

# **An analysis of diatoms as indicators of water quality in rivers of the Western Cape**

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OF

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**Oliver Slingers**

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## Abstract

In South Africa the systematic collection of water samples from surface waters and the collation of results is the responsibility of the Department of Water and Sanitation, previously the Department of Water Affairs and Forestry. Local authorities and private contractors are also responsible for conducting water quality tests for reporting purposes. In most cases, samples are collected by hand at predetermined sites and intervals across the country, and are tested for a standard set of parameters that covers various physical, chemical and bacteriological water quality measures. This approach and method of monitoring is time consuming, expensive and requires a high level of skills and capacity to achieve a representative and reliable sample. There is no immediate substitute, although there is a resurgence of interest in the use of bio-indicators in monitoring rivers. In this study, diatoms are investigated as an indicator of water quality because of the relative abundance of species and the ease with which they can be collected in the field, and the establishment of taxonomies of species that are aligned to pollution levels. There is limited knowledge about diatoms species found in lotic conditions, and in seasonal conditions in a Mediterranean climate where the surface water is slightly acidic. This study investigates the use diatoms in lotic waters of the Western Cape and compares this information to surface water quality measurements in the upper and middle course of the Berg River. The study commences by establishing baseline information of diatoms species for the upper to middle section of the Berg River which coincides with sites where surface water samples are regularly collected by the Department of Water and Sanitation. The study was conducted over a 12 month period in order to analysis the response of species to seasonal change and in relation to changing conditions at various point sources of where there is a known discharge into the Berg River. Species and population will be compared to the typological classification of diatom species and in relation to surface water quality. The study seeks to understand how diatoms respond to acidic waters of the Berg River; to measure the response of diatoms to changes in the water quality along the length of the river; and to determine the response of diatom species to point sources discharging water of varying quality into the river.

## Findings

Pollution tolerant genera like *Nitzschia* and *Navicula* show little seasonality along the Berg River and occurred in abundance through all seasons. *Nitzschia umbonata* (Ehrenberg) Lange-Bertalot contributed the highest number of taxon to the study. Winter showed the greatest variability amongst the study sites with the greatest diatom species range, while summer showed on average the highest concentration of pollution tolerant species. Winter and autumn had large values of *Gomphonema venusta* Passy, Kociolek & Lowe, a species associated with agricultural pollution. Species like *Craticula halophila* (Grunow) DG Mann, *Surirella splendida* (Ehrenberg) Kützing, and *Achnanthes oblongella* Østrup, show strong seasonality each preferring a different season. The diatom results vary by site but on average the Berg River gets more polluted as it enters the town (Paarl) and improves slightly as the river leaves it. A diatom baseline for Franschoek to Mbekweni was established totaling 148 different taxa.

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## Chapter 1

Some governments, particularly in the EU, have recognised the problem of poor water quality in some rivers, and are making an effort to monitor the problem using initiatives such as the Water Framework Directive (WFD) to guide their actions (Vorkamp et al., 2004; Sebastia et al., 2013 ; Ingleton & McMinn, 2012; Chave 2002). Attempts at quantifying the water condition, using physical monitoring techniques are successful in dealing with single parameters, but can only provide a fragmented overview of the state of the river (Taylor et al., 2007). The WFD, introduces biomonitoring as a tool for river assessment resulting in an improved understanding of the relationship between water quality, species and habitat (Vorkamp et al., 2004; Sebastia et al., 2013 ; Ingleton & McMinn, 2012; Chave 2002; Tornes et al., 2007)

These initiatives are an indication of the shift that is underway in finding new ways to monitor and restore polluted river systems (González et al., 2013; Everard & Moggridge, 2011; Bernhardt & Palmer, 2007; Giller, 2005; Walsh et al., 2005). River monitoring and ultimately restoration is a costly affair. The WFD aims, among others, to find effective ways of monitoring biological variables that affect water quality in freshwater systems. More specifically, researchers are turning to work that shows the potential for diatoms and other biotic flora to act as an indicator of water quality (Rimet, 2012; Alakananda et al., 2011; Smucker & Vis, 2011; Karthick et al., 2010; Taylor et al., 2007; Taylor et al., 2007 ; Taylor et al., 2005). Thus far, only a small amount of work has been done to strengthen the case for bio-monitoring techniques (Karthick et al., 2009; Karthick et al., 2010).

Attention is being given to the use of diatoms as monitoring organisms. Researchers are claiming that diatoms are stable indicators of water quality. Diatoms are ubiquitous (Sebastiá et al., 2013) and have been shown to adapt to changing water quality conditions (Taylor et al., 2007; Taylor et al., 2005), however it remains unclear how diatom species respond to urban river systems that are in close proximity to poor water quality resulting from urban runoff. The prevalence of pharmaceuticals, pesticides, chemicals and other urban pollutants within these river systems adds to the complicated nature of the system and resulting conditions in the receiving waters.

### **Project aims and potential outcomes**

The aim of this project is to establish the potential of diatoms as a reliable indicator of water quality along the Berg River in an urbanized region of Paarl, South Africa and in a seasonally fluctuating climatic zone.

The study examines the use of diatoms to determine seasonal changes in water quality conditions; to identify potential areas of concern within the Berg River; and to produce a reference of diatom species found with the Berg River.

These broad aims will be achieved by meeting the following objectives:

- An investigation of the potential and effectiveness of diatoms as a biological indicator of water quality in a riverine environment in the Western Cape
- A comparison of species community composition with surface water quality measurements
- An identification of species community composition in relation to point sources of discharge.

### **Rationale**

The aim of the study was to test the use of diatom species as an indicator of water quality in a slightly acidic river within the Western Cape. Diatoms are one of many kinds of bio-monitoring techniques that are available, but are selected in this study because of their known stable state and habitat from which to monitor communities in relation to tolerance of pollution and changes in water quality in the river system. Furthermore, diatoms are readily available and cells can be found in all river systems.

The study intends to investigate the possible effect that seasonal conditions has on diatom colonies with particular attention to changes resulting from pollution in the river. It should be possible to assess the effect of pollution on diatoms following incidents or point sources of discharge into acidic waters in the Mediterranean climate of the Western Cape.

### Research question

- How do diatom communities change in response to changing seasons; and how these changes align with changes in surface water quality conditions in the Berg River?

This study will therefore need to map the changing distribution and seasonal change in diatom distribution in the Berg River and to compare these changes with surface water quality measurements that span a period of 20 years or more.

### Methodology

Sampling sites were chosen at strategic positions based on point sources and accessibility along the Berg River. A reference site was identified upstream of the urban residential and industrial town of Paarl. At each point at least three diatom samples were taken, labelled and prepared for analysis in the Water Analysis Laboratory at UCT. The methodology seeks to understand how quickly diatoms respond to changes in water quality, and the effect of varying flow in acidic waters of the Western Cape.

Diatom samples were collected four times between July 2013 and June 2014, corresponding to the changing seasons. This sampling procedure aimed to collect a representative sample of the change in diatoms corresponding to periods of low flow, peak flow and average flow conditions. Diatoms were collected using the well-established methods of (Taylor et al., 2007; Taylor, et al., 2007). Once the sample was counted, the data was entered into a computer software database, Omnidia, from which several calculations were made using equations or using the sum of the water quality optima for all the species in the sample. The software has an inbuilt dataset for each species, which comprises taxonomically relevant data and indicative values for the calculation of the indices (Alakananda et al., 2011; Karthick et al., 2010). A variety of databases were developed and validated for use (Alakananda et al., 2011; Karthick et al., 2010). In the main, diatom species are assigned two values that reflect the tolerance or affinity of a diatom species towards a certain water quality and its relationship. These values were then weighted according to the abundance of the diatom in the sample (Alakananda et al., 2011; Karthick et al., 2010).

Index scores were calculated from Zelinka & Marvin (1961) summation formula. The index score reflected values between 0 and 20 providing an indicator position on a scale from pristine to highly eutrophic. Diatom identification were achieved from the works of Taylor et al. (2007).

The intention of this analysis is to use the presence of diatoms to make reasonably accurate predictions about water quality and to understand the ability of the selected river to recover from pollution events either from diffuse or points sources.

### **Scope**

The scope of the study is confined to monitoring two urban river systems, using diatoms as a monitoring tool, in the upper Berg River catchment, namely the Franschoek River, which is a tributary of the Berg, the middle reaches of the Berg River itself. Samples were taken seasonally over a year.

### **Limitations**

The project aims at showing diatom seasonality but being constrained to only one full set of season changes may not necessarily reflect the conditions long term. More studies are required at a later point to confirm the results. A further limiting factor is that there had been no previous studies diatom studies done on the Berg River specifically focussed on the urban area. Particularly with regard to species identification a follow-up study may be required to evaluate the accuracy of the species identified and authenticate diatom seasonal species. Furthermore no previous reference data means that the data generated from this study has no means with which to compare to.

### **Discussion**

Financial constraints linked to river monitoring schemes often affect the ability to obtain relevant results. Diatom analysis provides a cost effective solution to obtain water quality results at any point in time, minus the heavy financial burden required by other monitoring techniques. Although some diatom training is required, it does provide a good method for establishing water quality and is highly recommended.

Another aspect of this discussion should be concerned with the limited output of diatoms to provide a quantitative account of water quality. This creates a problem because water quality

monitoring measurements are most frequently reported in absolute values. There are limitations then in reporting taxonomic patterns that might be meaningless especially in the absence of a reference dataset from which to compare.

Traditionally, data acquisition results from governmental outsourcing of jobs to private consultants who use various techniques to source the data. In the case of water quality, the main technique currently being used is that of chemical analysis. Although this technique achieves the desired results in understanding water quality, it fails dismally in other areas. Chemical analysis tests are expensive and as a result, and only a limited number of tests can be performed. The inability to take data measurements frequently may lead to incorrect results because chemical testing responds to current water conditions and may not give an accurate reflection of conditions. Adding to the costs are the fact that certified testing labs are scarce and this means samples are often sent long distances further delaying the process. This would suggest the need to find an indicator that could be used for continuous assessment that is inexpensive and could represent water quality changes over a much longer period.

