula tenella	1.2		**5.9**	**17.9**
Navicula tenelloides	1.4	0.2	2.4	2.1
Nitzschia clausii	1.4	0.4	**28.2**	**22.4**
Nitzschia gracilis	**18.1**	0.2	0.4	2.7
Nitzschia kuetzingiana			0.2	**16.4**
Nitzschia linearis				0.6
Nitzschia palea	3.9	**18.2**	**8.4**	**6.9**
Nitzschia perminuta		0.4	0.6	1.5
Nitzschia umbonata		**74.7**		
Stauroneis merrimacensis	3.1			
Surirella angusta	1.2	0.8		1.8
Diatom metrics				
Total count	409	493	490	330
Species Richness	19	10	16	15
% N-heterotrophic species	4.3	92.9	9.2	9.3
% C-heterotrophic species	2.4	0.4	74.4	14.4
No. of dominants (> 5% of count)	**3**	**2**	**4**	**5**
No. of sub-dominants (> 3% of count)	6	0	0	3
Aggregate % of dominants	**59.1**	**92.9**	**88.6**	**75.7**

(Updated from Cholnoky 1970a, Archibald 1971)
Bold figures respresent data associated wtih dominant species

The diatom assemblage in the organically enriched dunder water drain at Site Non2 was typically dominated by nitrogen-heterotrophic Nitzschia species such as *Nitzschia umbonata* (Ehrenberg) Lange-Bertalot (74.7%) and *Nitzschia palea* (Kützing) W Smith (18.2%) constituting 92.9% of the total count. The dominant pollution-tolerant species at Site Non4, some 4.7 km downstream of the mill were similar to those of Non3 but there was a decrease in the relative abundance of *N.schroeteri* (46.1 - 12.1%) and *N. clausii* (28.2 - 22.4%), both of which were dominants favoured by sugar waste in the impacted reaches upstream at Site Non3 (**Table 7.2.1)**.

Table 7.3 Physico-chemical characteristics of the Lower Thukela River impacted by nitrogenous waste from a pulp and paper effluent (23/09/1999)

Water Quality Variables ▼	Sites ▶ / Units ▼	LT1 Upstream 0.5 km	LT 2 Downstream of discharge 0.2 km Impact zone	LT3 0.8 km Mixed zone	LT4 10 km Recovery Zone
Temperature	°C	25.2	28.6	25.1	26.8
pH value		8.2	6.9	7.1	7.5
Alkalinity	mg $CaCO_3 \ell^{-1}$				
Conductivity	mSm^{-1}	40.3	70.8	67.4	65.6
*TDS (calculated)	mg ℓ^{-1}	265.9	467.3	444.9	432.9
Suspended solids	mg $SS\ell^{-1}$	5.0	11.0	14.0	8.0
Chemical Oxygen Demand	mg $O\ell^{-1}$	9.9	112.0	95.0	47.0
Dissolved oxygen	mg$O\ell^{-1}$	8.5	4.9	4.7	6.3
DO % saturation	%	105.2	63.7	58.2	80.6
NH_4-N	µgN ℓ^{-1}	30	20	20	50
NO_3-N	µgN ℓ^{-1}	40	60	30	30
Kjeldahl-N	µgN ℓ^{-1}	912	1650	1100	650
TSP (soluble fraction)	µgP ℓ^{-1}	20	120	100	70

*Conversion factor for TDS mg ℓ^{-1} = Conductivity value (mSm^{-1}) x 6.6 [Dallas & Day, 1993]

The impact of Pulp and Paper Waste on the Lower Thukela River

The quality of the water in the Thukela River above the mill at Site LT1 was relatively good. The water was well oxygenated with an alkaline pH and relatively low alkalinity. Low nutrient concentrations were recorded (**Table 7.3)**. The temperature of the river water in the impacted reach at Site LT2, below the mill discharge, was elevated and sustained at 28°C during the milling phase because of the entry of the warm effluent. The concentration of total nitrogen, particularly the organic component, increased downstream of the effluent discharge. Changes in conductivity (265-467 mSm^{-1}) and pH values of the water (8.2-6.9) were recorded in the impacted reach but in the latter case a more alkaline condition of the water was restored further downstream. Similar water quality impacts from pulp and paper effluent have been reported previously in a study of the impact of liquid waste from such a mill discharge on benthic diatoms (Sudhakar *et al*. 1994) (**Table 7.3)**.

Table 7.3.1 Changes in diatom species composition (% Relative Abundance) at sites on the Lower Thukela River impacted by pulp & paper waste effluent (23/09/1999)

Sampling sites ► **Species composition ▼**	**LT1** Upstream Site 0.2 km	**LT2** Impact Zone 0.2 km	**LT3** Mixing Zone 0.8 km	**LT 4** Recovery Zone 10 km
		Distance downstream of discharge		
Achnanthidium exiguum	0.2	0.2	0.5	0.5
Achnanthidium minutissimum	1.0	1.9	**9.6**	0.2
Amphora pediculus			1.6	
Cocconeis placentula var. euglypta			1.3	0.2
Craticula cuspidata		1.3	0.7	0.4
Cyclotella atomus		0.4	1.3	
Cyclotella krammeri		1.5	2.8	0.4
Cyclotella meneghiniana	0.3	0.6	3.7	0.5
Cymbella aspera	0.5			
Cymbella tumida	0.3			0.2
Cymbella turgidula	**48.4**	4.2	**5.1**	0.7
Diploneis puella				0.4
Encyonopsis microcephala			1.0	
Eolimna subminuscula			0.4	
Fallacia tenera				**18.7**
Fragilaria nana		1.7	0.4	
Fragilaria ulna	0.2		0.4	
Gyrosigma rautenbachiae			0.2	0.4
Gyrosigma scalproides			0.5	
Hantzschia distinctepunctata	0.2	0.2		
Navicula cryptocephala	**11.7**	0.8	1.6	
Navicula cryptotenella	2.5			0.7
Navicula erifuga			2.8	
Navicula exigua				1.3
Navicula gregaria		1.5	1.0	
Navicula rostellata	0.5	2.5	1.9	1.5
Navicula schroeteri			0.2	0.4
Navicula subrhyncocephala	**12.5**	2.3	1.0	**5.2**
Navicula tenelloides	2.8			0.2
Navicula trivialis		0.4		0.2
Navicula vandamii	0.8	1.5	1.6	
Navicula veneta		1.5	1.6	4.3
Navicula vitabunda		0.4	1.0	
Nitzschia agnewii	1.0	0.4	2.9	0.4
Nitzschia archibaldii	3.5	**6.5**	2.5	**15.5**
Nitzschia bacilliformis			1.5	
Nitzschia bacillum	0.7	0.2		4.0
Nitzschia capitellata				0.5
Nitzschia desertorum		0.6	1.5	0.4
Nitzschia draveillensis	1.0	1.3	0.4	
Nitzschia filiformis	0.2	0.4		
Nitzschia gracilis			0.4	0.4
Nitzschia inconspicua			0.4	
Nitzschia intermedia		**11.8**	**11.3**	4.5
Nitzschia linearis			1.0	
Nitzschia palea	1.2	**42.2**	**24.5**	**10.8**
Nitzschia pumila	2.8			2.2
Nitzschia radicula	0.9		1.0	
Nitzschia umbonata		1.0	1.0	
Placoneis elginensis				1.1
Pleurosigma salinarum var. pusilla	1.4			
Rhopalodia gibba	3.5			
Sellaphora pupula		4.8	0.2	**20.2**
Tryblionella apiculata			0.7	
Tryblionella hungarica			0.7	
Other species	4.3	3.8	3.6	2.2
Diatom metrics				
Total count	577	475	412	552
Species Richness	24	27	39	30
No. of dominants (**>5%** of count)	**3**	**3**	**4**	**5**
No. of sub-dominants	2	2	1	3
Aggregate % of dominants	**72.6**	**60.5**	**50.5**	**70.4**

Bold figures represent dominant species

Diatom Responses to Nitrogenous-rich Pulp & Paper Waste

The diatom assemblage at the upstream site (LT1) was dominated by three species, *Cymbella turgidula Grunow* (48.4%), *Navicula subrhyncocephala* Hustedt (12.6%) and *Navicula cryptocephala* Kützing (11.7%) making up 72.6% of the total count (**Table 7.3.1**).

It was noted that the majority of species recorded in the upstream control site were present at the impacted site downstream of the discharge of effluent from the pulp and paper mill – an overlap of more than 50% of the species. However there were structural changes in the relative abundance of dominant species between these sites. The three dominant diatom species were *Nitzschia palea* (42.2%), *Nitzschia intermedia* Hantzsch (11.8%) and *Nitzschia archibaldii* Lange-Bertalot (6.5%). There was a concomitant reduction in the dominant species recorded at the 'control' site (LT1) upstream of the mill, namely *Cymbella turgidula* (48.4 to 4.2%), *Navicula subrhyncocephala* (12.6 to 2.3%) and *Navicula cryptocephala* (11.7 to 0.8%).

Four dominant species were recorded at the intermediate site, at least 1km from the effluent discharge, and these included *N.palea* (24.5%), *Nitzschia intermedia(11.3%), Achnanthidium minutissimum (9.6%) and Cymbella turgidula (5.1%)* making up 50.5% of the total diatom count*.* There was a further change in the community structure in the recovery zone site (LT4) such that the dominants were spread between four different species namely *S.pupula* (20.2%), *Fallacia tenera* (Hustedt) DG Mann (18.7%), *N.archibaldii* (15.5%) and *N.palea* (10.8%)- the latter being dominant at upstream sites as well. The major components of this assemblage made up 70.4% of the total count (**Table 7.3.1**).

The Impact of Sewage & Industry Waste on the Mbilo and Amanzimnyama Rivers

The river water in the upper reaches of the Mbilo River (Mb1) is well oxygenated, with an alkaline pH and low conductivity consistent with a low human disturbance. Organic matter and nutrients were also recorded at low concentrations. However the quality of water deteriorated downstream of the sewage discharge (Site Mb4) and there was a decline in oxygen saturation with a simultaneous increase in nutrients and chemical oxygen demand (COD) in the river water (**Table 7.4**).

High nutrient concentrations also persisted in the Amanzimnyama river water for the length of the river between Sites Amz1 and Amz2, a distance of only a few kilometres. This was ascribed to a reduced self-purification capacity in the concrete-lined river bed. The low oxygen content at the upper site was indicative of chemical contaminants from industry waste in a commercial area producing a high chemical oxygen demand and organic nitrogen content in the river water (**Table 7.4**).

Table 7.4 Physico-chemical characterististics of the Mbilo and Amanzimnyama Rivers impacted by domestic sewage and industrial waste (3/11/1998)

Water Quality Variables ▼	Sites ► Units ▼	Mbilo River 3/11/1998 Mb1 Upstream	Mb4 Downstream	Amanzimnyama River 3/11/1998 Amz1 Upstream	Amz2 Downstream
Temperature	℃	25.1	25.8	23.8	23.7
pH value		7.5	7.8	6.6	8.9
Alkalinity	mg $CaCO_3 ℓ^{-1}$	8.1	92.2	81.0	118.0
Conductivity	mSm^{-1}	4.3	48.0	47.0	60.0
Total Dissolved Solids	mgTDS$ℓ^{-1}$	28.4	316.8	310.2	399.9
Suspended solids	mg SS$ℓ^{-1}$	26	20	15	6
Turbidity	JTU	5.9	4.5	7.5	7.8
Chemical Oxygen Demand	mg O$ℓ^{-1}$	16.0	40.0	17.0	42.0
Dissolved oxygen	mgO$ℓ^{-1}$	7.6	7.1	2.4	7.2
Dissolved oxygen	% saturation	93.9	88.6	29.0	86.4
NH_4-N	µgN $ℓ^{-1}$	60	260	50	170
NO_3-N	µgN $ℓ^{-1}$	200	4700	4100	4700
Kjeldahl-N	µgN $ℓ^{-1}$	350	1300	10005	8600
TSP (soluble fraction)	µgP $ℓ^{-1}$	70	1600	1600	2300
Calcium	mg Ca $ℓ^{-1}$	1.9	18.0	20.0	27.0
Magnesium	mg Mg $ℓ^{-1}$	1.1	7.9	8.9	9.7
Sodium	mg Na $ℓ^{-1}$	4.5	95.0	70.0	85.0
Potassium	mgK $ℓ^{-1}$	2.7	7.6	4.8	5.4
M:D Cation Ratio		2.4	3.96	2.59	2.46
Sulphate	mg SO_4 $ℓ^{-1}$	2.9	29.0	48.0	55.0
Chloride	mgCl $ℓ^{-1}$	5.9	57.0	52.0	74.0

#Conversion factor for TDS (mg$ℓ^{-1}$) = Conductivity (mSm^{-1}) x 6.6 (Dallas& Day 1993)

Calculated M:D Cation Ratio = Monovalent cations : Divalent cations

Diatom Responses to Nutrient-rich Sewage and Industry Wastes

The headwater site of the Mbilo (Mb1) was dominated by 6 species namely, *Capartogramma crucicula* Grunow ex Cleve *(18.8%), Navicula gastrum* (Her.)Kützing *(8.8%), Navicula gregaria* Donkin *(7.4%), Navicula veneta Kützing (6.0%) Sellaphora pupula* Kützing *(5.9%) and Psammothidium oblongellum Oestrup (5.1%)* (**Table 7.4.1**). Three species dominated the downstream waters, namely *S.pupula (47.1%), N.palea* (16.4%) and *Nitzschia capitellata Hustedt* (5.3%). These species are characteristic of eutrophic conditions experienced downstream of a sewage discharge and constituted 68.8% of the diatom count. (**Table 7.4.1**).

Table 7.4.1 Changes in the diatom species composition (% Relative Abundance) at sites on the Mbilo River impacted by sewage wastewater (3/11/1998)

Sites ► **Species composition ▼**	**Mb1** **Headwaters**	**Mb4** **Impact Zone**
Achnanthes hauckiana		0.4
Psammothidium oblongellum	**5.1**	
Achnanthidium minutissimum	2.8	
Amphora fontinalis	2.8	
Amphora montana		0.6
Amphora pediculus		0.2
Capartogramma crucicula	**18.8**	
Craticula cuspidata		0.2
Cyclotella meneghiniana		1.6
Cyclotella pseudostelligera	2.4	
Cymbella aspera	1.2	
Cymbella reinhardtii	1.7	
Diadesmis confervacea		0.4
Diadesmis contenta	1.1	
Eolimna subminuscula		0.6
Eunotia minor	1.5	
Fallacia tenera	0.6	
Fragilaria pinnata	0.9	
Fragilaria ulna	0.6	
Frustulia vulgaris	0.6	
Gomphonema gracile	0.9	
Gomphonema parvulum	3.5	2.0
Hippodonta hungarica	4.4	
Luticola mutica		4.4
Navicula angusta	1.2	0.2
Navicula cincta	1.7	
Navicula cryptocephala	0.6	1.8
Navicula eidrigiana	3.5	
Navicula erifuga		1.8
Navicula gastrum	**8.8**	
Navicula gregaria	**7.4**	
Navicula reinhardtii	2.1	
Navicula rhynchocephala	0.6	
Navicula rostellata	4.0	1.2
Navicula schroeteri	0.8	0.6
Navicula tenelloides	1.1	0.2
Navicula vandamii	0.6	
Navicula veneta	**6.2**	1.0
Navicula viridula	1.4	
Nitzschia amphibia		0.2
Nitzschia capitellata		**5.3**
Nitzschia desertorum		4.8
Nitzschia dissipata	0.6	
Nitzschia frustulum	0.9	0.9
Nitzschia inconspicua		1.6
Nitzschia intermedia		1.2
Nitzschia palea		**16.4**
Nitzschia supralitorea		0.9
Pinnularia viridis	1.4	0.4
Placoneis elginensis	1.7	0.7
Sellaphora pupula	**5.9**	**47.1**
Sellaphora seminulum		0.9
Surirella angusta	0.6	0.4
Tryblionella hungarica		1.2
Diatom Metrics		
Total count	475	529
Species Richness	36	32
No.of dominants > 5% of of count	**6**	**3**
No.of subdominants > 3% of count	4	2
Aggregate % of dominants	**54.6**	**68.8**

Bold figures represent data associated with dominant species

Table 7.4.2 Changes in the diatom species composition (% Relative Abundance) at sites on the Amanzimnyama River impacted by industrial waste (3/11/1998)

Sites ► Species composition ▼	Amz1 Upstream	Amz2 Downstream
Achnanthidium exiguum		0.2
Caloneis bacillum	0.2	
Diadesmis confervacea	3.6	0.2
Eolimna minima	0.6	
Eunotia exigua	0.2	
Fallacia monoculata	1.0	0.2
Fragilaria ulna	0.6	
Gomphonema parvulum	0.4	3.2
Navicula cryptocephala	0.4	
Navicula rostellata	0.2	
Navicula veneta	0.4	4.2
Nitzschia amphibia	0.2	
Nitzschia clausii		0.2
Nitzschia frustulum	3.4	**8.0**
Nitzschia intermedia	0.6	**5.3**
Nitzschia palea	**13.6**	**14.0**
Nitzschia palea var. debilis	**44.4**	**48.7**
Nitzschia umbonata	1.7	0.6
Planothidium engelbrechtii		0.4
Sellaphora pupula	**27.7**	**14.8**
Sellaphora seminulum	0.8	
Diatom Metrics		
Total count	528	512
Species Richness	18	13
No. of dominants >5% of total count	**3**	**5**
No. of subdominants > 3% of total count	2	2
Aggregate % of dominants	**85.7**	**90.8**

Bold figures represent data associated with dominant species

The diatom assemblages obtained in the survey of the Amanzimnyama River exemplifies a river under stress from a mix of chemical pollutants and high nutrient concentrations (**Table 7.4.2).** *Nitzschia palea* W Smith var. *debilis* (44.4%) and *Nitzschia palea* (Kützing) W Smith (13.6%) together with *S. pupula* (27.7%) were the three dominants out of the 18 species in the sample. These species made up 85.7% of the count in the upstream sample.

The first impacted site (Amz1) is positioned in the upper reaches of the river and is typical of extreme disturbance, not only because of the poor quality water emanating from industrial processes but also because of the physical alteration to the structure of the river. *N. palea var. debilis (48.7%), S. pupula (27.7-14.8%) and N. palea (14.0%)* remained the dominant species downstream at site Amz2. *Nitzschia frustulum* (Kützing) Grunow (8.0%) and *N.intermedia* (5.3%) were also the dominants recorded downstream but with a reduced relative abundance. Five species made up 90.8% of the count in an assemblage of 13 species. The assemblage represents a mix of species indicating eutrophic waters with moderate to high electrolyte content (**Table 7.4.2**).

7.4.2 Diatom Assemblage Metrics

Changes in the %Relative Abundance of Pollution-Sensitive Diatom Species

The reduction in the relative abundance of the dominant species at a control site was recorded for each pollution impact scenario (**Table 7.4.3).** The pollution-sensitive dominant species were excluded completely, in some instances, for example, in the most severely stressed condition downstream of acid mine drainage. The dominant species in each pollution study case were eliminated at the impacted sites downstream and seldom recovered with one exception, that of *Achnanthidium minutissimum* in the recovery zone of the Tshoba River.

Changes in the %Relative Abundance of Pollution Tolerant Diatom Species

A suite of pollution-tolerant diatom species was also developed from the various rivers impacted by waste from human activities (**Table 7.4.3**).The most extreme pollution response was recorded in the acid mine drainage waters with a specific tolerance demonstrated by high numbers of *N. paleaeformis* (85%) in very acid waters. High percentage relative abundance values were also recorded for *N. schroeteri* (46%) and *N. clausii* (28%) in waters contaminated by sugar mill waste. A smaller increase was registered for *N.palea* (3.9-8.4%) and *Fallacia tenera* (1.2-5.9%). Similarly, increases in the relative abundance of *N palea (1.2-42.2%)* and *N.intermedia* (0-11.8%) were recorded in response to pulp and paper waste. Smaller increases in *N. archibaldii* (3.5-6.5%) were also recorded. Marked increases in the relative abundance for *S.pupula* (5.9-47%) and for *N.palea* (0-16.4%) was recorded in response to sewage waste in urban streams.

Responses of Acidophilous taxa

More than 85.5% of the diatom assemblage was made up of acidophilous diatoms, dominant in that part of the Tshoba stream exposed to extreme acidic conditions. High percentages of the dominant species, *N. paleaeformis*, representative of extreme acidic conditions, were only recorded from the upper Tshoba River The absence of these acidophilous species and the concomitant increase in diversity and abundance of other alkaliphilous diatoms was the first indication of recovery downstream within 10 km of the confluence.

Responses of Carbon heterotrophic taxa

C-heterotrophic taxa constituted more than 74% of the diatom community below the sugar mill discharge. It has been well documented that species such as *N. schroeteri* thrive in conditions enriched with carbonaceous material such as that which prevailed in the lower Nonoti River downstream of the sugar mill (Cholnoky 1970a, Schoeman 1971, Lange-Bertalot 1986).

Table 7.4.3 Summary of changes in the diatom response measures of <u>pollution-sensitive</u> and <u>pollution-tolerant</u> species to various human disturbance pressures in specific rivers of KwaZulu-Natal

Diatom Assemblage Attributes ▼	Acid Mine Drainage Tshoba River Alkaline **1R**	 Acid **2L**	 Recovery **3**	Sugar Mill Effluent Nonoti River Upstream **Non1**	 Impact **Non3**	 Recovery **Non4**	Pulp & Paper Effluent Thukela River Upstream **LT1**	 Impact **LT2**	 Recovery **LT4**	Sewage Effluent Mbilo River Upstream **Mb1**	 Impact **Mb4**	Industry Effluent Amanzimnyama River Upstream **Amz1**	 Impact **Amz2**
Metrics													
Species Richness	17	2	21	19	16	15	27	34	35	39	35	19	13
No. of Dominants >5%	5	2	3	3	4	3	3	3	5	6	3	3	5
Aggregate of Dominants (%)	80.9	99.9	72.6	59.1	88.6	75.7	72.6	60.5	70.4	54.6	68.8	85.7	90.8
Shannon Diversity Index H'	2.17	0.36	1.88	2.38	1.57	2.24	1.73	2.26	2.45	3.10	1.93	1.56	1.68
Pielou's J Evenness	0.77	0.52	0.62	0.81	0.56	0.83	0.52	0.64	0.69	0.85	0.54	0.53	0.63
Water Quality Indices													
IPS	15.5	11.4	14.1	12.6	8.9	8.3	14.3	4.3	8.5	14.7	6.6	3.6	2.2
BDI	15.2	14.0	15.1	14.3	7.9	10.1	13.2	8.2	9.9	11	6.2	6.4	6.3
TDI	30.0	80.2	39.9	55.2	76.2	77.3	49.4	83.2	83.3	68.4	89	97	95.4
%PTV	18.8	87.9	18.8	32.3	40.6	58.3	10.8	68.4	36.2	11.8	29	57.7	80.3
EPI-D	17.5	15.1	16.0	13.9	7.2	6.4	11.3	7.4	8.0	9.6	7.5	7.7	6.9
Pollution-sensitive species (% Relative abundance in assemblage)													
Achnanthidium minutissimum	**19.2**		**56.4**	**28.1**		3.8							
Brachysira vitrea	**25.2**		2.4										
Craticula buderi	**9.4**												
Encyonema cesatii	**17.7**												
Nitzschia nana	**9.4**												
Fragilaria intermedia				**12.9**									
Nitzschia gracilis				**18.1**		2.7							
Cymbella turgidula							**48.4**	4.2	0.7				
Navicula cryptocephala				3.6	0.2	1.2	**11.7**	0.8				0.4	
Navicula subrhyncocephala							**12.5**	1.0	**5.2**				
Psammothidium oblongellum										**5.1**			
Capartogramma crucicula										**18.8**			
Navicula gastrum										**8.8**			
Navicula gregaria										**7.4**			
Pollution-tolerant species (% Relative Abundance in assemblage)													
Nitzschia paleaeformis	0	**85.5**	0										
Stauroneis kriegerii	0	**14.4**	0										
Navicula schroeteri	0.7		1.5	0	**46.1**	**12.1**			0.4	0.8	0.6		
Navicula tenella				1.2	**5.9**	**17.9**							
Nitzschia clausii				1.4	**28.2**	**22.4**					0.2		
Nitzschia palea	2.4		1.5	3.9	**8.4**	**6.9**	1.2	**42.2**	**10.8**		**16.4**	**13.6**	**14.0**
Nitzschia palea var. debilis	0.4		3.0									**44.4**	**48.7**
Nitzschia archibaldii							3.5	**6.5**	**15.5**				
Nitzschia intermedia								**11.8**	4.5		1.2	0.6	**5.3**
Nitzschia capitellata									0.5		**5.3**		
Sellaphora pupula			0.8					4.8	**20.2**	**5.9**	**47.1**	**27.7**	**14.8**
Nitzschia frustulum										0.9	0.9	3.4	**8**

(**Bold figures** represent **%** Relative Abundance values of dominant <u>**pollution-sensitive**</u> and dominant <u>**pollution**</u> <u>**tolerant**</u> species)
Note: There was *no control site* for the Amanzimnyama River

A smaller percentage of the same species was recorded downstream of the pulp and paper waste discharge in the Thukela River and in the Mbilo River indicating a wider tolerance of *N. schroeteri* to other organic constituents in the presence of higher oxygen concentrations (Schoeman 1971, Taylor *et al*. 2007a) (**Table 7.4.3**).

Responses of Nitrogen heterotrophic taxa

Nitrogen heterotrophic species were highest in the waters of the lower Thukela River below the discharge of the pulp and paper effluent. These taxa were also present in waters contaminated by industry effluents in the Amanzimnyama River (**Table 7.4.3**).

Responses of Eutrophic and Polysaprobic taxa

The largest percentages of taxa associated with eutrophic contamination were recorded in the waters of the lower Thukela River, the Mbilo River and the Amanzimnyama River all of which are impacted by effluents from wastewater treatment plants and urban runoff (**Table 7.4.3**).

Changes in Diatom Metrics

Several different diatom metrics were generated out of the data obtained from the analysis of the diatom species composition for all pollution impact scenarios. The number of dominants for any given pollution scenario ranged from 2 – 6 species while the aggregate % relative abundance for a suite of dominants from an impacted site ranged from 60.5% (pulp and paper waste) to 99.9% (acid mine drainage). The effective reduction in species richness in response to various pollution impacts was greatest in the acid mine drainage study (17 species reduced to 2) representing nearly an 85% reduction in species richness **in the count** (**Table 7.4.3**). Smaller reductions in species richness were recorded at the impacted sites downstream of sugar mill waste discharges (19 to 16 species) and for sewage and industry wastes (39 to 35) and (19 to 13) respectively. An apparent reversal of this trend was recorded downstream of the pulp and paper discharge where species richness actually increased downstream of the pollution impact (27 species increased to 34). This suite of metrics is derived from direct observation and analysis of the species composition at impacted sites as opposed to using diversity and evenness indices based on theoretical interpretations (**Table 7.4.3**). The outputs generally conformed to conventional findings of reduced diatom species diversity and evenness in stressed conditions (**Table 7.4.3**).