## Chapter 2

### Introduction

Water resources in South Africa are scarce, often naturally ephemeral and are difficult to manage (Taylor et al., 2005). This makes it particularly difficult to obtain accurate water quality results and likewise to monitor in real time. This raises the need to establish a monitoring scheme that will allow for a more efficient way of water monitoring. South Africa has only 8.6% of the rainfall available as surface water (Walmsley et al., 1999). Large volumes of water are transferred from both inside and outside of South Africa to supply the steadily growing demands of industrial and urban centres (Taylor et al., 2007). In drier parts of the country borehole water points run dry and rainfall continues to become progressively erratic. Surface runoff has led to increased topsoil erosion and insufficient surface water infiltration has led to increased pressure on groundwater supplies. The scarcity of water is compounded by pollution of surface- and groundwater resources. Typical pollutants of South Africa's freshwater environment include industrial effluents, domestic and commercial sewage, acid mine drainage, urban expansion, agricultural runoff and litter. (SADC, 2008; DWAF, 1996). In South Africa, almost 70% of river sources are degraded to a point where it is no longer possible to rehabilitate ecological systems once present in these systems (SADC, 2008). It is difficult to establish a robust water quality database because of the geographical distribution and variability of rainfall in South Africa (SADC, 2008). Current monitoring techniques are struggling due to the financial expense of collection and in the cost of processing samples in laboratories where there is available expertise.

The Department of Water and Sanitation (DWS) recognizes the current shortcomings in monitoring rivers in South Africa (DWAF, 1996), and therefore, in conjunction with the Water Research Commission (WRC), have commissioned numerous studies in bio-monitoring techniques including the use of diatom algae as bio-indicators (Taylor et al., 2007; Taylor et al., 2005; Harding & Archibald, 2005; Bate et al., 2002). Bio-monitoring studies are proposed as a supplementary monitoring technique because of a range of the limitations of standardised monitoring techniques. These issues include the delay between data collection and processing, cost, and concerns that conventional sampling methods may not yield expedient information in the long term (Taylor et al., 2007).

The European Union implemented the Water Framework Directive (WFD) which, amongst other things, seeks to extend existing biomonitoring procedures for water quality assessments (Chave, 2002). The WFD requires biomonitoring techniques to be implemented and used in monitoring procedures. The result has led to a noticeable shift in scientific literature towards integrating current biomonitoring techniques with physical and chemical parameters (Bere & Tundisi, 2011 ; Tornes et al., 2007; Duong et al., 2006; Ingleton & McMinn., 2012)

In South Africa, as with many other developing countries elsewhere, the management of aquatic ecosystems requires a well-designed and validated tool that is simple and inexpensive for the assessing and monitoring of ecosystems to diagnose the causes of degradation and to track responses. For this purpose, diatoms are claimed to be robust bio-indicators (Beyene et al., 2014; Blanco et al., 2012). Moreover, diatoms are freely available bio-organisms found in all types of stream. They are able to adapt to changing water conditions and are ideal monitoring source for rapidly changing environments in an urban context.

### **Urban stream syndrome**

The “Urban Stream Syndrome” is a conceptual framework for describing observed ecological degradation of streams in urban landscapes (Walsh et al., 2005). The increase in the number of deteriorating streams within the urban catchment has resulted in further research into urban ecologies (Walsh et al., 2005). Attention is drawn to re-evaluating the connection between urban land-use and stream ecological function and structure (Walsh et al., 2005). Land-use and increased human activities have degraded the way in which urban streams drain into their catchments (Walsh et al., 2005). The result is observed in ‘flashier’ hydrographs, elevated nutrient contents, altered morphologies, reduced biotic richness and an increase in dominance of more tolerant species which are all symptoms of river degradation (Walsh et al., 2007).

Factors such as changes in hydrology due to channel incision, or the increase in impervious channels along the catchment altered observed hydrographs (Walsh et al., 2005). Increased impervious channels results in increased erosion of stream channels particularly during times of peak flow. Typical stormwater infrastructure and management results in an end-of-pipe runoff control procedure, not taking into account the impact this has on the ecological benefits of storm

water on the system (Walsh et al., 2005). Impervious channels decrease the amount of infiltration due the hardened surfaces which allow nutrients to be easily transported downstream (Walsh et al., 2005). A factor of further concern is that sewerage leakages from WWTWs, for example, have lasting implications on underlying water chemistry problems. Improving stormwater management and runoff is critical to improving water quality across the range of precipitation variability (Walsh et al., 2005).

The high levels of pollution in urban areas affect nutrient cycling (Pickett et al., 2011). Increased concentrations and loads of chemical pollutants in streams appear to be universal in river systems (Walsh et al., 2005; Walsh et al., 2005).

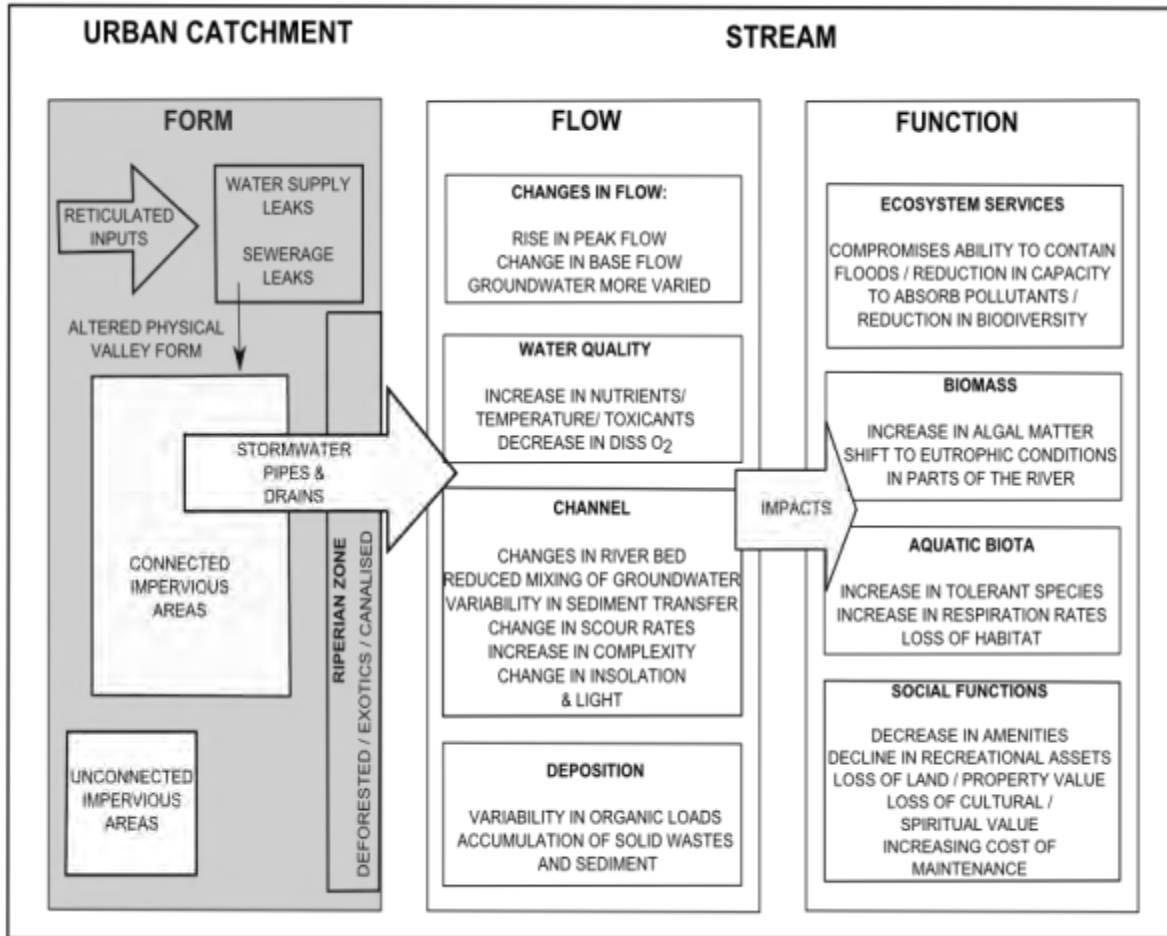


Figure 1. Urban Stream Syndrome Conceptual Model on urban river impacts adapted from (Walsh et al., 2005).

The primary influences behind the Urban Stream Syndrome are based on sources of stress within the catchment, particularly that of stormwater within the system. Some effects of urbanisation only affect subsets of the stream. A possible way of monitoring certain aspects of river health is by using different trophic levels in the food chain. Bacillariophyceae is a vital part of this system and provide essential feedback on how other systems are functioning based on species availability and abundance (Walsh et al. 2005). The inputs, such as forest loss, the built infrastructure and water supply, as outlined in Walsh et al. 2005, have specific outcomes based on different stream variables within a river system. These outcomes, including flow, water quality, morphology, light, organic matter, are dependent on the interactions within the river

system and particularly changes to riparian vegetation and catchment areas (Walsh et al., 2005). Water quality as a variable is strongly linked to other variables within the stream system. Furthermore stream biota are largely dependent on the availability of these variables at any particular time. Multiple outcomes are responses to changing water quality, relating not only to stream variables, but also to the biota (Walsh et al., 2005). These changes are both chemical and biological. In particular, biological biota respond well to nutrient content within river systems. Biological abundance are affected by the availability of certain nutrients in the river (Walsh et al., 2005). Impervious surfaces including pipes and stormwater systems provide a new dynamic to the system since these bypass the "catchment and "riparian zones", and directly impact the variables within the river system (Walsh et al., 2005). This provides an uneven balance in dealing with flow and possible infiltration and nutrient transportation. Stream outputs are closely monitored in various aquatic species and algae. Algal biomass provide an understanding of numerous factors within a river systems including stream flow, water quality (nutrients, temperature etc.), light availability and organic matter (Walsh et al., 2005). These factors can be judged based on production, nutrient uptake, abundance and other factors. Thus diatoms have been singled out as a potential algal source to measure these factors. Furthermore biological biota are easily adaptable and respond well to infrequent "pulsed" events as described by Collins et al. (2008). These events are difficult to map due to their unpredictability, but factors associated with the "pulsed" event are a lot easier to map. This makes the Urban Stream Syndrome framework useful in dealing with water quality issues.

## Diatoms

Diatoms are a commonly occurring assemblages of algae belonging to the Bacillariophyceae group (Taylor et al., 2009). Diatom cell walls ('skeletons') are made of silica, much like a glasshouse. The construction of the cell wall, called the frustules, consists of two halves, known as 'valves', that fit into each other (Martin & de los Reyes Fernandez, 2012; Taylor et al., 2010; Van Vuuren, 2007). It is reported that their varied shapes and the complex ornamentation of their cell walls made the study of the diatoms and related siliceous organisms a favourite pursuit of the microscopical pioneers. The frustules can persist in the environment long after the organisms have died (Taylor et al., 2009; Van Vuuren, 2007) and are easily identifiable at species level (Martin & de los Reyes Fernandez, 2012). This has resulted in the creation of numerous diatom-based

indices of water quality. In practice, the use of diatom indices involves making a list of the taxa present in a sample, along with a measure of their abundance. The index is expressed as the mean of the optima of the taxa in the sample, weighted by the abundance of each taxon. The indicator value acts to further increase the influence of certain species (Kelly, 1998). "Diatoms found in rivers grow on a variety of substrata including submerged aquatic plants, emergent macrophyte plants, rocks, in-stream detritus and debris, sediments, and may even grow in abundance on other groups of algae" (Taylor et al., 2009).

Periphyton, that is the biotic group containing diatoms, are valuable indicators of environmental conditions in streams and rivers. It has been argued that the predictability of periphyton to ecological tolerances is favoured as a water quality indicator (Biggs, 1989; Dela-Cruz et al., 2006; Li et al., 2010). Periphytons provide a reliable environmental indicator for estimating eutrophication in river systems (Kitner & Poulickova, 2003). They have rapid reproduction rates and very short life cycles and therefore can be expected to reflect short-term impacts and sudden changes in the environment. Periphyton attach to a substrate, where they grow and can respond directly and sensitively to many physical, chemical and biological variations in the stream (or river). An example of such variation is reach, which includes temperature, nutrient levels, current regimes and grazing (Li et al., 2010). Patterns of benthic diatom communities are responsive to the nature of the physical and chemical characteristics of lotic systems. They respond rapidly to degradation of water quality, often changing in both taxonomic composition and biomass where even slight contamination occurs (Round, 1991). Diatoms are reliable indicators of specific water quality problems such as organic pollution, eutrophication, acidification and metal pollution, as well as for general water quality (Karthick et al., 2010). Thus, pollution control and monitoring programmes routinely include the examination of diatoms to investigate the ecological status of lotic systems (Round, 1991). The unique composite picture of ecosystem conditions provided by the diatoms can only be replicated by intensive chemical monitoring studies.

### Bio assessment capabilities

Water quality describes the aesthetic, biological, chemical, as well as the physical properties of water that determine the sustainability and protection of aquatic ecosystems (DWAF, 1996). However De la Rey et al. (2008) suggest that "there are several factors that contribute to the decline in water quality, the most important being industry, intensive and careless agricultural practices and the population explosion, which increases the demand for domestic water supply ". De Villiers (2007) concurs by suggesting that the most likely sources of pollution in the Berg River occur from agricultural runoff, effluents from residing informal settlements and wastewater treatment works. Effluent exposure, whether treated or not, can reduce the functioning of river systems and cause significant eutrophication (Giller, 2005). Walsh & Wepener (2009) account for the changing diatom taxonomies based on varying land-uses in the Vaal catchment. They have shown that varying chemical concentrations, due to agricultural runoff in the river system, have affected the species present in their study. Chemicals and chemical compounds constantly fluctuate in the river system: they are broken down, and dissolved by environmental conditions such as light and heat energy; and they are also constantly removed from the system via uptake by organisms and sedimentation (Taylor et al., 2007). Furthermore, chemical components in a river system may also be diluted by inflows of rainwater or augmented from runoff from point (mine, sewage, storm water drainage) and diffuse sources (agricultural runoff, groundwater seepage from settling ponds), or become concentrated during times of drought and low flow.

The "importance of freshwater systems in the provision of ecological services and diverse habitats for a huge range of species, highlights that there is a clear need for restoration that can maintain sustainable ecological services, whilst reinstating ecosystem function and habitat range" (Giller, 2005: 201). In order to prevent further water quality problems, these issues would need to be monitored, with particular emphasis placed on water quality. Knowledge of diatom distribution in South African rivers could be used to infer water quality (García-Rodríguez et al., 2007). Diatoms are a good indicator of water quality as they are responsive to changes in river

chemistry particularly that of salinity (Bate & Smailes, 2013; Harding & Taylor, 2011; Van Vuuren, 2007; Harding & Archibald, 2005; De la Rey et al., 2004).

A variety of techniques and tools have been used in monitoring water quality including "direct measurements of plants, invertebrates, fish and microorganisms" (Harding & Taylor, 2011). Biomonitoring and physico-chemical monitoring can be run concurrently and effectively (Matlala et al., 2011). Biomonitoring is a site-specific quantitative or qualitative process describing the biological status of aquatic systems, based on the reference, which are used to identify the unimpacted condition of the biological communities inhabiting a specific site (DWAF, 1996). Some authors agree that chemical analysis on its own is not sufficient to give a proper reading of water quality as it merely shows results as a 'grab shot' for a given period (Matlala et al., 2011; Taylor & Harding, 2011; Taylor et al., 2007; Harding & Archibald, 2005). Diatoms have shown to be good indicators as they are readily available and easily acquired. "Algae (including diatoms) and other microscopic organisms attached to submerged surfaces occur in most shallow aquatic habitats where there is sufficient penetration of light" (Matlala et al., 2011).

Diatom indices were developed to determine water quality in a South African context (Taylor et al., 2005; Taylor et al., 2005; De la Rey et al., 2004). Up to 70% of what occurs in water quality tests are reflected in diatom assemblages (Van Vuuren, 2007). The diatom in abundance will represent a specific chemical component and will give a reflection of the abundance of that chemicals presence. Harding & Taylor (2011) concede that the shortcoming of the method is that it requires the researcher to have an in-depth knowledge of the "autecology of individual diatom species" to draw accurate environmental conclusions based on diatom community composition. De la Rey et al. (2004) suggest that results from biological monitoring are more cost effective when compared to chemical monitoring and the results can be obtained rapidly.

Periphyton respond differently due to ranging anthropogenic and biological factors in the upper Berg River (Ewart-Smith & King, 2012). The areas measured in the 2012 study conducted by Ewart-Smith & King correspond to "natural" conditions outlined by River Health Programme Report (River Health Programme, 2004). Further data corresponding to urbanized areas are not

reflected in this study, however an abundance of N and P in the river system appear to reflect positive diatom growth conditions in the river systems. This study reflects periphyton changes due to seasons, with particular variability noted during flooding season. During the first annual growing season, the spring & summer peak was dominated by diatoms, particularly the pollution sensitive diatom, *Eunotia rhomboidea* (Ewart-Smith & King, 2012).

Diatoms have been successfully mapped along points of the Franschoek River and the point at which a river returns to its normal state prior to a point source pollution spike was noted (Frick, 2010). Diatoms also exist in canalized rivers in urban environments, where diatoms attach themselves to the hard canal floor. Harding & Taylor (2011) further describe diatoms as being “uniquely suited as indications of water quality in urban waterways”. The relative abundance of diatoms in all river systems and their ability to respond to changing water conditions is the reason for this.

Results obtained from biological monitoring are claimed to be cost effective, rapid and easily accessible (De la Rey et al., 2004). The current biological monitoring systems that are used in South Africa are the SASS (South African Scoring System) or the FHI (Fish Health Index) systems, which uses numerical indices for biotic macro-invertebrates as an indicator of water quality. However, Round (1991) lists several reasons why animal components of an ecosystem may not provide a satisfactory index system. The reasons are linked to the complexity of reproduction cycles and difficulty in monitoring some species, particularly monitoring the system across the entire stream. Resh (2008) required the removal of fish from a monitoring programme because of the extensive time it took to collect representative fish samples.

Aquatic invertebrates studies have been shown to be influenced by flood, drought, hydrology and seasonal variations in invertebrate occurrence (Beyene et al., 2009). Invertebrates are weak indicators of eutrophication and diffuse and point source impacts which may be identified by direct measurements of diatom associations (Harding & Archibald, 2005). Unlike macro-invertebrates and fish, diatom species occur in a wider variety of waters and their distribution is cosmopolitan (Potapova & Charles, 2007). Diatoms are also suited for monitoring very heavily impacted systems where other types of organisms are absent (Taylor et al., 2007). The study of diatoms for monitoring the integrity of rivers has increased because of the limitations of macro-

invertebrates and fish as indicators and significant improvements in technologies for diatom assessment that increase the information per cost ratio. As diatoms are relatively immotile and can possibly be found under almost any conditions, they are good indicators of pollution levels among heavily impacted sites where macro-invertebrates are absent. Therefore, diatoms are the preferred bio-indicators for monitoring urban-impacted and seriously stressed rivers and to examine pollution gradients and impacts of specific pollution sources (Beyene et al., 2009).

In freshwater systems where changes in hydrology are rapid and difficult to estimate, biological monitoring has proven to be very useful due to its integrative nature (Soininen & Eloranta, 2004; Soininen & Könönen, 2004)

### Diatom indices

There is plenty of support to indicate that diatoms are reliable indicators of water quality (Bere et al., 2014; Blanco et al., 2012; Delgado et al., 2012; Ector et al., 2012; Alakananda et al., 2011; Taylor et al., 2007; Taylor et al., 2005; Bate et al., 2002; Dell'Uomo, 1996). Complexity in measuring multiple water variables has resulted in many diatom-based indices being developed in Europe (Lecoite & Coste, 1993) and North America (Potapova & Charles, 2007). Indicators such as Specific Polluosensitivity Index (IPS), CEMAGREF (1982), Pampean Diatom Index (IDP) (Gómez & Licursi, 2001), Van Dam (Van Dam et al., 1994), Trophic Diatom Index (TDI) (Kelly & Whitton, 1995.) are some of the available indicators.