Results generated from the suite of water quality indices also demonstrated the impact of pollution irrespective of the index or the type of pollution. The change from a high ecological quality to a lower status was evident in all reported index values calculated from impacted sites, irrespective of the nature of the pollution. Calculated values ranged from <14 to 2.2 in the worst case scenario (**Table 7.4.3**).

7.5 Discussion

These ad hoc surveys provided an opportunity to re-examine whether there were groups of diatom species with specific responses to each of the extreme human impact conditions experienced in KwaZulu-Natal rivers (e.g. severe pH changes from acid mine drainage). The occurrence of certain diatom taxa may be highly correlated with a water quality parameter such as pH in acid contaminated streams (Pan *et al.* 1996, Verb & Vis 2000) or heavy metals (Sabater 2000). However a short-coming of these particular ad hoc surveys was the lack of a long term data series on the potential effects of seasonal variation in each case study. Furthermore, once-off surveys only provided one set of measures for any given metric measure vis-á-vis changing river conditions. This made statistically sound interpretation of changes in the various metric values, such as species diversity indices, unreliable hence more significance was placed on the broader and more comprehensive response measures obtained from changes in diatom species composition for a given human disturbance scenario.

Changes in species composition induced by Acid Mine Drainage (AMD)

The **pollution-sensitive** dominants upstream of the acid mine drain were all characteristic of conditions favoured by neutral, alkaliphilous species with the exception of *C. buderi* which has been found previously in mine effluent with a relatively high electrolyte content (Taylor *et al.* 2007a).This assemblage was regarded as the baseline target community characteristic of the water quality in the uncontaminated section of the upper sub-catchment of the Tshoba River.

Two dominant species showed a tolerance of the harsh acidic conditions and made up almost 100% of the total count in the acidic feeder stream and downstream at the impacted reach. The dominance of the acidobiontic species, *Nitzschia paleaeformis* Hustedt (85.5%) and to a lesser extent *Stauroneis kriegerii* Patrick (14.4%) was almost complete in this assemblage. The former species has previously been recorded from sulphuric acid ponds associated with coal mining operations (Krammer & Lange-Bertalot 1986) whereas the latter species has been reported to occur in waters around a neutral pH value (van Dam *et al.* 1994) but it has also been registered in a database of diatoms occurring in acid mine drainage waters (De Nicola 2000a, pers. comm. 2010).

A resource gradient across the 20 m wide stream at Site Tsh2 translated from an extreme acidophilic condition on the left bank, through an intermediate acidic midstream to an alkaliphilous condition in the right channel. Species composition changes and water chemistries were most marked in this component and reflected mixing and dilution of the acidic stream in mid-stream between the left and right channels. More importantly, the diatom population structure at Site 3 was characterized and dominated by non-acidophilic species indicating a measure of recovery within 15 - 20 km of the upstream acidic conditions at Sites 1L and 2L. The presence of *A. minutissimum* was indicative of the improved good water quality characterized by well-oxygenated conditions while a low relative abundance of *G. parvulum* indicated some mild nutrient pollution at this site.

Changes in species composition induced by discharges of sugar mill wastes

The presence of *Nitzschia gracilis Hantsch (18.1%)* as a dominant indicated the slightly polluted conditions upstream of the mill (Krammer & Lange-Bertalot 1988, Taylor *et al*. 2007a). The autecology of *Fragilaria intermedia* Grun. (12.9%) remains uncertain although it was grouped with the *Achnanthes* complex (Cholnoky 1970a). *Achnanthidium minutissimum* is a species frequently recorded from well oxygenated alkaline waters (Taylor *et al*. 2007a). *N.schroeteri* has been listed in South African rivers as a species particularly tolerant of carbonaceous-rich waters (Cholnoky 1968a, Schoeman 1971).The remaining opportunistic dominant was *Navicula tenella* (syn. *N.cryptotenella,* Krammer & Lange-Bertalot 1986) (5.9%) which frequents waters with very high or very low electrolyte content (Taylor *et al*. 2007a).The river bed at Site Non3, immediately below the mill was smothered by large growths of sewage fungus ('*aufwuchs*') while the water showed severe oxygen depletion (<50% O_2 saturation). This stressed condition was sustained for several months during the winter low flow period while the mill was operational. The high aggregate relative abundance of *N.umbonata* and *N.palea* in dunder effluent drain water, linked with reduced species richness, was also a good indication of a stressed diatom community in the drain water. The water quality some 5 km downstream of the mill at Site Non4 showed some recovery despite the confounding contamination by the dunder water. The larger number of species observed some distance downstream and the more equitable distribution of the count between species indicated an assemblage with greater diversity in a recovery zone. This phenomenon is generally associated with more stable conditions and is consistent with a diatom community which is subjected to less environmental stress (Cairns *et al*. 1973). An increase in *Nitzschia kuetzingiana* count Hilse (syn. *N.pusilla*, Krammer & Lange-Bertalot 1988) to16.4%, and the presence of *N.palea* (6.9%) indicated a persistence of some eutrophication from the secondary dunder water effluent from the mill in the recovery zone at Site Non4. The presence of *Navicula tenella* (17.9%) which is favoured by eutrophic conditions also supported the contention that recovery was not complete in the recovery zone (van Dam *et al*. 1994, Taylor *et al*. 2007a).

Changes in species composition induced by Pulp & Paper Waste

The discharge from the pulp & paper mill was continuous but the natural flow regime of the large river was reduced during the winter period thus exacerbating the effects of the effluent on the river biota. This was a worst case scenario given that dry conditions also resulted in abnormal additional abstractions from the river upstream of the mill. However the low flow regime downstream of the effluent discharge created a more stable environment favouring a reliable and distinct diatom response to the prevailing environmental conditions. *C. turgidula* has been reported to have a preference for weakly alkaline fresh waters, oligo-mesotrophic conditions and a tolerance of waters with low electrolyte content (van Dam *et al*. 1994, Taylor *et al.* 2007a) (**Table 7.3.1**).The majority of species in this community have a similar ecological characterisation and tolerance profile.

N.palea was the dominant species recorded at the impacted site below the mill discharge and it is well documented as an indicator of waters with elevated organic nitrogen content (Krammer & Lange-Bertalot 1988). The species is characterised as being an obligatory nitrogen-heterotroph, i.e. utilising organic nitrogen as a preferred source of nitrogen when living in polysaprobic conditions (BOD_5 >22 mg/*l*) and tolerating oxygen saturation levels as low as 30% saturation (van Dam *et al*. 1994). The remaining two dominant species, *Nitzschia intermedia* Hantzsch and *Nitzschia archibaldii* Lange-Bertalot find their optimal growth conditions in waters with a pH around neutral values and are considered as tolerant of β-mesosaprobic and eutrophic conditions (BOD_5 2-4 mgℓ^{-1}) (Krammer & Lange-Bertalot 1988, van Dam *et al.* 1994). The pollution tolerant species of *N.palea* (24.5%) *and N.intermedia* (11.3%) were the most abundant species. *S. pupula* is characterised as a freshwater taxon, favouring neutral pH waters, but also tolerant of waters with organic contamination i.e. α-mesosaprobic conditions (BOD_5 4-13 mgℓ^{-1}) (Krammer & Lange-Bertalot 1988, van Dam *et al*. 1994).Two other sub-dominant species, *N.intermedia* and *Navicula veneta* Kűtzing had a relatively high abundance in the community of the recovery zone and both are tolerant of pollution. The sub-dominant species of *Cyclotella meneghiniana* Kűtzing is characterised as preferring alkaline freshwater, and is recorded in the class of facultative nitrogen heterotrophic diatoms tolerant of α-mesosaprobic conditions (BOD_5 4-13 mgℓ^{-1}) associated with low oxygen saturation requirements of less than 30% (van Dam *et al.* 1994). *N.subrhyncocephala* is recorded as a cosmopolitan species with a preference for waters mainly with high electrolyte content (Krammer & Lange-Bertalot 1986). *A.minutissimum* in contrast, is more frequently associated with alkaline freshwaters having a high level of oxygen saturation. It is also a nitrogen autotroph (i.e. it is unable to utilise organically bound nitrogen as its nitrogen source) although it has been found in β-mesosaprobous waters (BOD_5 2-4 mgℓ^{-1}) (van Dam *et al*. 1994).This apparent anomaly in the occurrence of this species in mildly polluted conditions has been reported previously (Eloranta & Soininen 2002, Acs *et al.* 2004).

In some cases the ambiguities in the purported ecological preferences is linked with uncertainties of accurate identification of this small diatom and is a subject of continued debate (Acs *et al.* 2004, Potapova & Hamilton 2007, Ector 2009, van de Vijver 2009, Wojtal *et al*. 2011).

Changes in species composition induced by Sewage and Industry Waste Water

The sewage and industrial waste discharge was continuous during the winter low flow regime – a worst case scenario in the Mbilo River. However, in this case the river condition was measured in the latter part of summer to determine the diatom responses when river dilution was likely to have been most beneficial – a best case scenario. This assemblage is a mix of species tolerant of mild pollution and moderate electrolyte content indicative of pollution from a small urban environment. The most significant change in the structure of the diatom community at the lower site (Mb4) was the increase to those species characterised as obligate or facultative nitrogen heterotrophs tolerant of meso- to polysaprobic conditions.

Changes in diatom assemblage metrics induced by pollution impacts

Changes in species richness, diversity and evenness were recorded where pollution impacts had been measured chemically and by analysis of diatoms species composition changes (**Table 7.4.3**). The inadequacy of this data set for statistical assessment of diatom assemblage metrics has been alluded to previously. The direction of change is not always as critical as an observation that a change in itself, as manifested in a shift in the pattern of various diatom metrics, is a function of a pollution impact (Stevenson 2006).

Indices of species diversity and evenness are two commonly reported measures of the structural attributes of diatom assemblages. Such specific metrics have been used to compare attributes of upstream and downstream communities to infer impacts of pollution and also to show recovery from a polluted condition (Verb & Vis 2006, Bray 2007, Ni Chatháin *et al.* 2007, Porter *et al.* 2008, Zalack *et al*. 2010). There is an expectation of a decrease in measures of diversity and evenness indices of a diatom community along a pollution gradient of an impacted river in response to increases in human disturbances (Gaufin & Tarzwell 1952, Patrick *et al.* 1954). The most commonly used diversity measure is the Shannon Diversity index (Clarke & Warwick 1997). The direction of change was consistent with the expectation of a decrease in diversity indices scores downstream of a pollution impact with the exception of the Lower Thukela River study. Pielou's 'J' Evenness Index is also a commonly used measure of '*equitability*' or evenness of the distribution of individuals among the species making up an assemblage (Clarke & Warwick 1997). The same trend and pattern of decreasing evenness measures were observed in all the ad hoc investigations.

Reduction in species richness was less marked in the case of sugar mill waste impacts on the diatom community (19 species reduced to 16) – a reduction of only 15% in the species richness in the total count. Recovery in species richness was not evident (16 species versus 15) some 4.7 km downstream at Site Non4. The conditions in the receiving water below the pulp and paper effluent discharge were such that there was a reversal of this trend and a slight increase of 25.9% in the species richness (27 to 34 species) was recorded at the impacted site LT2. However a concomitant decrease in pollution-sensitive species can also be taken as a measure of the impact of pollution stress on species composition as a whole. It is noteworthy that with only one exception (*Achnanthidium minutissimum)* in the Tshoba River) none of the dominant pollution-sensitive species was able to regain a degree of dominance downstream of the impact, even in the recovery zone.

Each river condition retained an apparent unique suite of species making up the assemblage at the upstream control site. There was an apparent reversal in the premise of a reduction in species richness at an impacted site in the case study of the pulp and paper effluent discharge.

It may have been related to dilution of the effluent by a large river and to the presence of a much greater diversity of micro-substrates and therefore a greater number of opportunistic species prevailed. The phenomenon of a reversal of the expected trend with an increase in species richness downstream of a pollution impact has also been reported previously for other situations (Round 1991, Clarke & Warwick 1997).

Relatively small reductions of 11% (39 species to 35) and 27% (19 species to 13) in species richness were measured in response to impacts from sewage and industrial waste respectively when upstream-downstream comparisons were made separately for the Mbilo River and the Amanzimnyama River

The recovery potential of most of the impacted rivers was also demonstrated by an increase in both the Shannon Diversity Index and Evenness measures downstream of the impacted sites. A test of significant differences in the Shannon Diversity indices (Solow 1993) between the upper 'control' site and the impacted site immediately downstream of an effluent discharge was made for each case study. It was shown that in all cases the measures at the impacted sight were significantly different from those at the upstream 'control' site except for the reversal in this trend observed downstream of the impacted site in the lower Thukela River case study.

Diatom Water Quality Indices

Several water quality indices and some other metrics are routinely produced when diatom species composition data are processed using the Omnidia software program. This interactive database holds autecological data of diatom species and was designed initially to produce water quality indices for monitoring rivers in France (Lecointe *et al*. 1993). These are very informative outputs and useful in the assessment of general water quality conditions prevailing at a river site. The absence of an established diatom water quality index for South African rivers required that a choice of appropriate diatom indices had to be made.