Diatom indices, each with varied calculation systems measuring selected variables (i.e. pollution indices), reflect water quality and pollution in a river system. Certain diatoms are more responsive and tolerant to changes in pollution than others (Tornés et al., 2007). For example, *Navicula veneta* Kützing is a cosmopolitan species found in heavily eutrophic waters and is highly tolerant of pollution. *Navicula cryptotenella* Lange-Bertalot is only moderately tolerant to pollution and found in freshwater biotopes ranging between oligotrophic to eutrophic (Taylor et al., 2007). *Eunotia bilunaris* (Ehrenberg) Mills is found in acidic, flowing or standing waters (Taylor et al., 2007). Species composition and abundance can provide critical information on how water quality is changing in a river.

Some index systems are designed around Zelinka and Marvan's formulae. Index values are obtained using relative abundance, pollution sensitivity indices and species indicative values that are used to calculate organic pollution. Indicator values range from 1 to 5, with 5 representing heavily polluted until and 1 representing no pollution. Specific Polluosensitivity Index (IPS) is very sensitive to organic pollution used by various authors (Elias et al., 2012; Duong et al., 2006). IPS is effective in dealing with monitoring of a streams ecological quality throughout the year without having to worry about interference of the natural temporal variability of diatom communities (Elias et al., 2012; Jüttner et al., 2012). IPS has been shown to link very well to the Trophic Diatom Index (Kelly and Whitton 1995) but this index system reflects chemical conditions less well (Jüttner et al., 2012).

Eutrophication and Pollution Index- Diatoms (EPI-D) is an index system that uses species relative abundances and is calculated using Zelinka & Marvin's formula. Values for each sampled site are graded and interpreted based off of values ranging from 'excellent' water quality to 'very heavily polluted', ranging on a scale from 0 to 4 respectively. EPI-D and other European diatom indices seem to link very well together and in particular translate well to other regions of the world (Torrise & Dell'Uomo, 2006). The EPI-D index when compared with the chemical data and the general situation of the examined watercourses showed an excellent capacity for use in Italian rivers.

Diatom Index of Saprobity and Eutrophication (IDSE) (Le Clercq, 2008), is a diatom index representing percentage composition of pollution tolerant species and used to calculate organic pollution. Values range from 0 to 100, representing 'no pollution' to 'very high levels' of pollution. Index systems that were borrowed from other regions of the world have been shown to be applicable because many widely distributed diatom species have similar environmental tolerances to those recorded for these species (Bere & Tundisi., 2011). However in Turkey, due to the fact that geo-geographical characteristics are different, saprobity and trophic values of organisms must be modified for Turkish conditions and their indication weights must be accounted for again (Kalyoncu & Şerbetci, 2013). For the South African context, A South African Diatom Index (Harding & Taylor, 2011.) has been adapted from IPS and developed in an attempt at preventing this sort of problem.

Van Dam (Van Dam et al., 1994), is representative of multiple variables including pH, salinity, nitrogen uptake, oxygen requirements, saprobity, moisture and trophic status. It is different to the above-mentioned indices in that it measures nutrients within a river system as opposed to organic pollution. Oxygen requirements are shown using continuously high (100%) to very low (<10%).

Another diatom index is Pampean Diatom index (IDP) (Gómez & Licursi, 2001). Diatom variables are put into four categories all showing reality stages of organic pollution which has been strongly recommended method for use in Zimbabwean rivers (Bere et al., 2014). The Pampean Diatom Index yielded results suggesting minimal fluctuations seasonally with results lying between polluted to heavily organically polluted conditions. Trophic Diatom index (Kelly & Whitton, 1995) can be used as a measure for organic pollution and links well to IPS (CEMAGREF, 1982.), but shows results similar to both EPI-D (Dell'Uomo & Torrisi, 2011) and IPS (Bere et al., 2014).

It is important when choosing the right metric as these respond differently over time, however it has been shown that diatom indices responded homogeneously in Mediterranean climates (Tornes et al., 2007).

### **Diatom studies in South Arica**

There have been many studies on the diatom flora of South Africa. In the 1960s, a large volume of work was done by (Giffen, 1975; Giffen, 1971; Giffen, 1970), (Cholnoky, 1968.; Cholnoky, 1958b; Cholnoky, 1958a) and (Schoeman & Archibald, 1976-80). Recently, Bate et al. (2002) suggest that some authors such as (De la Rey et al., 2008; Taylor et al., 2007; Taylor et al., 2007; De la Rey et al., 2004) have focused more on ecological interpretations of diatoms in river systems.

In 1972, Archibald attempted to relate observed diversity in diatom communities to water quality. Similarly, Schoeman, (1976) used diatom indicator groups in the assessment of water quality. He simplified the community analysis method of Cholnoky by dividing diatom associations into four groups, each with their own particular ecological requirements. Giffen focused attention on mapping diatoms in Cape Town, Saldanha Bay and Gordon's Bay (Giffen, 1975; Giffen, 1971; Giffen, 1970). In 1979 a monitoring system was developed by Lange-

Bertalot and was based on groups of diatom communities that share the same pollution tolerances. Diatoms, as indicators of water quality, were again investigated in depth in South Africa by Bate et al. (2002). The investigation attempted to relate a descriptive index, based on a dataset for the environmental tolerances of diatom species found in the Netherlands, to water quality in South Africa. Thereafter, Taylor et al. (2007) produced a comprehensive manual to identify and sample diatoms in the South African river system.

Much focus in South Africa the possibility of using diatoms as river bio-indicators (De la Rey et al., 2008; Harding & Archibald, 2005). A distinct advantage of this bioindicator is that limited time is required to gain the required skills to use this as a suitable bioindicator (Taylor et al., 2009). The possibility of using epiphytic diatoms to assess water pollution has also been investigated (García-Rodríguez et al., 2007). European diatom indices have been tested in South Africa (Taylor et al., 2007) with IPS giving the best results (De la Rey et al., 2004). It is recognised that certain species are endemic to South Africa and an indicator species emphasize that several diatom species are endemic to South Africa and thus a South African diatom index system should be adapted (Taylor et al., 2007). In particular the use of benthic diatoms as bio-indicators in running waters has increased in recent years due to their broad distribution and the variation in species composition that can be attributed to ecological variations (Soininen & Eloranta, 2004)

### **Diatoms analysis in Mediterranean climate**

Freshwater systems are naturally self-regulating. With increased pressure caused by the discharge of wastewater into rivers and expanding settlements, river systems impacted by surface runoff as well as point-sources entering the rivers. For example, Waste Water Treatment Works (WWTW) increase the risk to river systems. Monitoring aquatic resources forms the basis of constructing and implementing successful management plans for river systems. With changing hydrology as a result of impervious surfaces, water quality becomes difficult to estimate. Biological monitoring has proven to be very useful method due to its integrative nature (Delgado et al., 2012) using benthic biota to indicate water quality and habitat integrity has certain advantages. Aquatic organisms respond to the physical and chemical properties of water as well

as providing an indication of the impact of various pollutants on the river system, not simply just focusing on the concentration of an effluent, but its actual ecological impact on the environment (Taylor et al., 2005; Archibald & Taylor, 2004). Diatoms pose a possible solution to the bio-monitoring problem as known species are tolerant of pollution spikes.

The WFD that was drawn up in the European Union has seen a strong emphasis placed on the need for biological monitoring, and has seen an increase in the number of diatom studies done in the region. Diatom sensitivity and its ability to adapt to changing water condition proposed a potential solution to monitoring rivers in the region. Diatom assessment techniques have been tested and there has been an attempt at integrating a diatom-monitoring index into the WFD (Tornés et al., 2007).

Diatom response to changing water conditions are largely due to their species sensitivity to a change (Rimet, 2012). In that way some diatom communities react to specific changes in its environment and will become more abundant. Diatom response times vary from a few hours to a few days (Rimet et al., 2009; Rimet, 2009). When polluted site were compared to unpolluted sites, diatom species composition where always different (Rimet et al., 2009; Rimet, 2009). Diatom monitoring has been shown to be a main contributing factor in monitoring human impacts on river systems (Smucker & Vis, 2011; Tornés et al., 2007). Acid mine drainage and urban industrial pollution are common problems in the interior of South Africa. Taylor et al. (2007) suggests that some *Tabularia* and *Nitzschia* Species respond well to industrial pollution whereas some acidophilious species such as *Stauroneis kriegerii* Patrick respond better to acid mine pollution. Some diatom species like *Eunotia microcephala* Krasske also has been considered a taxon of good ecological quality in France and in the Iberian Mediterranean rivers (Rimet et al., 2009; Rimet, 2009; Tornés et al., 2007).

The type-specific taxa from near-natural streams are coincident with the indicator taxa for high ecological status (Tornés et al., 2007). Human impact reduced the typological heterogeneity of the diatom community composition. Overall, the diatom communities in NE Spain exhibit a regional distribution pattern that closely corresponds with that observed in river systems elsewhere. Physiographical differences are only evident in undisturbed sites, while nutrient

enrichment and other human disturbances may mask the regional differences in the distribution of diatom communities (Tornés et al., 2007).

The diatom communities' composition and the characteristic species of each group of sites in Spain closely corresponded with those observed in other geographical areas (Tornés et al., 2007). Benthic diatom assemblages are controlled by multiple factors reflecting land use and site-specific conditions at various temporal and spatial scales. Diatom distribution is to the biogeochemical characteristics of the waters, their nutrient content, velocity and substratum type (Tornés et al., 2007). Certain Taxa such as *Nitzschia capitellata* Hustedt, *Nitzschia palea* (Kützing) W Smith, *Nitzschia frustulum* (Kützing) Grunow and *Navicula veneta* and were type-specific for river sections affected by intensive agricultural and industrial activities.

An obvious consequence of pollution influences is that differences in diatom assemblage composition are more evident among relatively undisturbed sites than among sites severely affected by nutrient enrichment. One of the strong inferences that may be drawn from the present results on diatom studies is that disturbances lead to the homogenization of the diatom community composition over wide areas (Tornés et al., 2007).

### **Diatom response to direct changes**

Biological monitoring techniques have been introduced as part of routine monitoring programmes due to certain shortcomings in standard physical and chemical methods (Harding & Taylor, 2011; Matlala et al., 2011; Taylor et al., 2007). Diatoms can be used on its own (Taylor et al., 2007) as a pollution-monitoring tool or in conjunction with other monitoring techniques. The use of biological variables in biomonitoring appears to be quite cost - effective, shows a wide range of response, and thus may be very useful (Ramakrishnan, 2003). Biological monitoring techniques and chemical monitoring are statically significant (Ramakrishnan, 2003). Diatoms were useful in showing strong pollution gradient between upstream monitoring sites with strong agricultural influences downstream (Tornés et al., 2007).

Some authors have attempted to address eutrophication and nutrient concentrations in river systems (Ingleton & McMinn, 2012; Duong et al., 2006; Kitner & Poulickova, 2003; Fawzi et

al., 2002; Kelly, 2002; Kelly, 2001; Kelly & Whitton, 1998). This has been largely successful when use in conjunction with another biomonitoring tool. Diatom species favour conditions with higher concentrations of heavy metals (Ingleton & McMinn, 2012). Seasonality is apparent in diatom composition (Elias et al., 2012; Chatháin & Harrington, 2008; Coste, 1993), but this does not affect the ability of diatoms to reflect water quality. Diatom monitoring is branching out into a multidisciplinary field incorporating many resources to monitor rivers (Ingleton & McMinn, 2012; Lavoie et al., 2008). Diatoms have been coupled with remote sensing technologies to assess the effect thermal changes may have on water sources (Ingleton & McMinn, 2012). Diatom assemblages shifted from control to affected site.

Waste water treatment works in urban centres often deposit effluence directly into river systems due poor regulation and management. The WFD has strict measures in place for dealing with discharge into river systems (Tornes et al., 2007). With the increased strain expanding cities have placed on WWTWs by law are required to monitor the output of nutrients entering the river system. Developing countries such as Ethiopia have struggled to monitor STWs outflow due to poor management (Beyene et al., 2014; Beyene et al., 2009). This has resulted in spillage of raw sewerage into the freshwater systems and calls for greater enforcement of laws (Bere & Tundisi, 2011; Beyene et al., 2009).

Some work has been done in attempting to understand the effect organic pollution has on diatom colonies in different parts of the world. In studies that contained strong pollution gradients (Tornés et al., 2007; Dela-Cruz et al., 2006; Duong et al., 2006), it was seen that diatom assemblages shifted to species that were more tolerant of pollution. Duong et al. (2006) too noted an increase in the amount of brown algae present along the pollution gradient.

Sebastiá et al. (2013) attempted to understand what effect seasonality has on nutrient concentrations from stream discharge. Nutrient fluctuations and strong diatom blooms were strongly linked to rainfall events and in one case up catchment snowmelt. Seasonality amongst diatoms was shown to be prevalent, but had no effect on results obtained when dealing with IPS or other diatom indices (Gómez & Licursi, 2001; Kelly and Whitton, 1995; CEMAGREF, 1982). Furthermore it has been shown that diatoms sensitivity to changes in pollution and temperature

can be used to effectively monitor climate change (Ingleton & McMinn, 2012; Alakananda et al., 2011). There is a greater need to monitor and properly manage STW discharge into open water sources (Beyene et al., 2009; Chave, 2002; Fawzi et al., 2002; Kelly, 2002).

The latest development in diatom monitoring has seen the introduction of GIS as an indicator classification tool (Naumoski & Mirceva, 2011). GIS-based management schemes are being developed to effectively monitor and water quality.

Predictable changes in diatom assemblages do occur in response to organic and nutrient loading gradients in Mediterranean climates (Delgado & Pardo, 2015). Diatoms are widely used for the bio-assessment of permanent rivers and streams due to their broad distribution and their ability to integrate changes occurring in water composition and quality. Seasonal changes are visible in diatoms with different taxon present in the headwaters (Delgado et al., 2012). Diatom indices show strong correlations with potentially toxic elements such as heavy metals as well as being sensitive to the concentration of fats, oils and trichloroethene (Blanco & Bécares, 2010). Diatom studies are widely used within the European Union but little work has been done on temporary streams in the Mediterranean (Delgado & Pardo, 2015). The greatest seasonal variability is seen in the headwaters, with much greater seasonal changes noted (Delgado & Pardo, 2015; Blanco & Bécares, 2010; Tornés et al., 2007). There is a need to develop a diatom index system that does not require a reference site, particularly as these reference sites become harder to find as you near the coast (Blanco et al., 2012; Blanco & Bécares, 2010).

## Chapter 3

### Introduction

Diatoms are known to respond to changing water qualities based on species sensitivities and tolerances (Harding & Taylor, 2011; Harding & Archibald, 2005). In this study sampling sites were chosen to correspond to known point sources pollution sources, stormwater drainage systems, and reticulation outflows (Frick, 2010; Winter & Mgese, 2012).

Diatom analysis relies on the ability to correctly identify diatom species and apply indices to establish water parameters. Effectively identifying diatom species and relating it to a specific index will determine the effectiveness and accuracy of diatoms to assess water quality.

Diatom studies have focused mainly on assessing water variables to diatom composition. Diatom seasonality has been covered briefly by Tornés et al. (2007) and Sebastia et al. (2013), but was never the focus of studies elsewhere, but limited to present an understanding of conditions in a Mediterranean climate and an urban environment.

### Site description

The project was designed to assess 12 sites along the Berg and Franschoek Rivers. Two sites on both rivers were chosen as references points with the aim of showing the presence of diatoms and their taxonomies at sites less affected by pollution. The next 10 sites corresponded to points of potential pollution which included stormwater drains, conduit canals and a polluted tributary. Furthermore these sites also corresponded to changes in land-use in the range from agriculture, periurban to urban.

Samples were taken seasonally enabling the researcher to establish a comprehensive seasonal diatom catalogue. Seasonality in diatom assemblages are then compared to surface water qualities during the corresponding seasons over time.

Table 1: Site Co-ordinates.

Site number	Site Over view	Land-use	Latitude	Longitude	Y	X
1	Franschhoek Reference	Undeveloped Residential	33°54'21.64"S	19° 6'7.09"E	-33.90601111	19.10196944
2	Rickety Bridge 1	Vineyards	33°53'59.62"S	19° 5'36.88"E	-33.89989444	19.09357778
3	Rickety Bridge 2	Vineyards / Pastures	33°53'55.01"S	19° 5'32.93"E	-33.89861389	19.09248056
4	Paarl Reference	Pastures	33°45'46.85"S	18°58'26.86"E	-33.76301389	18.97412778
5	Paarl Boat Club	Residential	33°44'12.92"S	18°58'16.00"E	-33.73692222	18.97111111
6	Paarl Industrial	Mixed Industrial	33°42'44.69"S	18°58'22.54"E	-33.71241389	18.97292778
7	Instream Conduit	Informal Residential	33°41'50.79"S	18°58'49.65"E	-33.69744167	18.98045833
8	Paarl WWTWs 1	Recreational/ Mixed	33°41'52.91"S	18°58'48.93"E	-33.69803056	18.98025833
9	Paarl WWTW 2	Mixed Industrial	33°41'47.49"S	18°58'46.16"E	-33.696525	18.97948889
10	Mbekweni Instream	Informal Residential	33°40'15.37"S	18°59'5.33"E	-33.67093611	18.98481389
11	Mbekweni 1	Informal Residential / Recreational	33°40'16.73"S	18°59'5.24"E	-33.67131389	18.98478889
12	Mbekweni 2	Informal Residential / Recreational	33°40'14.39"S	18°59'4.34"E	-33.67066389	18.98453889

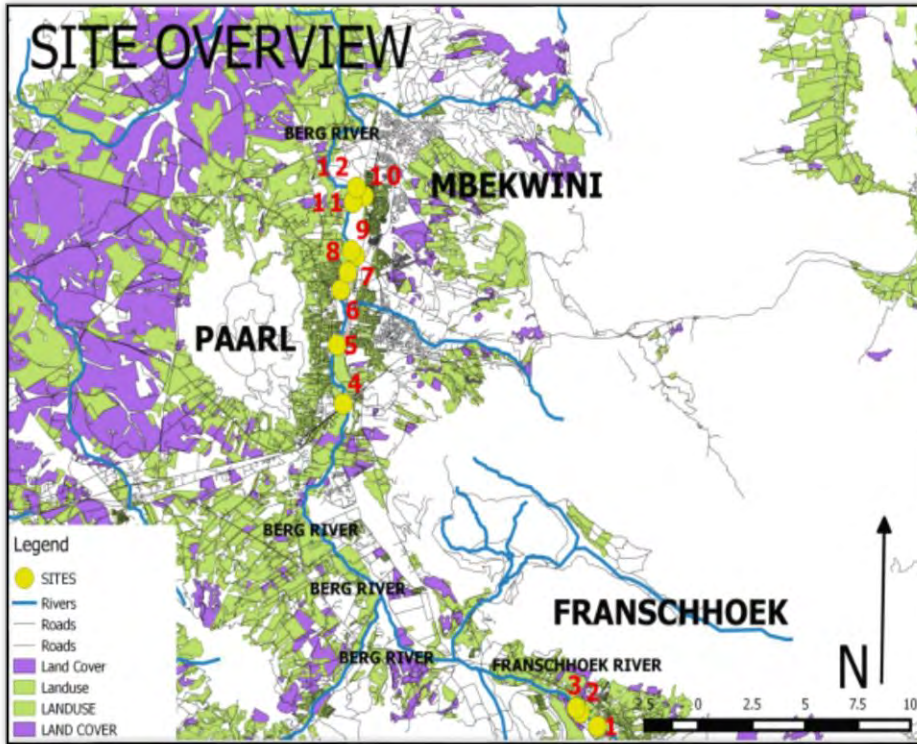


Figure 2: Location of Sites Overview.