The IPS Index (**I**ndice de **P**ollusensibilité **S**pecifique (Tison *et al*. 2007) or **I**ndex of specific **P**ollution **S**ensitivity was selected as the most appropriate index for general assessment of water quality in local rivers. The logic for this decision is based on the knowledge that this component in the Omnidia database is underpinned by the autecology of the greatest number of species (~2 035 species). Furthermore the autecology data of many of these species, identified previously from local rivers, is included in this database using information from investigations of South African Rivers (Cholnoky 1968a). The efficacy of the IPS general water quality index has also been tested recently using an updated version of Omnidia and it was shown to be the most suitable and appropriate index for this application in South African Rivers (De la Rey *et al*. 2004, Taylor 2004a, Taylor 2004b).

The IPS index values in the impacted reaches downstream of pollution sources were always less than a threshold value of 10 which was taken as the **upper boundary for poor water quality** associated with human disturbance pressures. All the calculated values of the IPS index decreased downstream of the pollution impacts, relative to the upper control site for all case studies, regardless of the nature of the pollution. These findings confirmed the poor water quality conditions downstream of the pollution impacts in every case study. In many instances the **control sites** recorded IPS values of less than 15 indicating that intermediate or moderate water quality conditions existed at these 'control' sites rather than being true reference sites, necessarily free of human disturbance.

Summary

Comprehensive structural changes were observed in diatom assemblages downstream of all pollution impacts. There were distinct and different measurable responses of the diatom assemblages vis-á-vis the different types of human disturbance covering examples of acid mine drainage, sugar mill effluent, pulp and paper waste, sewage discharges and industry waste.

- Sensitive species were eliminated or much reduced (in terms of % relative abundance) while the dominance of different species **tolerant** of specific pollution types increased in terms of the percentage relative abundance of these species.
- The direction of change in some metrics derived from a diatom assemblage **(i.e. a decrease in the diversity index and a concomitant decrease in species richness)** was consistent with conventional findings except for one case study.

- A discernible biological signal of pollution impacts followed by a reversal indicating recovery potential downstream was demonstrated in terms of some diatom metrics e.g. (i) species composition changes and changes in relative abundance of species (ii) species richness.
- The interpretation of the metrics associated with species diversity and evenness was considered to be unreliable because of the ad hoc nature of each case study.
- The various values of the water quality indices that were generated from these data all showed a similar trend indicating disturbance to the river water quality to which the diatom assemblages respond although the absolute values were different because of varying spatial and temporal scales.
- An analysis of collective responses of species, making up the diatom assemblages, in the form of relative proportions of pollution indicators could potentially be considered as a useful pollution impact assessment tool (Raunio & Soininen 2007) as distinct from those assemblages associated with near-natural conditions.
- Diatoms are therefore held to be useful indicators of changes in river conditions based on their ability to differentiate between the human disturbance impacts on water quality of a river.
- The relative abundance of specific groups of diatoms that are adapted to environmental extremes can be used to diagnose stresses caused by highly acidic conditions, high salinities, low dissolved oxygen and high nutrient and organic contamination (Stevenson & Smol 2003) (See Table 7.4.3).

Diatom metrics are reliable measures of river impact and recovery despite uncertainties that may arise from variability in methodologies of the diatom assessment protocol. Standardisation of many aspects of a surveillance protocol would improve comparability between studies both local and international. The sub-cosmopolitan nature and discrete autecological characteristics of diatom species is reflected in the remarkable similarities between the species tolerant of different types of pollution stressor in local rivers and those recorded elsewhere in the world. There is an expectation of a demonstrable change in the assemblage structure from increases in the relative abundance of pollution **tolerant** species which have a capacity to survive and multiply under external stresses (Cholnoky 1968a, Cairns *et al.* 1973). The exclusion and absence of some **pollution**-**sensitive species** and the proliferation of **pollution-tolerant** species is a result of competition along a resource gradient. This leads to measurable and distinctive changes in the species composition in terms of relative abundance of species and other metrics associated with an assemblage.

CHAPTER 8

SUMMARY AND FINDINGS

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SUMMARY AND FINDINGS

8.1 Aims of the Research Programme

A headwater stream that is embedded in a watershed is characterised by its climate, location, geology, water quality, physical microhabitats and biota. This embraces the totality of the environmental condition of a river site to which the patterned response of a diatom community conforms, under the universal axiom that the "*valley rules the stream"* (Hynes 1975).The river network of South Africa is large and encompasses diverse climatic, geomorphological and geological features and vegetation types, making it difficult to establish reference conditions that are applicable across the country (Palmer *et al.* 2003). It is divided naturally into a western winter rainfall area and an eastern summer rainfall region separated by the very arid central Karoo Desert but is linked by the Orange River basin which traverses the interior from east to west. The great diversity of river and landscape features led to the realisation that it would be beyond the scope of the limited resources of this investigation to attempt a definition of diatom reference state conditions for rivers on a national scale. The study area was confined, therefore, to the network of rivers on the eastern seaboard of South Africa located within the Province of KwaZulu-Natal (**Figure 1**).

The essential purpose of this investigation was to develop a protocol for the identification of diatom reference state conditions for a part of the country where there has been little or no human disturbance for centuries. The quest to identify diatom reference conditions was premised on the expectation that the appropriate reference state sites would engender diatom assemblages of high ecological status, representative of a near-natural reference state. A post World War II development trajectory for the Province of Kwazulu-Natal was established in order to validate the interpretation of reference conditions for the major river systems. The 1950-1955 period is regarded as the baseline or threshold for intensification of industrialization and urbanisation of the region. This was a period prior to significant increases in nutrient and other chemical contamination of local rivers, estuaries, reservoirs and lakes. However most of the disturbance-free headwaters of the large economically important rivers of this province originate in the adjacent conservancies of the Ukhahlamba World Heritage Site and the Royal Natal National Park. These protected areas were established in the early part of the 20th Century and therefore also represent unique, present-day river conditions, free of human disturbance pressures. (See also Appendix III).

The specific research objectives of this investigation were focused on deriving diatom reference state communities for type-specific conditions prevailing in these headwaters. The challenges involved identifying, describing and characterizing the responses of present-day and historic diatom assemblages for these rivers. The refinement of the spatial scale, as conceived for this investigation, resulted in the delineation of adjacent longitudinal geographical spatial zones incorporating the headwaters of the Montane Uplands, Interior Midlands and Coastal Lowlands, each drained by a different category of river. The delineation of the spatial zones and the consequent river classification system produced a scientifically valid and logical '*a priori*' grouping of the headwaters of the river systems, essentially based on the geomorphological and geological features peculiar to the region.

8.1.1 Previous Diatom Research in South Africa

The context of this research effort is contrasted against previous diatom research in South Africa when wide-ranging river surveys were concentrated mostly on taxonomic studies in the period between 1950 and 1981(Cholnoky 1956-1963, 1968a, 1970a; Schoeman 1971, Schoeman & Archibald 1976, Archibald 1972, 1981). The outputs from this early research led to the establishment of the South African Diatom Collection, the largest of its kind in Africa (Harding *et al.* 2005). Several publications were produced, culminating in a final major work on diatom ecology (Cholnoky 1968a). A foundation in river health assessment in South Africa had also been established from investigations using diatoms to assess **disturbed** river systems[8] (Archibald 1972, Schoeman 1979). Much of the taxonomic work remains relevant in key reference literature today (Schoeman & Archibald 1976), yet subsequent inactivity led to the near-demise of diatom research as a whole in the country between 1985 and 2000. The value of this early work for bio-monitoring applications was also seemingly misunderstood and underestimated by river health practitioners and water regulatory authorities in South Africa.

The imperatives of the national regulatory requirements embodied in the National Water Act in 1998 (Act 36 of 1998) ushered in renewed interest and support for diatoms in river bio-monitoring in South Africa. The new provisions of the RHP Implementation Document specifically required an assessment of the integrity of river fauna and flora. The initial revival of interest in the value of diatoms, was however, manifested in investigations of the '*a priori'* correspondence between water quality and diatom responses from a selection of rivers (van der Molen 1998, 2000), and in the more recent application of a 'new' generation of diatom water quality indices in river 'health' assessments (Taylor *et al*. 2004a, 2004b; Taylor 2006, Taylor *et al.* 2005-2007, Archibald &Taylor 2007).

More importantly, there was also a concomitant, crucial, philosophical and operational shift to the adoption of **a reference state approach** in river bio-monitoring

[8] (Shadbolt 1854, Grove 1894) – the earliest known references to diatom research in KwaZulu-Natal Rivers

(DWA&F 2008) without being able to stipulate threshold values for river conditions. The efficacy of this approach is heavily dependent on the availability and suitability of appropriate type-specific reference state sites in near-natural river conditions. This notion of naturalness requires a suitable group of sites in headwaters, evidently free from human disturbance pressures, from which candidate reference sites are most likely to be identified. This investigation therefore followed a dual approach which gave access to a **present-day** pool of protected river headwater sites together with a pool of similar valid **historic sites**, the data from which corroborated the high ecological status expected at a reference state (Cholnoky 1956, 1957 1960b, 1968b, 1970a).

8.1.2 The Utility of Historic Diatom Data

The scientific value of the apparently unique set of historic diatom data obtained from headwaters of rivers of the study area more than 50-60 years was highlighted and emphasised even more by the paradigm shift to a requirement for a biota-based reference state approach. The diatom material in the South African Diatom collection was therefore revisited after lying dormant for several decades, with the key objective of evaluating the historic environmental condition of the headwaters of rivers, **in the absence of relevant adequate water quality data**, using information derived from these diatom assemblages.

"A historic condition of a stream is defined by some previous point in time. "*Such a condition may provide an accurate representation of biological integrity if the historical point chosen is* <u>*sufficiently well described prior to known human interventions*</u> *e.g. Pre-agriculture, pre-tourism, pre-human occupation"* (Stoddard *et al*. 2006). The data used in this investigation therefore was putatively of great scientific value in the context of describing the biological integrity of 'near natural' biological quality elements, for several reasons:-

- Firstly, no other biological component of rivers in the country was so expertly documented and comprehensively described as the early work on the diatoms of KwaZulu-Natal River systems, covering a period of nearly twenty years between 1950 and 1970 (Cholnoky 1956,1957,1960b,1968a,1968b,1970a).
- Secondly, the naturally high variability of water quality variables, together with the absence or lack of adequate detailed reporting of corresponding historic water quality data of these rivers (Cholnoky 1968a, Porter *et al*. 2008) enhanced the unique value of the historic diatom data which encapsulates the historic river water quality status retained in the diatom communities..
- Thirdly, interpretation of the historical river conditions does **not** require that recent autecological ratings of the species be known at the time of the historic surveys and

therefore does not invalidate retrospective interpretation of historic water quality conditions of candidate reference sites. The historical information, from this perspective, therefore takes on even greater importance and relevance in providing a scientifically valid expression of a biological benchmark of the reconstruction of reference state conditions of river water quality of 50-60 years ago.

- Fourthly, the independent and objective manner of the historic investigations, at that time, was such that there was no presumption or preconceived notion of what constituted a diatom reference assemblage because the goals of the original research were different from the specific objectives of the present-day investigation.
- Fifthly, the diatom data was generated at the species level which is close to the highest taxonomic resolution especially in species-rich genera with a wide range of tolerance among such species (Chessman *et al*. 2007a). It is the most detailed and minimum level of accuracy of identification required for this work and therefore provides the best opportunity to interpret diatom-water quality relationships.

No more than 2% of the species in the data set were found to be either endemic and /or without an autecological rating for application of present-day diatom water quality indices. Furthermore these were mostly rare species with low relative abundance scores if and when these were captured in the counts. Finally, the species guilds that were recorded from the headwaters collectively contained a suite of species that showed responses consistent with similar responses of diatom dominants recorded from similar environments in rivers elsewhere in the world. This implied that the application of present-day diatom water quality indices, developed from species guilds with similar responses and autecological ratings from other parts of the world, may be transferrable. This allowed for retrospective interpretations of prevailing water quality conditions and comparison with present-day findings in the quest to identify diatom reference state assemblages from the same rivers.

8.2 Protocol for the Derivation of a Diatom Reference State Community

This investigation has been built on the strong foundation of the early taxonomic and ecological investigations of rivers by the original leaders in diatom research in South Africa. The study makes some important advances towards developing a protocol for establishing reference state communities. It enhances the cause for diatoms being re-established as important biological elements that provide accurate measures of the integrity of biota at near-natural river reference state. The high ecological status ‘signatures’ derived from the structural attributes of assemblages in the headwaters were premised on the responses of diatoms conforming to the natural river templates (Cholnoky 1963, Hynes 1975, Townsend & Hilldrew 1994). The complexity of the variation in these diatom responses within a range of natural templates was assessed and reduced by using ordinations in relation to unmeasured environmental gradients. The correspondence between the near-natural templates, classified within different spatial zones, and the diatom assemblages was the basis for defining the reference state communities of the study area. "*The position of the sites along the gradient of maximum variance is determined by the diatom community structure and is **independent** of measured environmental variables"* (Grenier 2006, Lavoie 2006, 2009).

The expectation was that diatom assemblages of undisturbed near-natural headwaters would be more similar within the zones than between zones, if this is primarily due to differences in sub-regional geology and the associated water qualities (e.g. Montane Uplands versus Coastal Lowlands communities). A corollary to this principle was the expectation that differences would be more distinct between undisturbed sites and contaminated sites if human impacts mask the influence of the near-natural template.

The National Spatial Biodiversity Assessment (NSBA) initiative describes in quantitative terms how much degradation has occurred in river systems of South Africa (Nel *et al.* 2007). Much of the relatively small percentage of unimpacted river reaches remains in the protected headwaters of Zone 3 Rivers of KwaZulu-Natal. This information highlights the uniqueness of the remaining river components located in the near-natural protected headwaters of the study area, confined within the Ukhahlamba World Heritage Site and the Royal Natal National Park.

A stepwise protocol was followed for the establishment of diatom reference communities of the important rivers of the Province of KwaZulu-Natal.

► Establishment of an human development trajectory for the region in order to validate a baseline for historic reference conditions.

► Provision of substantive evidence (expert opinion, narrative and quantitative data) to demonstrate that the present-day headwaters correspond with undisturbed reference conditions.

► '*a priori'* definition of small scale headwater zones within the confines of river basins as a function of geomorphological and geological features of the province.

► Classification of diatom assemblages based on relative abundance data at the species level followed by an 'a posteriori' interpretation of environmental gradients to identify the diatom communities associated most closely with reference conditions. The communities at the lowest end of the environmental gradient were representative of the near-natural reference conditions.

- ► Confirmation of the ecological status of the reference communities using a suite of commonly used and widely tested diatom water quality indices. High ecological status values associated with reference conditions are associated with an Ecological Quality Ratio value close to 1 and deviations due to human disturbance downstream are represented by values close to zero.
- ► Derivation of metrics of diatom assemblages that characterise the differences between reference assemblages and those impacted by human-induced stressors.

8.3 Findings

The reference condition approach for bio-monitoring of rivers is now widely promoted in Europe and the United States and has also been introduced in several other countries, including South Africa. The modus operandi followed in this thesis is therefore relevant and timeous in relation to river management directives in South Africa (DWAF 2008). It is also very much aligned to the Biological Condition Gradient (Davies & Jackson 2000) which espouses the notion of '*naturalness' at the one end of the biological condition gradient as opposed to heavily altered condition at the most impacted end of the gradient.*

The findings of this research reinforce the notion of the predictive power of diatoms as biomonitors of river condition because a good correspondence between a range of environments and diatom responses was established. Several case histories (Chapter 7) also demonstrated the specificity of the diatom responses to different pollution types. Guilds of pollution sensitive species and pollution tolerant species were recorded with their associated metrics.

The main findings from this study determined that:-

[i] *A dual 'a priori and an 'a posteriori'* chemistry-free approach was an objective practicable, relevant and tractable scientific protocol for identifying reference state conditions.

The interpretation of the response patterns of diatoms to hypothetical near-natural environmental gradients was achieved without recourse to the autecological ratings of the diatom species to avoid circularity of argument. These ratings were however only used in the penultimate step to confirm the high ecological status of the river sites – a condition expected in undisturbed environments. **This approach is an advance on previous diatom research protocols in South Africa**.

It is objective because it does not entertain any prior assumptions as to the environmental factors influencing the response patterns of the diatom communities. It is relevant because the protocol is aligned to the most recent regulatory directives and ecological perspectives of a biological condition gradient.

[ii] Structural attributes of diatom assemblages are fundamentally informative measures of environmental conditions, the status of which can be distinguished using the attributes and metrics of pollution-tolerant and pollution-sensitive guilds.

Measures of the relative abundance of the dominant species in a diatom community are regarded and have been promoted as cogent quantitative biological measures of river conditions (Cholnoky 1968a, Round 1991, Stevenson 2006). It is an operational principle that differs fundamentally from the concept of using floristic changes in diatom communities alone (e.g. species richness, evenness and diversity) to measure increasing human disturbance (Patrick 1949, 1973). The appropriateness of this latter approach has been questioned by observations on the St Lawrence River (Lavoie 2006, Lavoie *et al.* 2009).

Diatom attributes and metrics associated with reference state conditions were generated for different categories of river. It was shown that species richness was highly variable but distinct for a given condition. The trend in increasing diversity with increasing ecological status was consistent in most cases and vice versa, irrespective of the preferred diversity index. Species diversity and evenness indices were also variable even in unpolluted headwaters which would generally be expected to support a species-rich diverse community. High altitude sites, characterised by low ionic concentrations and low nutrient and organic contamination, actually produced low species richness, low species diversity and a highly uneven spread of individuals per species. This apparent reversal and contradiction of initial principles may be explained by the severe constraints or intense stress (e.g. nutrient deficiency, extremes in flow regime) experienced by communities at high altitude despite the prevailing high quality water conditions.

Only a limited number of species are favoured by these extreme conditions resulting in lower diversity (Stevenson 1984, Round 1991). Furthermore, the high altitude streams are in fact exposed to more regular **natural** disturbance of the regime, as distinct from **human-induced** disturbances. The location and near-natural state of high altitude stream sites is therefore apparently a condition predisposed to quite intense natural disturbance and stresses with a consequent reduction in the diversity of species (Connell & Orias 1964).

A similar situation has been reported in the near-natural upper reaches of streams of the Canadian Shield where a low number of taxa (low species richness) was also contrasted with a higher diversity in the impacted reaches of the lower St Lawrence River (Lavoie *et al.* 2009). It has been shown previously that species diversity reaches optimal levels at intermediate levels of stress (Archibald 1972).

The importance of taxonomic distinctness, differentiation and identification to species level is demonstrable when added-value scientific interpretations are required to determine the possible cause of the impact in bio-monitoring. This capability gives greater predictive power to the species level information because the researcher can generate hypotheses and reasoning relating to the causes of a disturbance. This is an improvement on the invertebrate data (SASS protocol) which **does not** provide information on the causes of an impact. "*In addition to the universality and transferability of ecological ratings of indicator species**,** the identification of local species as indicators of specific pollutants is very important for diatom applications. Diatom taxonomy is achieving finer resolution of* ***species*** *with the concomitant effect of finer resolution of its biomonitoring capacity The state of knowledge of diatoms in South Africa is thus better than many other countries because of* ***species*** *level information* (J.John, pers.comm. 2011).

If this research is to provide a benchmark for reference conditions in KwaZulu-Natal Rivers, the higher level of resolution is justifiable and scientifically more accurate. Genus level resolution may therefore not be sufficiently appropriate or diagnostically distinctive for establishing **reference conditions.** Rapid assessment technologies restricted to genus level identification may suffice for larger routine monitoring programmes of environmental degradation to obviate interpretation of species level uncertainties, but more information from advocates of this approach is required (Kahlert et al 2007, Kelly 2009). It has been however also been reported previously in a marine invertebrate study that taxonomic distinctness decreases along a gradient of increasing environmental contamination and stress (Warwick & Clarke 1995).

[iii] Investigations of the undisturbed chemically dilute headwaters of local rivers also revealed the characteristics of the target reference state taxa of diatom assemblages associated with sites of good ecological status.

Several of the dominant reference diatom taxa identified from headwater zones of local rivers (e.g. *Tabellaria flocculosa, Achnanthidium minutissimum, Achnanthidium crassum, Psammothidium oblongellum, Cocconeis placentula*) have been reported from comparable type specific water quality conditions reported in the literature. There were marked similarities in composition and magnitude (% dominance of the count at a site) of key dominant species from the identified reference sites with those recorded elsewhere in the world. The species composition of the diatom assemblages derived from the different sets of **headwater reference sites** was markedly different and separated physically into distinct geographical groups :-

[a] **Montane Uplands Spatial Zone (Zone 3 Rivers) – (High Altitude Group)**
These are from the headwater sites originating in the source zone of the main Thukela River.

[b] **Montane Uplands Spatial Zone (Zone 3 Rivers) – North-east and North-west Groups 1 & 2.**
These are the headwater sites from catchments of large rivers originating in the North-east and the North-west including the main tributaries of the Thukela River (the Buffalo River tributaries in Sub-catchment 2) **(Figures 1.1, 1.2**).

[c] **Montane Uplands Spatial Zone (Zone 3 Rivers) – (Western, South-western and Southern Groups 3, 4 & 5.**
These are the sites from the headwaters and tributaries from the catchments of large rivers originating in the West and South-west of the study area (e.g.Thukela Mkomazi, Mgeni, Mzimkulu) **(Figures 1.1, 1.2)**.

[d] **Coastal Lowlands Spatial Zone (Zone 1: Rivers) –** (**Eastern Group Figure 1.1)**
These are sites from the headwater sites of small rivers originating in catchments to the east of the axis of tensional folding.

[iv] The application of water quality indices, which were developed outside of the study area, may still be sufficiently valid in specific rivers of South Africa in the absence of a locally-derived diatom water quality index. The threshold value for high ecological quality river conditions was determined as >15.6.

The reference condition approach produced sites, selected from the headwaters of Zone 3 and Zone 1 rivers, the great majority of which were rated to be of high ecological status (i.e. Water Quality Index values > 15); a threshold condition expected of least disturbed headwaters of rivers. However, water quality indices alone provide no information on the structure and composition of the diatom guilds. It was demonstrated that water quality indices with high ratings can result from several different structural combinations of diatom assemblages (Historic versus Present-day). This was of particular significance in the comparison of temporal variations between historic and present-day assemblages of Zone 3 headwaters, each engendering a high water quality index.

This is the first set of diatom metrics for KwaZulu-Natal Rivers of its kind. The data provides an early benchmark for the 21st century for future research applications and comparison involving diatoms (Appendix II). The historic data was not originally processed in this way because software routines were not available at that time and this data may never be re-visited again. It was therefore deemed useful and practical to include examples of generic indices of general water quality as well as representation from indices based on species level identifications to reflect the degree of contamination of the local river waters in the absence of data from a formal accredited South African index

The inclusion of GDI metrics allows for comparison with similar diatom indices because there is no other such published data for reference conditions in local rivers. Recent findings in South Africa have confirmed the similarities in values produced by a suite of diatom water quality indices for some other rivers (Taylor 2004b, Taylor *et al.* 2007c, 2007d). Furthermore the consensus from these findings, adopted by the South African River Health Programme, was that the IPS Water Quality Index is a metric that should be taken as the 'benchmark' to provide some standardisation in reporting diatom water quality index results in the absence of an accredited South African Diatom Water Quality Index (DWAF 2008). This situation may be remedied when an exclusively South African version of a Diatom Water Quality Index is produced and accredited as the standard (J.Taylor *pers.comm*. 2010).

The inclusion of the GDI index results with the other listed metrics was based on the knowledge that research elsewhere had shown that genus level information had some merit, particularly when large monitoring programmes involve several technical staff with different skills in routine applications (Kelly *et al*. 2008). However a distinction and choice has to be made between the resolution of identification needed for large routine monitoring programmes and the accuracy of scientific data required for establishing diatoms reference conditions. It has been acknowledged that the determination of ecological status is crucially based on measures of the diatom assemblage structure and functioning of an ecosystem (Stevenson *et al*. 2008).

Species level data was therefore considered essential to establish a reference baseline of diatom assemblages to allow clear ordinations of the data by comparison with what should be expected in a reference state community (Yallop *et al.* 2009).

Other less well known indices produced by Omnidia software were not included in this investigation because of infrequent reference to these in the literature across several continents (USA, Australia, Southern Africa and to a lesser extent Central - Eastern Europe**).** The applicability and transferability of these relatively unknown indices (possibly untested outside the country of origin) was uncertain compared with the more established and frequently quoted suite of indices used in this thesis (Grenier *et al*. 2006, Lavoie *et al*. 2006, 2009).

8.4 Discussion

8.4.1 Limitations

Headwater spatial scales and Heterogeneity within physical environments

A reference site is designed to be representative of the river type for which it fulfils a reference state condition and is therefore normally selected within a spatial framework. Data from several reference sites that are located in the same spatial unit as a potential monitoring site will improve the level of confidence one can place in that reference state. In some instances the reference condition can only represent an approximation of expected natural reference conditions (e.g. Zone 2 Rivers).

A potential limitation to the diatom reference condition approach is the incongruence between diatom responses and the variability of geological and water quality templates across large scale eco-regions. The appropriate scale of operation therefore has been reported by several previous findings elsewhere as showing a low correspondence between larger scale eco-regions and diatom community responses (Weilhoeffer & Pan 2006, Metzeltin *et al*. 2006). Furthermore, large scale eco-regions have been shown to have limited use in the specification and definition of a reference template to be matched against expected reference state communities (Hawkins *et al*. 2000).

The difficulty in finding sites that are consistent with undisturbed habitats has been reported previously in several papers because there are varying perceptions of the degree of refinement required to qualify as an undisturbed state (Eloranta & Soininen 2002). This was particularly true of local Zone 2 rivers in which the headwaters are situated within well developed catchments. Smaller spatial zones for headwaters of rivers, with similar reach level characteristics, as conceived and proposed in this investigation were therefore considered to be more likely to provide much better correspondence with diatom responses. Appropriate refinement of the spatial scale by initial '*a priori*' classification of river systems would therefore diminish the possibility of incurring a Type I error giving a '*false positive*' finding (i.e. identification of impairment when it does <u>not</u> exist), a result which may penalise a water management agency with high costs of remedial actions. Similarly, an appropriate scale classification would lessen the likelihood of incurring a Type II error giving a '*false negative'* finding (i.e. a non-identification of impairment when it does actually exist) – a result which may compromise protection of the biological integrity of an aquatic resource.