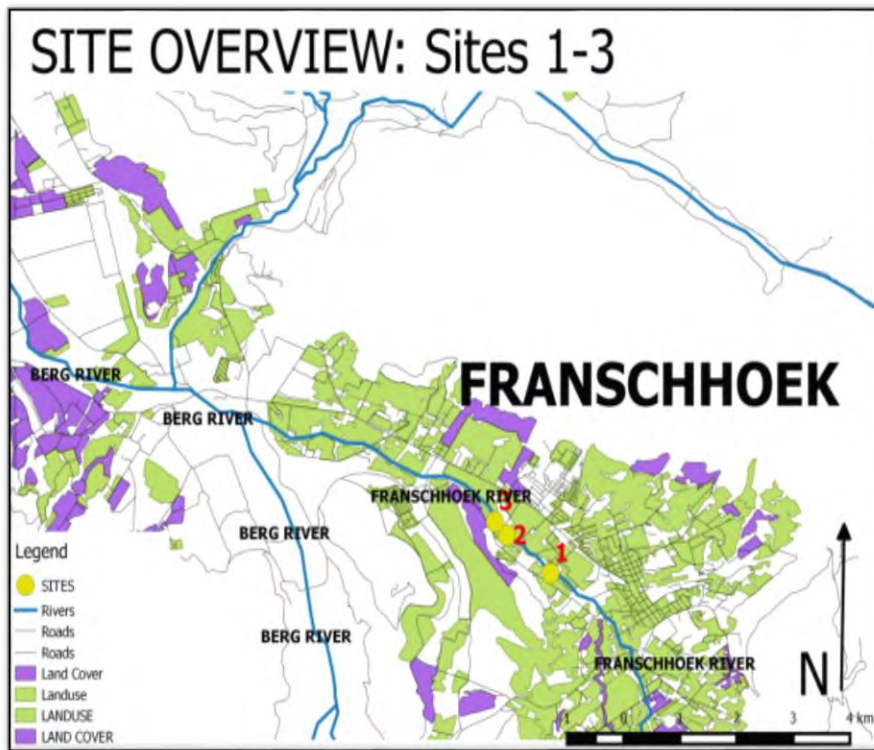


Figure 3: Location of Sites 1-3.

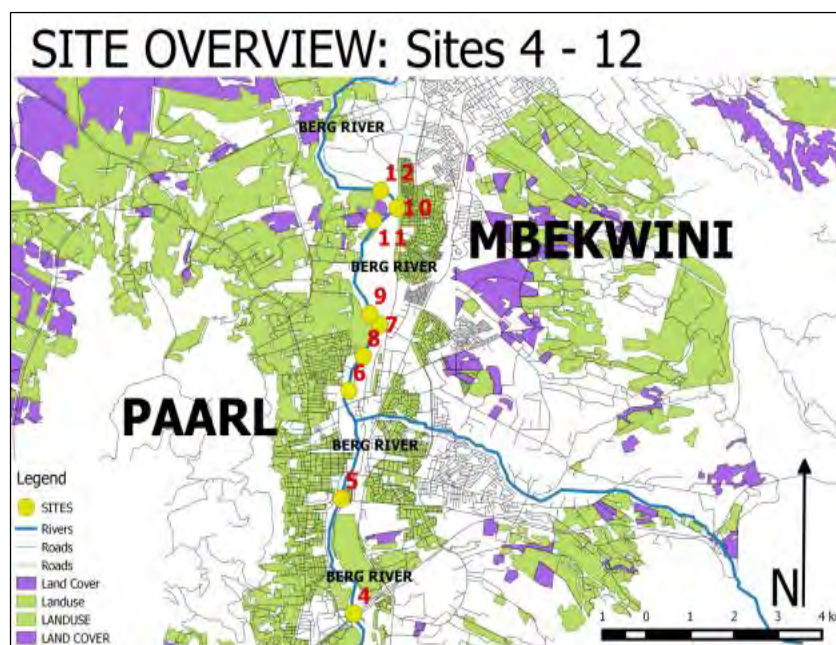


Figure 4: Location of Sites 4 - 12.

## Research methods

### Sampling criteria

Sampling was undertaken based off the recommendations from Taylor et al. (2007; 2007). Glass test tubes were pre-labelled and rinsed with distilled water. Three samples were taken at each site as described earlier, with each test tube representing a separate substrate within the allotted sample area. This was done to get an average representation of the diatom colonies growing in this region. Diatom samples were collected off rocks, plants or other hardened substrata within a 5x5m predetermined area. Surfaces were scraped using a toothbrush and concentrated sample stored in glass test tubes with rubber stoppers in a portable test tube rack. Water quality data was obtained from the DWAS water quality data sets. Water quality data was used to show how nutrient concentrations fluctuate with the change in seasons and how this relates to changes in diatom concentrations.

Diatom samples were collected and placed inside glass test tubes on site. On completion of the days field work, which took approximately 7 hours, samples were treated with  $H_2O_2$  and HCL,

and left to stand overnight in accordance with recommendations from Taylor et al. (2007). Samples were then placed into water-filled glass beakers and allowed to boil on a hot stove top and excess organic material was removed. Caution was taken to avoid any spillage and loss of diatom material.

Each sample was then rinsed with distilled water three times until the sample pH became neutral. In preparing the slides, a cover slip was gently heated on a stove inside a fume cabinet and a drop of concentrated diatom sample was placed onto the surface. The excess moisture in the solution was evaporated and Pleurax mounting fluid was added. The slide was then lowered and placed on the cover slip and immediately inverted. Once the Pleurax was sufficiently distributed and any gaseous odours were removed, the slide was allowed to rest and dry off inside the fume cabinet. A total of three slides per sample were made for each site resulting in 108 slides per season.

Sampling was undertaken over a period of 12 months, and divided into four quarters to be representative of the change in seasons. Seasonality is important to gain an understanding of how diatoms naturally respond to change in seasons. Sample dates were pre-determined to give an indication of seasonality. Sampling days occurred on the following days: 23 July 2013; 5 October 2013; 13 January 2014; 17 March 2014. These dates correspond to the different seasons.

### **Sample analysis**

Diatom samples were placed onto slides and analyzed using a Zeiss Axiostar microscope. Each slide was used to count diatom frustules. Three horizontal tracks per slide representing more or less 100 frustules per track were recorded following the standard methods according to Taylor et al. (2007). Once a diatom slide was selected and approved for use, the lens was set 400x magnification and a quick scan through of the slide was done, recording the different species present. The objective lens was then set at 1000x and the counting tracks began. Each diatom frustule was recorded on a separate piece of paper. On conclusion of the track, the lens was set to 400x and any frustules that may have been missed were included. This process was repeated three times to give a final count of 300 frustules or more.

Omnidia software was used to tabulate and sort each sample set. Omnidia contains a diatom database that allows for the quick assimilation of diatom data and index calculation. Diatom frustules will be calculated and identified, and captured and placed in the database. Omnidia was used to calculate water associated variables using various built-in water quality indices.

### Statistical

Analysis was conducted to establish the relationships between diatom abundances and further establish statistical significance between sites. To establish statistical significance between sites, a 1-way ANOVA was undertaken. It was initially planned to use all diatoms as part of the ANOVA, but it was found that the low abundance outliers served to skew the results and retrieved very high f statistic values. It was decided then that that only diatom counts that were found at 5 or more sites were considered. At this point two ANOVAs were considered. The first was to show a relationship between the reference site at 1 and sites 2 and 3, and the reference site at 4 and the rest of the sites. This ANOVA will be used to show seasonal changes and which sites it affects. The second ANOVA will relate all sites to site 1, giving an indication on how various urban zone relate to the reference site. The ANOVAs will indicate significant sites based on abundance comparisons and significant sites will mean significant differences between the reference and the site in question.

Cluster analysis was also used to group diatom species based on abundances and allow for further water quality relationships to be made. Diatoms are clustered based on species that contribute similar numbers of taxon to the sample pool. This gives a good indication of which species or clusters are contributing the most to a seasons water quality. Clustering will also give an indication of what cluster groups are able to handle similar water qualities. Seasonal cluster groups can be compared and seasonal species contributions can be determined.

The aim of this study was to establish how well diatoms relate to seasonal changes in water quality. The ANOVA statistics will allow for seasonal differences between site to be established based off diatom abundances. If The ANOVA returns the same results each season then it has been established that seasonality has little effect of diatom populations. Cluster analysis will highlight similar groups (based off abundance counts) and help to establish how diatoms relate to each other seasonally.

## Chapter 4

### Results

#### Introduction

The chapter is structured the following way. The chapter presents a 20 year record of seasonal changes within nutrients on the Berg River (Figures 5-10). It is hoped that that a strong seasonality in the data is seen. Diatom counts will then be clustered (Figures 11-14) and the most abundant species are recoded. Table 12 is found as part of Addendum D and has a complete list of diatom assemblage counts for each season. This is proceeded by ANOVA tables (Tables 2 to 5), taken from site 1 and 4 which represent the reference sites, used to show significant differences between means of the reference site and the other respective sites. Establishing significant differences will give an indication of how each site responds based on seasonality. Further ANOVAs (Tables 6 to 9), taken from site 1 will be used to show how the variance of the means are affected by seasonality and changing land-uses. A section on diatom seasonality follows where diatom indices are graphed (Figures 15 to 16) showing pollution fluctuations along the river, pollution seasonality and site similarities. This section is followed by Table 10, a table consisting of diatom indices found at each site with a brief summary relating site conditions to the indices.