Concepts of moderate and poor status are not articulated in the RHP documentation for diatoms. The classification of ecological status for diatoms was based therefore initially on data from countries in which species responses to water quality condition gradients were similar to those in South Africa (Lange Bertalot 1979, Prygiel & Coste 1993, Kwandrans *et al.* 1998, Soininen 2002) and more recently from research on local rivers (Taylor 2004b). The rationale for establishing local ecological status categories was developed further from the Ecological Quality Ratio concept (Wallin *et al.* 2003) and the Biological Condition Gradient (Davies & Jackson 2006) in the absence of defined quantitative classes by the River Health Programme for diatoms (See also Chapter 5). *Poor* and *Moderate* class boundaries were established after setting the lower limit for a '*High Ecological Status'* based on the 25th IPS percentile for high quality headwaters of Zone 3 Rivers (Table 5.2). Likewise the upper boundary for the '*Poor class'* was established from the 25th IPS percentile of contaminated Coastal Lowland Rivers. *"Once the correlation between environments and diatom assemblages is established the predictive power of diatoms alone can be used as monitors of the conditions of river (J.John – pers.comm 2011).*

High variability of water quality variables and limited data sets for headwaters

An appropriate set of water quality measurements was difficult to obtain for this study in relation to the diatom sampling strategy because the sites were located in the undisturbed remote headwaters where monitoring agencies do not operate routine chemical monitoring programmes.

The need to identify 'near natural' sites as potential reference sites dictated that these sites would necessarily and logically be physically located in least impacted reaches of the respective headwaters of each of the representative groups of small, medium and large rivers of KwaZulu-Natal (Chapter 2). This, by definition, restricted the pool of sites to the headwaters of the rivers within the respective spatial zones. A potential limitation was the relatively small number of sites from both historic and present-day series, although the initial scoping of the data included several hundred sites. The scale of the study was such that replication of the headwater communities was obtained from adjacent river systems rather than from several samples from one location. It is undeniable that a more intensive sampling strategy within the defined spatial scale would improve the confidence level (Cassie 1969).

Several issues relating to the key findings serve to underpin the improvement of our understanding of the response of diatoms. Future extensions of this work should focus on regional refinement and calibration of ionic proportions in relation to diatom indicator species. Applicability and reliability at a national scale will only increase with the introduction of more regional data sets covering a full range of environmental gradients and conditions beyond the scope of this investigation.

Sub-regional Reference State conditions

Reference state conditions for rivers of KwaZulu–Natal are applicable and appropriate to these systems and would not necessarily be applicable elsewhere. The focus was on the description of communities expected of a river water typology (alkaline, low nutrient, chemically dilute conditions) rather than that of a more restrictive site specific condition.

Comparison between headwater sites and lower reach sites of a long river that traverses more than two spatial zones is inappropriate. Changes in the geological templates longitudinally downstream in KwaZulu-Natal Rivers (Figure 2, Table 2.1) lead to natural increases in ionic concentration in the waters by increased dissolution of chemicals from rocks (Kemp 1969). Some research has shown that diatom responses may conform to such gradients in alkalinity (Kelly *et a*l. 2008) and ionic composition (Potapova & Charles 2003) for unimpacted streams. These changes in water quality are reflected in different diatom assemblages with increasing ‘distance’ from the reference condition even in the absence of human disturbances. However the possibility of increases in alkalinity and total dissolved solids being derived from sources other than natural geological influences may obscure the natural gradients (Kelly *et al*. 2008).

The reference condition approach adopted in this thesis is premised on **type specific** conditions, as recommended in EU Directives (Wallin *et al.* 2003) and the South African River Health Programme (DWAF 2008) rather than **site specific** conditions. This implies that comparison or assessment of deviations from reference conditions is only valid if the **type specific** conditions are being compared **within the same spatial zones.**

Application and Transferability of Water Quality Indices

Diatom water quality indices are useful measures of the general water quality condition of rivers. The use of the IPS index is recommended in the absence of an improved index (J.Taylor pers.comm. 2009) with an upper threshold value of 15 (high ecological status) and a lower threshold value of 10 (poor ecological status) indicating a need for remedial measures. This is a more pragmatic and sensible approach than delineating subcategories based on narrative descriptions which are difficult to interpret consistently.

The intermediate range of 10-15 would represent sites in need of intensive monitoring. Human disturbances from diffuse sources "*beyond the margins of a stream*" (e.g. *atmospheric and agricultural impacts, urban runoff) and from point source discharges (e.g. industry and sewage effluents) frequently result in disturbance to the near-natural stream dynamics* (Likens 1984). These external influences may disrupt or distort the linkage between the river basin and the responses of stream biota.

Taxonomic Interpretations

Accurate taxonomic interpretation and autecological ratings of species and the direct correspondence between diatoms and the chemical environment of a reference state evokes the very real possibility that the diagnostic power of diatoms can be used effectively for defining historic and present-day near natural conditions in a river (Kociolek & Stoermer 2001). Characterisation of diatom communities from headwater sites, at the **species level**, resulted in more meaningful definition of diatom structural responses and ecological interpretations at this level of diagnostic resolution. Genus level diagnosis was discouraged because the perceived taxonomic challenges of species level identifications are relatively easily overcome with modern Image Analysis software and networking between taxonomists (Round 1991). A compelling motive for resorting to species level diagnosis is the benefit to be derived from the inclusion of a wider range of data of sensitive or pollution tolerant species of the larger commonly occurring taxa such as *Navicula* and *Nitzschia.* Both of these taxonomic entities have the greatest number of species some of which have very different ecological preferences and ratings which may be obscured at genus level.

8.4.2 Future Challenges

Some issues require more intensive research to refine and add to the present findings that were made during this investigation.

- There is considerable scope to improve on the coverage of endemic diatoms still undiscovered in rivers of the protected areas of the Province. The inclusion of these rarer species in a South African based diatom water quality index would be a challenge for future diatomologists. The common and dominant species encountered in historic and present-day samples are well documented in an illustrated guide to common diatom species from South Africa (inclusive of KwaZulu-Natal rivers) as a primary reference manual (Taylor *et al* 2007). The historic data sets were also audited against the available slide material and type specimens contained in the South African Diatom Collection held in Durban.

The characteristic features of very small diatoms or the more subtle divergence within a species, however, is sometimes not easily distinguished even at 1000x magnification. The use of SEM (Scanning Electron Microscope) images to resolve these diagnostic characteristics is not normally routinely available to an ecologist because of high costs. There have also always been differences in taxonomic concepts between scientists despite standard methods for sampling and analytical procedures, leading to an unresolved question as to whether fine-level taxonomic discrimination is really necessary for ecological applications (Kelly 2009).

Taxonomic errors may influence the results produced from Omnidia software in some unusual cases if apparent divergent forms of a species have two very different ecological tolerance ratings and the individuals are 'binned' *inconsistently or incorrectly* (Kociolek & Stoermer 2001). This possibility relates to some known examples which are well documented in the literature (Acs *et al.* 2004, Potapova & Hamilton 2007, Kahlert *et al.* 2007, Ector 2009, Wojtal *et al*. 2011). Taxonomic challenges in the case of two species in South African rivers were resolved in recent joint papers (Taylor *et al.* 2005b, 2010). The results presented in this thesis were not affected by the caveats alluded to above. The *Achnanthidium* 'divergent forms' (Potapova & Hamilton 2007, Ector 2009, Wojtal 2011) are most apparent when waters are mildly polluted and the two forms occur together in different proportions. This was not the case in this study where the dominants in samples were primarily drawn from uncontaminated headwaters. A similar situation is documented for two divergent forms of *Gomphonema (viz. G.parvulum Kützing and G.parvulum var. exilissimum Lange Bertalot & Reichart) (Kahlert et al. 2007)* and the additional variability within the genus of individuals from other South African rivers *(*Passy *et al. 1997)*. However the relative abundance of the divergent forms was also low in all analysed samples.

The species names assigned to the historic data were updated and presented as the accepted modern concepts recorded in recent literature. However, the Omnidia software accommodates older and more recent names as well by internal linkages and cross referencing of coded acronyms and therefore the likelihood of errors of interpretation are considerably reduced. Furthermore a default option to genus level is available in the programme such that average values for a genus are assigned to specimens which cannot be readily identified to species level.

- There is a need for a more focused water quality monitoring programme to produce antecedent water quality data sets prior to sampling a headwater reach. This would markedly improve our understanding of the relationships between diatom responses and the prevailing water quality of a region. This is of particular relevance where the geological template differs between headwaters at a sub-regional level. It would also facilitate application of constrained multivariate procedures (e.g. Canonical Correspondence Analysis) to define the environmental factors that may further explain the distribution and responses of these diatom communities.

■ Measurement of spatial and temporal heterogeneity of river systems using diatom responses is central to the proper classification of reference sites and conditions. A key question relates to improved understanding of the extent of temporal and spatial variability of these responses at the habitat scale within the different sub-regions. Improvement of the associated diatom assemblage metrics will lead to refinement of the criteria for more accurate classification of a diatom reference condition.

8.5 Conclusions

► The emergence of potential reference state sites from the diatom species composition gradients illustrates the utility of bio-indication derived from present-day and historical diatom data. The fundamental concept of the river reference state is based on the "*naturalness of diatom communities from undisturbed lotic systems*" engendering a high ecological status of headwater sites of the study area. The ecological status was shown to change with downstream gradients of human impacts as the structure of the diatom assemblages respond and deviate from a high ecological status reference state community of the headwaters.

► A group of sites within different sub-regions, sufficiently similar in composition and with a high Water Quality Index rating (> 15), was identified objectively and these represent a benchmark to judge the differences between the 'expected and observed' states within different categories of river.

► Natural variation was assessed by extensive sampling of reference sites with low levels of human disturbance in regions where such sites still exist in KwaZulu-Natal. Other approaches are required in sub-regions where near natural conditions are not evident.

Diatom metrics change with increasing and different human impact pressures but the direction of change may not always be regarded as an obvious signal of human disturbance.

► Diatom assemblages are crucial structural biological quality elements that give 'response signatures' at sites 'free of human disturbance' distinct from those of impacted sites – a key operating principle in the execution of a bio-monitoring programme.

- Reference sites that fulfil abiotic and biotic criteria should therefore surely be protected heritage areas as refugia for reference diatom communities representative of uncontaminated headwaters. This is aligned with a goal of the National Spatial Biodiversity Assessment of rivers dealing with the conservation of headwaters of tributaries of impacted rivers being retained and protected as refuges to maintain biodiversity. The diatom data produced from historic and present day findings will provide an additional resource as a benchmark for future NSBA assessments of the status of KwaZulu-Natal Rivers – a key water resource for South Africa.
- Restrospective analysis of historic data was found to provide valuable information of the pre-existing river conditions captured and retained by the diatom assemblages. This enabled valid conclusions about the existence of historic high ecological status reference communities commensurate with a reference state.

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Appendix I

SITE REFERENCES

Zone 3: **Montane Upland Sites**

Zone 2: **Interior Midland Sites**

Zone 1: **Coastal Lowland Sites**

A1. Site codes and Reach co-ordinates of Zone 3 River headwaters originating in the Montane Uplands

River System	Tributaries	Site Codes: Historic Cholnoky (1960b) (Resource A1-A3)	Site Codes: Present-day 2006-2009 (Resource A4)	River Reaches: Latitude	River Reaches: Longitude
Pongola	Headwaters		PON1A	*-27.400S*	*30.284E*
Mkuze	Headwaters		MKU1,Z3Mk1	-27.731S	*31.095E*
Mfolozi	Black		BMF01	*-27.804S*	*31.050E*
	White	389,390	WMF1	*-27.709S*	*30.636E*
Thukela (Source)	MontAuxSources	MAX 1,3, 5,6	TMX 482,487,492	*-28.764S*	*28.884E*
Thukela (Gorge)	Headwaters	TUG 1, 4-6	THUK1, Z3THK2	*-28.743S*	*28.918E*
Thukela (Upper)		Z3RN1,Z3TG81,35		*-28.733S*	*28.921E*
		TUG2,6		*-28.712S*	*28.932E*
	Mehai	Z3Me48,50,52	Meh1		
	Golide	Z3Fg1,2.6		*-28.661S*	*28.935E*
	Gudu	Z3Gu1		*-28.673S*	*28.922E*
	Venvaan	Z3Vv1,Vv2	vev1	*-28.711S*	*28.920E*
	DevilsHoek		dhk1	*-28.711S*	*28.921E*
	Inn	IGE1,3		*-28.671S*	*28.953E*
	Mlambonjwa	ML111,112,306, 307	Z3MJ1,mlj1,	*- 28.949S*	*29.185E*
	Oqualeni		Z3OQ1,oqu1	*- 28.951S*	*29.185E*
	Nhlonhlolo	308		*- 28.949S*	*29.207E*
	Thlwazini	LC 302,304	thl1	*- 28.931S*	*29.281E*
	Sterkspruit	318,323	stk1,stk2	*- 28.051S*	*29.388E*
	Bushmans	BU385,386,387		*- 29.269S*	*29.520E*
		GC330,333,337,338		*- 29.265S*	*29.518E*
Mvoti			Z3MV1	*- 29.186S*	*30.352E*
Mkomazi	Headwaters	348,351	MKO1	*-29.537S*	*29.444E*
	Umkomazana	349		*-29.589S*	*29.295E*
	Polela	354		*-29.703S*	*29.417E*
	Amanzimnyama	LO345,346		*-29.278S*	
	Inzinga	347		*-29.470S*	*29.699E*
	Loteni	LO352,346,354	Z3LO1,lot1	*-29.436S*	*29.517E*
Mgeni			Z3MGE 08,09	*-29.506S*	*29.903E*
Mzimkulu	Main headwaters			*-29.736S*	*29.188E*
	Ingwagwane			*-29.884S*	*29.279E*

Site Codes or Site Numbers for historic sites as given in Cholnoky (1956, 1957, 1960)
Latitude and Longitude are given in decimal format
Co-ordinates are given to 3 decimal place accuracy

B1. Site codes and Reach co-ordinates for Zone 2 headwaters originating in the Interior Midlands