## Nutrient record

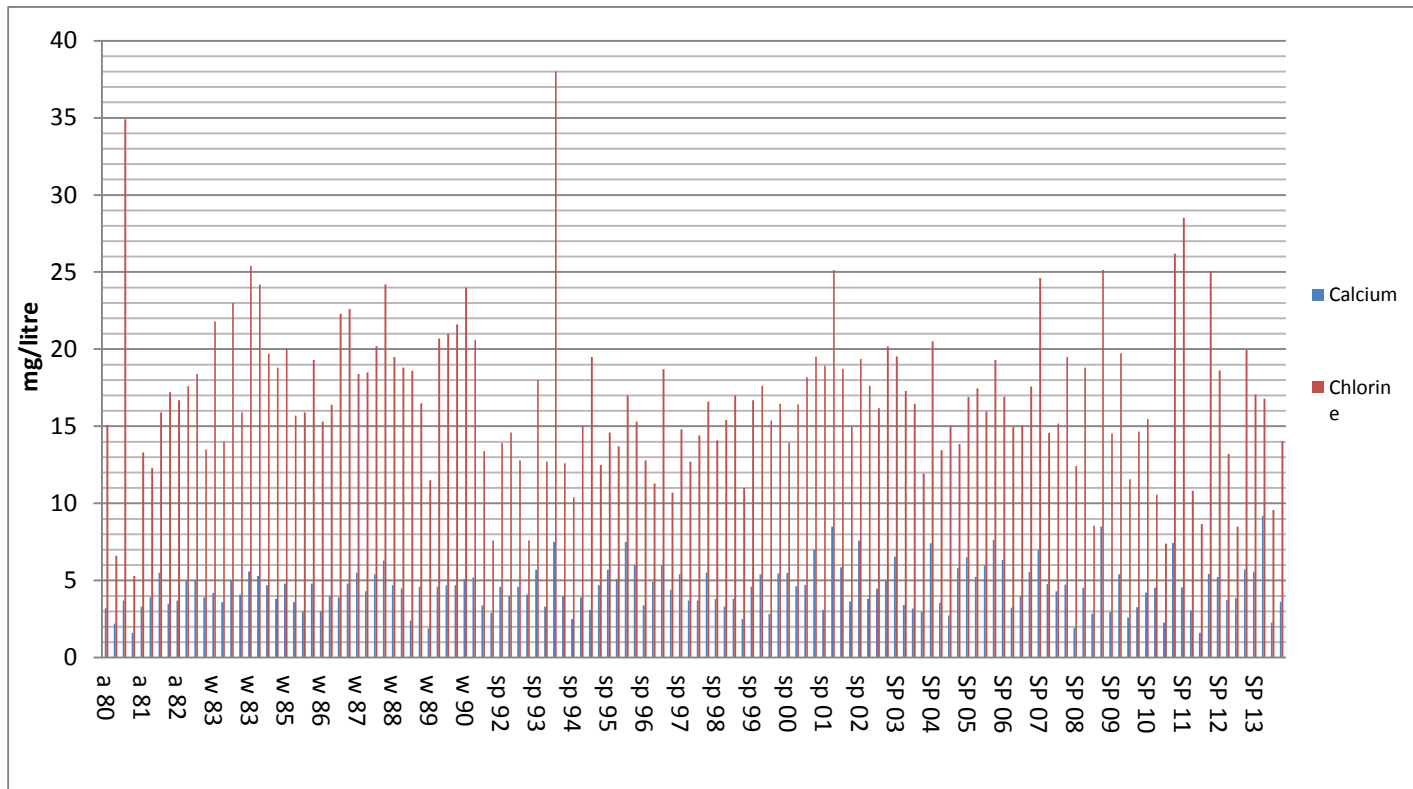


Figure 5: 20 Year average seasonal water quality record: Calcium & Chlorine.

Figure 5 represents calcium, chlorine and fluorine levels along the Berg River over a 20 year period, each take at different points along the river. Calcium levels fluctuate drastically over this period. Figure 5 and 6 show strong linkage between each other as ionic levels align themselves. Figure 5 and 6 show strong seasonality with the calcium readings however increases in calcium concentrations are not season specific. A similar result is noted for chlorine levels.

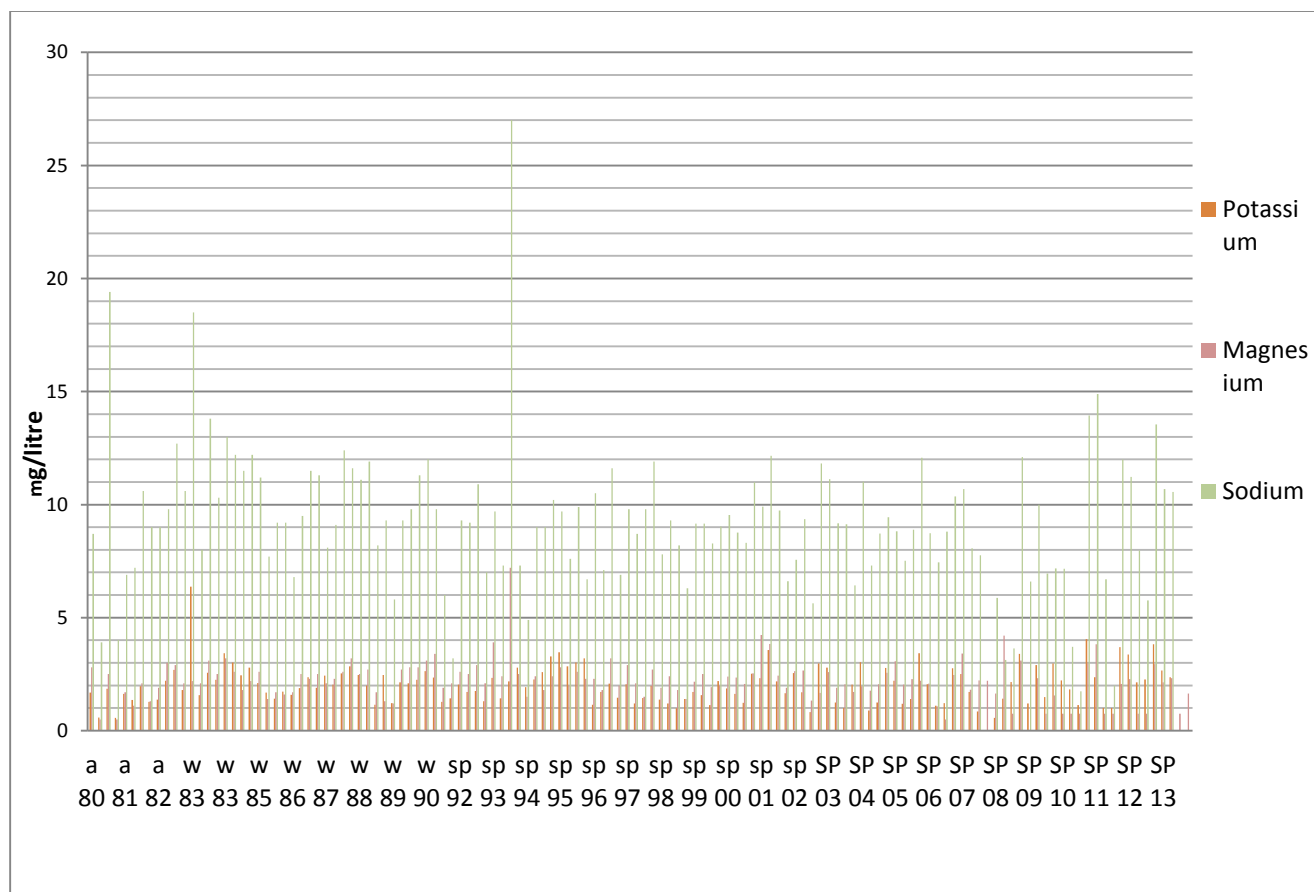


Figure 6: : 20 Year average seasonal water quality record: Potassium, Magnesium & Sodium.

Figure 6 represents fairly constant levels of potassium, magnesium & sodium. A few spikes in sodium are noted in spring 1993 and autumn 1980. Elevated potassium levels seem to correspond well with elevated sodium levels as seen in the graph.

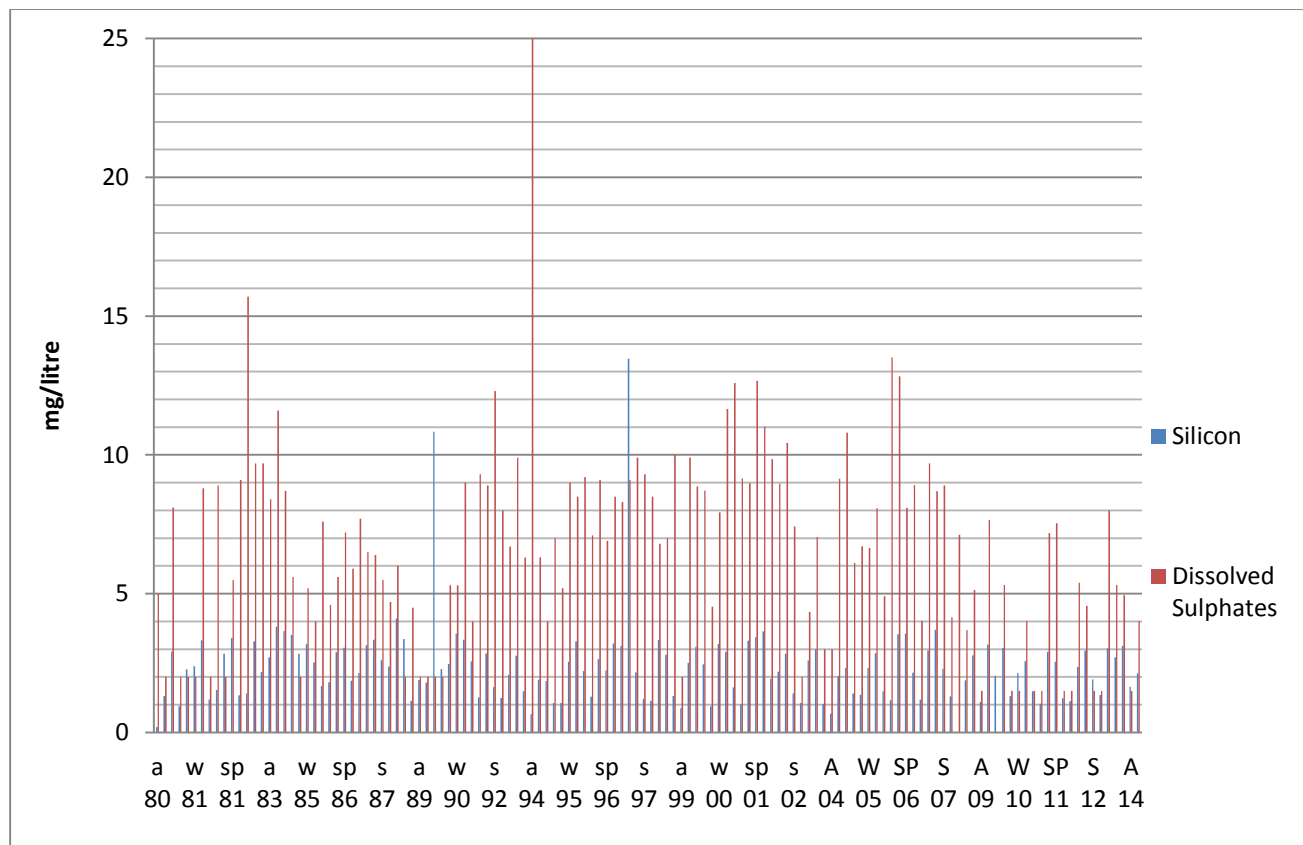


Figure 7: 20 Year average seasonal water quality record: Silicon and Sulphates.