		Site Codes		River Reaches	
River System	Tributaries	Historic	Present-Day	Latitude	Longitude
		Cholnoky (1960b)	2006-2009		
		Resource B2	Resource B3		
Thukela	Mooi (Inyamvubu)	Z2NY1		-29.180S	30.338E
	Jameson's Drift Stream	Z2JA2		-28.762S	30.893E
Amatikulu				-29.783S	30.170E
Mvoti	Mbalana	Z2MB1		-29.291S	30.686E
	Mvozana	Z2MV1		-29.063S	30.689E
Tongati				-29.421S	30.898E
Mgeni	Nagle stream	Z2UM1	Mge1	-29.587S	30.622E
	Karkloof		Kar1	-29.301S	30.050E
Mlazi			Mlz1	-29.761S	30.205E
Lovu			Lov1	-29.783S	30.170E
Mpambinyoni			Mpa	-30.235S	30.221E
Ifafa			Ifa1	-30.309S	30.336E
Mtwalume			Mtw1	-30.320S	30.209E
Mzumbe			Mbe1	-30.240S	30.146E
Mzimkulu	Ixopo	Z2MK2	Ixo1	-30.165S	30.093E

Latitude and Longitude are given in decimal format
Co-ordinates are given to 3 decimal place accuracy

C1. Site Codes and Reach co-ordinates of Zone 1 headwaters originating in the Coastal Lowlands

River System	Tributaries	Site codes: Historic	Site codes: Present-Day	River Reaches: Latitude	River Reaches: Longitude
		Cholnoky (1960b, 1968b)			
		(Resource C1,C2)	(Resource C3)		
Pongola	Magudu	MA295		*- 27.423S*	*31.628E*
Black Mfolozi	Bekamuzi stream	BM289		*- 28.195S*	*31.421E*
St Lucia	Mtutuba Stream	SL221		*- 28.369S*	*32.275E*
Mhlatuzi	Eshowe stream	MH230		*- 28.690S*	*31.486E*
Mgeni	Drummond stream	Z1DR255		*- 29.752S*	*30.711E*
	Nagle stream (1)	Z1UG271	nga217	*- 29.580S*	*30.688E*
	Nagle stream (2)	NA274		*- 29.682S*	*30.638E*
	Molweni		Mol89	*- 29.791S*	*30.790E*
	Mzinyathi		Mzi31	*- 29.640S*	*30.895E*
Nonoti		Non1		*- 29.215S*	*31'225E*
Ohlanga				*- 29.677S*	*30'946E*
Mbilo			MBI124	*- 29.794S*	*30'829E*
Mhlatuzana			MHL19,154	*- 29.756S*	*30'728E*
Mbokodweni			Mbk177	*- 29.876S*	*30'560E*
Amanzimtoti			TOT122	*- 30.013S*	*30'784E*
Hlongwane			HLO10	*- 30.207S*	*30'931E*
Mzinto				- 30.264S	30'516E
Mzimkulu	Mzimkulwana	MZ363		- 30.707S	30'270E
	Harding Stream	HA358		- 30.380S	29'931E

Latitude and Longitude are given in decimal format

Co-ordinates are given to 3 decimal place accuracy

Appendix II

DIATOM ASSEMBLAGE ATTRIBUTES AND METRICS OF HEADWATER SITES

A. Zone 3: Montane Upland Sites

B. Zone 2: Interior Midland Sites

C. Zone 1: Coastal Lowland Sites

A1. Diatom metrics derived from present-day diatom assemblage data of sites in headwaters from Zone 3 rivers originating in the Montane Uplands of KwaZulu-Natal

Location	Mkomazi			Mgeni	Thukela Southwest headwaters				Thukela (Source Zone headwaters)					Thukela East (Headwaters)			Mvoti	Mfolozi		Mkuze	Pongola
River	Loteni	Mkomazi	Mkomozana	Mgeni	Mlambonjwa	Oqualeni	Thlwazini	Sterkspruit	Main channel			Vevaan	Mehai	Ngagane	Horn	Ngogo		White	Black		
Site code	**LOT1**	**MKO1**	**MKZ1**	**MGE09**	**MLJ1**	**OQU1**	**TLZ1**	**STK1**	**TMX482**	**TMX487**	**THUK1**	**VEV1**	**MEH1**	**NGA1**	**HOR3**	**NGO1**	**MVO1**	**WMF1**	**BMF01**	**MKU1**	**PON1A**
Date	20/07/2008	17/06/2006	19/06/2006	6/06/2009	30/08/2006	30/08/2006	29/08/2006		14/06/2008	14/06/2008	28/05/2008	28/05/2008	13/05/2008	21/06/2007	21/06/2007	20/06/2007	26/07/2008	19/06/2007	19/06/2007	19/06/2007	5/06/2009
Assemblage Attributes																					
Species Richness	16	25	13	15	13	13	11	19	9	20	13	14	10	30	39	35	21	16	35	26	30
No. of dominants	3	4	5	3	3	3	2	4	3	2	4	4	3	2	3	3	3	3	7	5	5
Aggregate % dominants	77.7	70.1	88.4	77.8	91.1	90.4	92.5	91.5	96.9	70.3	90.2	69.3	93.9	81.1	57.9	61.5	83.7	86.7	49.9	78.1	76.4
Diversity Index																					
Shannon H'	1.44	2.36	1.62	1.78	1.26	1.37	0.93	1.70	1.11	1.67	1.49	1.62	1.01	1.50	2.48	2.29	1.55	1.18	3.10	2.25	2.39
Simpson's D	2.35	7.23	3.83	4.16	2.35	2.90	1.95	4.41	2.55	2.93	3.16	3.74	1.95	2.82	5.63	4.45	3.08	2.31	19.05	6.20	7.70
Margalef	2.43	3.95	1.43	2.26	1.97	1.95	1.47	2.47	1.30	3.14	1.81	1.94	1.46	4.49	6.32	5.65	2.79	1.98	5.30	4.17	4.71
Berger Parker	0.64	0.24	0.39	0.38	0.62	0.48	0.67	0.34	0.55	0.55	0.41	0.42	0.69	0.46	0.38	0.45	0.45	0.61	0.10	0.32	0.21
Evenness Index																					
Pielou's J	0.52	0.73	0.70	0.66	0.49	0.53	0.40	0.61	0.51	0.56	0.60	0.63	0.44	0.44	0.68	0.64	0.53	0.46	0.89	0.69	0.70
Simpson's E	0.15	0.29	0.38	0.28	0.18	0.22	0.19	0.28	0.28	0.15	0.26	0.29	0.20	0.10	0.14	0.13	0.17	0.18	0.58	0.24	0.26
Water Quality Index																					
IPS	15.1	16.9	19.5	18.3	19.6	19.7	16.7	18.8	19.4	19.3	18.7	17.8	19.8	18.2	12.2	13.3	19.2	19.3	11.5	17.2	17.7
BDI	13.5	15.1	16.0	17.0	17.5	17.7	14.2	16.6	17.2	17.8	15.3	14.5	17.7	15.2	12.9	13.2	17.7	17.2	12.1	18.0	16.5
GDI	13.7	15.7	16.4	17.1	17.4	17.1	14.4	16.1	18.9	16.8	16.4	15.4	17.5	16.1	12.5	12.7	17.1	17.6	10.7	15.9	14.3
Nutrient Index																					
EPI-D	15.3	14.7	16.3	17.1	16.9	17.1	14.6	15.0	16.8	17.0	15.1	15.2	16.6	15.6	10.1	7.0	15.9	16.8	10.0	15.8	15.7
TDI	47.2	43.3	38.1	27.1	35.6	19.6	57.5	46.4	25.1	25.7	48.9	51.6	31.3	40.2	61.1	69.9	28.2	28.5	66.6	23.8	34.9
%PTV	0.2	3.2	0.4	0.1	0.1	0.1	0.2	0.9	0.1	0.1	0.1	0.1	0.1	0.4	9.3	51.1	2.7	1.2	31.2	9.6	0.9

Resource : A4 (2006-2009)

TDI Scale (0 - 100) Low ▸ High nutrient contamination

%PTV Scale (0 - 100) Low ▸ High Proportion of valves tolerant of organic pollution

IPS,BDI,GDI, EPI-D Scale: (0 - 20) = Poor ▸ High general water quality

A2. Diatom metrics derived from historic diatom assemblage data of sites in headwaters of Zone 3 rivers originating in the Montane Uplands of KwaZulu-Natal

Site Code	LOT346	LOT347	MKZ349	MKO351	LOT352	LBU329	BUS330	BUS333	BUS337	BUS338	LIN114	EMM302	LOS324	LOS325	STK116	STK321	STK323	MAT111	MAT112	NHL309	TZW304	BUF395	BUF388	BUF386	MFZ390
Location	MKOMAZI River catchments					THUKELA-South catchments : Bushmans River					THUKELA - West catchments : Sterkspruit - Little Tugela River							THUKELA - S West catchments - Mlambonjwa River				THUKELA- East catchments - Buffalo			MFOLOZI
River	Amanzimnyama	Inzinga	Mkomozana	MKOMAZI	Loteni	Little Bushmans	Bushmans				Lindeque	Emmaus	Loskop	Loskop	Sterkspruit			Matsugwana		Nhlonthlo	Thlwazini	Buffalo		Rorkes	White Mfolozi
Survey Date	18/07/1958	18/07/1958	19/07/1958	19/07/1958	19/07/1958	17/07/1958	17/07/1958	17/07/1958	17/07/1958	17/07/1958	2/07/1956	14/07/1958	16/07/1958	16/07/1958	4/07/1956	16/07/1958	16/07/1958	2/07/1956	2/07/1956	14/07/1958	14/07/1958	25/07/1958	24/07/1958	24/07/1958	25/07/1958
Assemblage Attributes																									
Species No.	13	13	14	26	25	12	30	35	35	25	26	16	20	27	31	27	34	11	12	32	32	19	23	23	29
No. of dominants	2	2	3	2	3	2	4	5	8	8	3	5	3	3	2	2	5	1	1	4	5	3	5	3	2
Aggregate % dominance	87.0	80.4	94.0	78.5	80.8	88.0	70.0	53.3	69.4	82.9	84.2	95.9	87.6	75.1	71.6	72.9	59.3	91.5	88.7	69.7	68.0	82.3	88.1	83.1	60.1
Diversity Index																									
Shannon H'	0.83	1.11	1.18	1.51	1.56	1.07	2.36	2.94	2.91	2.55	1.52	1.19	1.43	1.91	1.68	1.51	2.77	0.47	0.56	2.19	2.39	1.76	1.90	1.57	2.03
Simpson D	1.49	1.78	2.52	2.37	2.41	1.99	6.44	14.13	13.37	9.67	2.64	2.00	2.70	3.62	2.43	2.14	10.27	1.20	1.27	4.00	5.26	4.14	4.26	2.68	3.37
Margalef	2.02	2.03	2.19	4.22	4.11	1.85	4.92	6.17	6.33	4.32	4.12	2.49	3.16	4.45	4.95	4.30	5.74	1.66	1.81	5.20	5.23	2.99	3.72	3.81	4.65
Berger Parker	0.82	0.75	0.49	0.63	0.63	0.68	0.31	0.14	0.18	0.21	0.57	0.69	0.55	0.48	0.63	0.68	0.23	0.91	0.89	0.48	0.41	0.37	0.42	0.58	0.53
Evenness Index																									
Pielou J	0.32	0.43	0.45	0.46	0.49	0.43	0.69	0.83	0.82	0.79	0.47	0.43	0.48	0.58	0.49	0.46	0.79	0.20	0.23	0.63	0.69	0.60	0.60	0.50	0.60
Simpson E	0.11	0.14	0.18	0.09	0.10	0.17	0.21	0.40	0.38	0.39	0.10	0.13	0.14	0.13	0.08	0.08	0.30	0.11	0.11	0.13	0.16	0.22	0.19	0.12	0.12
Water Quality Index																									
IPS	17.7	17.9	18.5	17.3	16.5	13.5	14.7	15.0	14.3	16.5	14.4	15.3	16.5	17.2	16.0	17.1	12.4	18.5	17.9	17.0	14.1	14.9	15.1	16.0	13.3
BDI	17.2	17.5	17.5	17.1	17.4	14.3	15.8	14.3	13.8	15.2	17.3	16.4	16.8	15.3	16.9	16.5	12.8	17.6	17.5	17.1	16.3	14.1	13.9	13.6	15.8
GDI	17.0	16.6	16.5	16.6	16.5	16.5	14.5	11.9	12.2	12.1	13.1	15.8	17.1	16.9	15.0	15.3	12.2	16.8	16.9	15.3	14.1	16.6	16.7	17.7	14.4
Nutrient Index																									
EPI-D	17.3	17.1	17.9	16.7	16.9	17.3	14.3	12.0	10.9	13.1	17.1	16.1	17.2	15.2	15.0	16.6	13.6	17.5	17.4	15.1	14.8	15.9	16.4	15.8	14.8
TDI	27.9	30.4	27.6	31.0	31.6	43.5	43.5	43.3	52.3	55.2	36.5	39.4	28.5	31.1	36.2	32.4	68.8	27.7	26.8	39.3	45.1	17.8	13.0	11.1	37.4
%PTV	0.3	3.5	1.3	3.7	3.2	0.1	8.2	26.6	29.8	10.5	1.6	12.0	4.4	5.2	14.1	6.6	33.0	2.5	0.7	22.1	12.5	6.3	4.0	0.9	10.5

[Resource A3 : (Cholnoky 1960b)]

TDI Scale (0 - 100) Low ▸ High nutrient contamination

%PTV Scale (0 - 100) Low ▸ High Proportion of valves tolerant of organic pollution

IPS,BDI,GDI, EPI-D Scale: (0 - 20) = Poor ▸ High general water quality

B. Diatom metrics derived from historic diatom assemblage data in river sites for Zone 2 rivers originating in the Interior Midlands of KwaZulu-Natal

Location	MZIMKULU	MKOMAZI		THUKELA										MVOTI				
River ▶	Machlateen	Ixopo	Umzimhlanga	Ceciliaspruit	Muden	Jameson	Mpanza	Greytown	Mpanza	Fabeni	Kwiti	Czwaka	Sampofu	Inyamvubu	Umvozana	Rooispruit	Mbalana	Goedgegun
Code ▶	**MAC2**	**MKO2**	**IXO1**	**CEC1**	**MUD1**	**JAM2**	**MPA1**	**MPA2**	**MPA3**	**FAB1**	**KWI1**	**CZW1**	**SAM1**	**INY1**	**UMV1**	**MVO2**	**MBA1**	**PON3**
Date ▶	5/07/1956	5/07/1956	21/07/1958	4/07/1956	4/07/1956	21/07/1957	21/07/1957	21/07/1957	24/07/1956	21/07/1957	21/07/1957	24/07/1958	24/07/1958	26/07/1957	21/07/1957	26/07/1957	26/07/1957	28/07/1957
Assemblage Attributes																		
Species Richness	32	29	34	17	20	30	19	30	34	33	16	16	13	17	33	34	21	32
Dominants	5	3	5	2	1	3	4	5	6	5	4	4	3	4	4	4	3	6
Aggregate % dominance	59.3	83.0	47.9	83.5	81.2	66.2	93.4	75.1	71.2	66.1	88.1	91.5	91.3	85.3	51.4	65.6	79.7	66.8
Diversity Index																		
Shannon H'	2.72	1.60	3.02	1.19	0.90	2.18	1.57	2.41	2.66	2.63	1.56	1.23	1.39	1.40	2.84	2.53	1.62	2.65
Simpson's D	9.12	2.61	16.04	1.83	1.49	4.54	3.64	7.33	9.88	9.44	2.94	2.03	3.07	2.31	10.10	7.29	2.76	8.15
Margalef	5.18	4.48	6.05	2.60	3.09	5.00	3.00	4.91	5.56	5.52	2.51	2.50	2.04	2.72	5.48	5.64	3.31	5.56
BergerParker	0.26	0.60	0.13	0.74	0.82	0.43	0.36	0.22	0.20	0.22	0.54	0.69	0.40	0.64	0.25	0.26	0.58	0.30
Evenness Index																		
Pielou's J	0.79	0.48	0.86	0.42	0.30	0.64	0.53	0.71	0.75	0.75	0.56	0.44	0.54	0.49	0.81	0.72	0.53	0.76
Simpson' E	0.29	0.09	0.47	0.11	0.07	0.15	0.19	0.24	0.29	0.29	0.18	0.13	0.24	0.14	0.31	0.21	0.13	0.25
Water Quality Index																		
SPI	11.7	14.7	10.3	18.6	18.9	14.5	11.7	7.4	11	11.3	14.2	7.4	14.7	16.1	14.1	11.7	14.7	15.8
BDI	12.2	15.0	13.5	17.2	15.5	16.3	13.7	8.3	12.6	13.1	12.1	16.7	13.0	17.0	15.2	13.6	16.4	16.7
GDI	10.0	14.0	8.1	16.0	13.4	14.1	15.5	10.6	11.7	11.8	16.9	12.7	18.0	15.9	12.9	11.7	14.0	15.6
Nutrient Index																		
EPI-D	13.7	16.9	10.6	16.1	18.2	14.9	15.4	9.0	13.9	12.8	15.1	17.0	16.3	16.7	13.2	10.0	16.8	15.8
TDI	55.2	34.9	63.1	28.3	3.2	42.5	62.8	56.2	53.7	42.8	24.1	76.5	12.9	35.3	50.7	66.4	30.3	33.7
%PTV	24.6	14.9	27.8	8.1	4.4	11.2	37.3	19.5	11.1	16.6	3.3	0.2	1.7	0.8	15.2	11.7	2.2	1.1

[Resource B2: Cholnoky 1960b]

TDI Scale (0 - 100) Low ▶ High nutrient contamination

%PTV Scale (0 - 100) Low ▶ High Proportion of valves tolerant of organic pollution

IPS,BDI,GDI, EPI-D Scale: (0 - 20) = Poor ▶ High general water quality

C1. Diatom metrics derived from present-day diatom assemblage data of sites in headwaters of Zone 1 rivers originating in the Coastal Lowlands of KwaZulu-Natal

Location	Rural			Urban Residential			Urban Central				Rural				
River	Hlongwane	Ngane	Umgababa	L.Amanzimoti	Amanzimtoti	Mbokodweni	Mhlatuzana			Mbilo	Molweni		Mshazi	Nkutu	Mzinyati
Site Code	**HLO10**	**NGA216**	**BAB219**	**LTO183**	**TOT122**	**MBK177**	**MHL149**	**MHL19**	**MHL54**	**MBI124**	**MOL26**	**MOL89**	**MSH102**	**NKU101**	**MZI31**
Date	20/06/2007	4/05/2009	15/05/2009	15/05/2009	8/05/2009	25/052009	26/05/2009	27/05/2009	10/03/2006	26/06/2009	20/06/2007	31/07/2009	10/08/2009	14/08/2009	20/06/2007
Assemblage Attributes															
Species Richness	23	24	22	23	17	20	10	35	13	27	32	20	23	30	36
No of dominants	4	3	5	4	2	3	3	4	2	2	7	2	2	3	5
Aggregate % of dominants	83.4	68.7	82.6	77.4	91.7	87.0	90.0	78.7	93.2	76.1	77.9	78.7	69.9	72.5	60.7
Diversity Index															
Shannon H'	1.91	1.90	2.07	1.77	0.80	1.18	1.29	2.07	0.59	1.47	2.52	1.41	1.44	1.92	2.71
Simpson's D	4.24	3.10	5.29	2.81	1.46	1.82	2.61	4.21	1.31	2.06	8.69	2.26	2.32	3.32	8.81
Margalef	3.66	4.12	3.48	3.67	2.66	3.09	1.47	5.55	2.00	4.27	5.18	3.09	3.60	4.78	5.91
Berger Parker	0.39	0.55	0.31	0.58	0.82	0.74	0.57	0.44	0.87	0.69	0.21	0.65	0.63	0.52	0.27
Evenness Index															
Pielou's J	0.61	0.60	0.67	0.57	0.28	0.39	0.56	0.58	0.23	0.45	0.73	0.47	0.46	0.56	0.76
Simpson's E	0.18	0.13	0.24	0.12	0.09	0.09	0.26	0.12	0.10	0.08	0.27	0.11	0.10	0.11	0.24
Water Quality Index															
IPS	13.1	14.0	15.3	11.9	14	14.1	13.4	15.3	19.1	15.6	17.2	14.0	17.8	14.9	17.4
BDI	11	12.7	12.6	10.5	11.3	11.5	16.8	12.9	20.0	12.4	15.7	12.1	16.5	11.1	14.2
GDI	12	12.4	14.0	12.5	12.8	12.6	16.0	15.7	13.5	16.2	14.8	13.0	16.1	15.7	14.6
Nutrient Index															
EPI-D	12.4	15.8	15.6	10.8	14.7	13.8	19.4	17.8	18.8	18.8	16.7	12.0	16.5	18.0	13.4
TDI	58.3	55.1	38.1	57.9	51.3	51.2	9.1	35.5	96.2	14.2	40.1	49.5	32.9	32.8	44.4
%PTV	15.1	8.7	0.1	31.1	2.2	3.7	5.3	7.0	0.5	5.2	18.2	8.2	4.0	6.8	14.3

Resource : C3 (2006-2009)

TDI Scale (0 - 100) Low ▶ High nutrient contamination

%PTV Scale (0 - 100) Low ▶ High Proportion of valves tolerant of organic pollution

IPS,BDI,GDI, EPI-D Scale: (0 - 20) = Poor ▶ High general water quality

Appendix III

THE NEAR-NATURAL REFERENCE CONDITIONS OF HEADWATERS OF KWAZULU-NATAL RIVERS

a. **Land-use**

b. **Vegetation**

c. **Water quality**

Historical context

A context was created for the interpretation of reference conditions from a broad based development trajectory for the Province of KwaZulu-Natal. The post World War II period (1950-1955) was taken as a threshold historic period prior to significant development in KwaZulu-Natal. It is based partially on narrative and quantitative evidence confirming that the historic period was considered free of human disturbance in the headwaters of KwaZulu-Natal Rivers when the original diatom data was generated (Cholnoky, 1956, 1957, 1960).

Preamble on the present condition of South African Rivers

"The status of the main rivers in South Africa was based on the extent to which each ecosystem had been altered from its natural condition The state of main river ecosystems in South Africa is dire. The data shows that 84% of the ecosystems are threatened, with a disturbing 54% critically endangered, 18% endangered, and 12% vulnerable (Nel *et al.* 2007). Only 50% of rivers within protected areas are intact providing some of the first quantitative data on the positive role protected areas can play in conserving river ecosystems. Main river ecosystems are in a much worse state than terrestrial ecosystems. Ecosystem status is likely to differ with the inclusion of tributaries, since options may well exist for conserving critically endangered ecosystems in **undisturbed headwater tributaries**, which are generally less regulated than main rivers.

"The NSBA river component study highlights the importance of healthy tributaries for achieving river conservation targets, and the need for managing main rivers as conduits across the landscape to support ecological processes that depend on connectivity. A paradigm shift is required in the way protected areas are designated, and integrated river basin management plans are required to meet targets, and strategies for conservation of freshwater ecosystems and their ecological services." (Nel *et al.* 2007)

The identification of diatom assemblages, representative of reference conditions in the protected headwater tributaries of the largest rivers of KwaZulu-Natal Province is therefore more than just an academic exercise. It has practical benefits as an input to the assessment of biodiversity of South African rivers. Diatom diversity is important for supporting biological functions in river ecosystems. Accurate characterisation of diatom assemblages and species composition is useful for assessing biological condition and diagnosing stressors in aquatic ecosystems (Stevenson *et al.* 2008).

a. Land-use

The distribution and density of the population in the Province of KwaZulu-Natal is strongly governed by a variety of physical and human factors which influence settlement and land-use patterns. The physiography provides a division into natural topographic units, an appreciation of which is necessary to the understanding of the population distributions. The Drakensberg is a mountainous borderland with a rigorous climate and topographical irregularities which make it a thinly populated area and much of it is completely uninhabited (Thorington-Smith 1952).

b. Vegetation (See also examples of disturbance free headwaters in colour plates below)

Historical picture

"*The effects of man on the vegetation were probably small, since few Bushman roamed the country randomly in small clans and built no permanent habitations, living on various plants and hunting. Overgrazing effects of herbivores (elephant, buffalo, zebra) were transitory and minimal (Barter 1852). Extracts as quoted (Edwards 1967).*

"The headwaters of the mountain region remain free of depletion of soil and vegetation resources. Because of the major contribution to the water resources of the Thukela River basin, conservation of the Mountain Region is essential and with its spectacular scenery it serves as an unspoiled area. The most appropriate form of land use for the High Drakensberg covering 7% of the headwater areas is conservation. (Edwards 1967).

The Mountain Veld is situated in the higher altitudes with shorter denser grasses. This area is of the utmost importance to the country as a whole. The sources of large rivers all fall within Ecoregion No 15 and this vegetation type must be managed to ensure that a permanent supply of water to these rivers is assured. It was considered to be of little value for farming because of the steep slopes and poor grazing capacity *(Thorington-Smith 1952).*

Subalpine region

A description of the prevailing vegetation of the headwaters of major rivers in the 1950's (referred to as the historic condition) is given by Oliff (1960). In common with many other swift flowing rivers in Africa the flora associated with the river channel is relatively poor. This is ascribed to the high rate of flow, and the seasonal fluctuation of water levels. "*In the higher source zone (Mont-aux-Sources) the streams are fringed by stream bank grasses which trail in the water but there is no submerged aquatic vegetation*".

"*In the foothill torrent zone of the headwaters, the in-stream vegetation is composed of Cyperus marginatus*, *Pennisetum natalensis*, and *Ornithogolum zeyheri where the water flows rapidly, together with riparian grasses such as Hyperhaenia glauca and H.hirta.*

Occasionally patches of Phragmites communis occur where the water flows slowly.

c. Water quality

The most comprehensive of the earliest records of water quality were given by Oliff (1960) drawing on the research of the chemical constitution of these rivers (Caplan et al. 1952).

*"Dissolved solids in the rivers were comparatively low at all times. Bicarbonates of calcium and magnesium formed the bulk of the material, with lesser amounts of sulphates, chlorides and sodium salts" (*Oliff 1960*). These conditions reoccur from year to year* ([#]Caplan *et al*. 1952).

The dilute chemistries of the headwaters was confirmed by the low concentrations of several constituents, for example, nitrates ranged between 0.01-0.04mg$NO_3$$\ell^{-1}$ and phosphates ranged between 0.007-0.020mgPℓ^{-1}while alkalinity ranged from 30 – 35mg$CaCO_3$ ℓ^{-1}.

[Note*:* [#]Several efforts were made to locate the Caplan reports from the CSIR Library Services but these were all unsuccessful.]

[A] **Peripheral vegetation in disturbance-free sub-catchments of different near-natural headwater reaches of major rivers of KwaZulu-Natal**.