Figure 7 shows erratic changes in water quality along the Berg River. The period between spring 1993 and autumn 2007 showed an elevated nutrient trend. After 2007 the sulphates in particular begins to trend downward. Silicon remains relatively constant over the period with spikes noted during the autumn 2009 and spring 1996. Figures 6 and 7 relate particularly well with sulphate spikes which often correspond to sodium spikes.

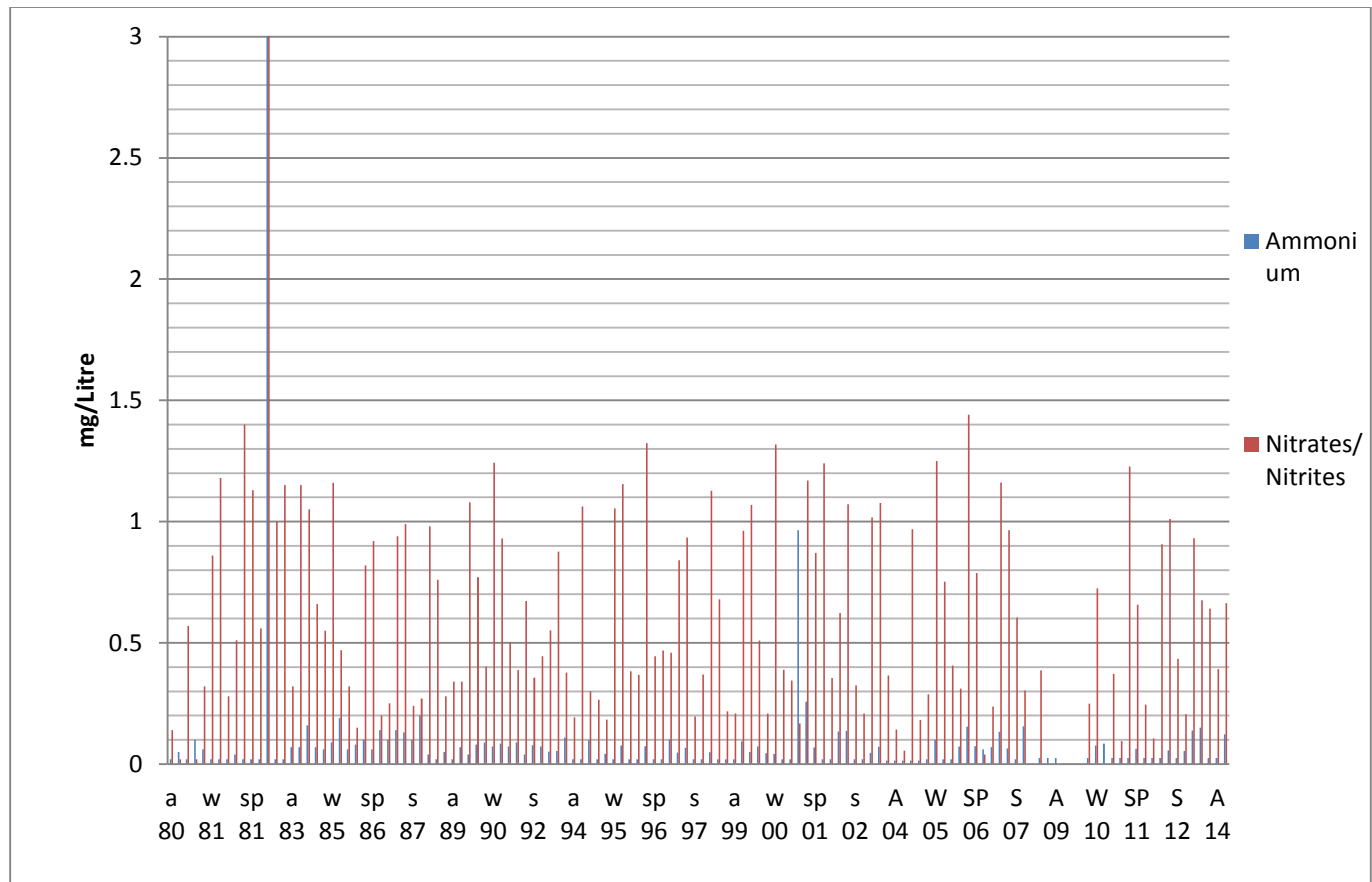


Figure 8: 20 Year Average Seasonal Water Quality Record: NH4 and Nitrites & Nitrates.

Nitrites and to an extent ammonium show a seasonal trend in water quality. Winter corresponds to a period that shows general nitrate increase although there are exceptions to this. Ammonium shows seasonality a particularly over a period between the summer 1983 to autumn 1990.

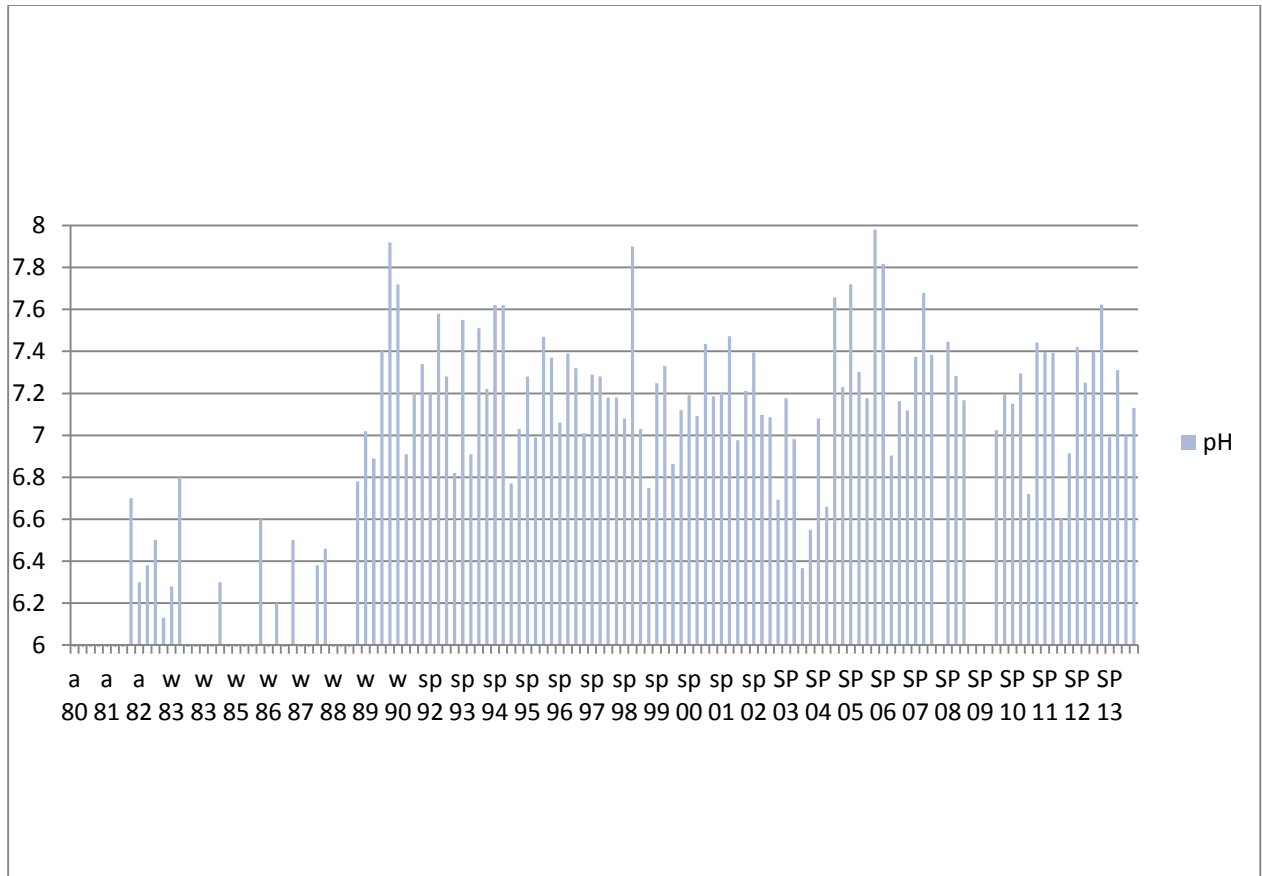


Figure 9: 20 Year average seasonal water quality record: pH.

A strong fluctuation of pH values is noticed in Figure 9. PH values seem to fluctuate between alkaline and acidic conditions, but not necessarily according to season. The pH too seem to fluctuate per decade as during the 1980's it seem to reflect acidic conditions and from the 1990's onwards the pH seem to reflect a more neutral to alkaline condition.

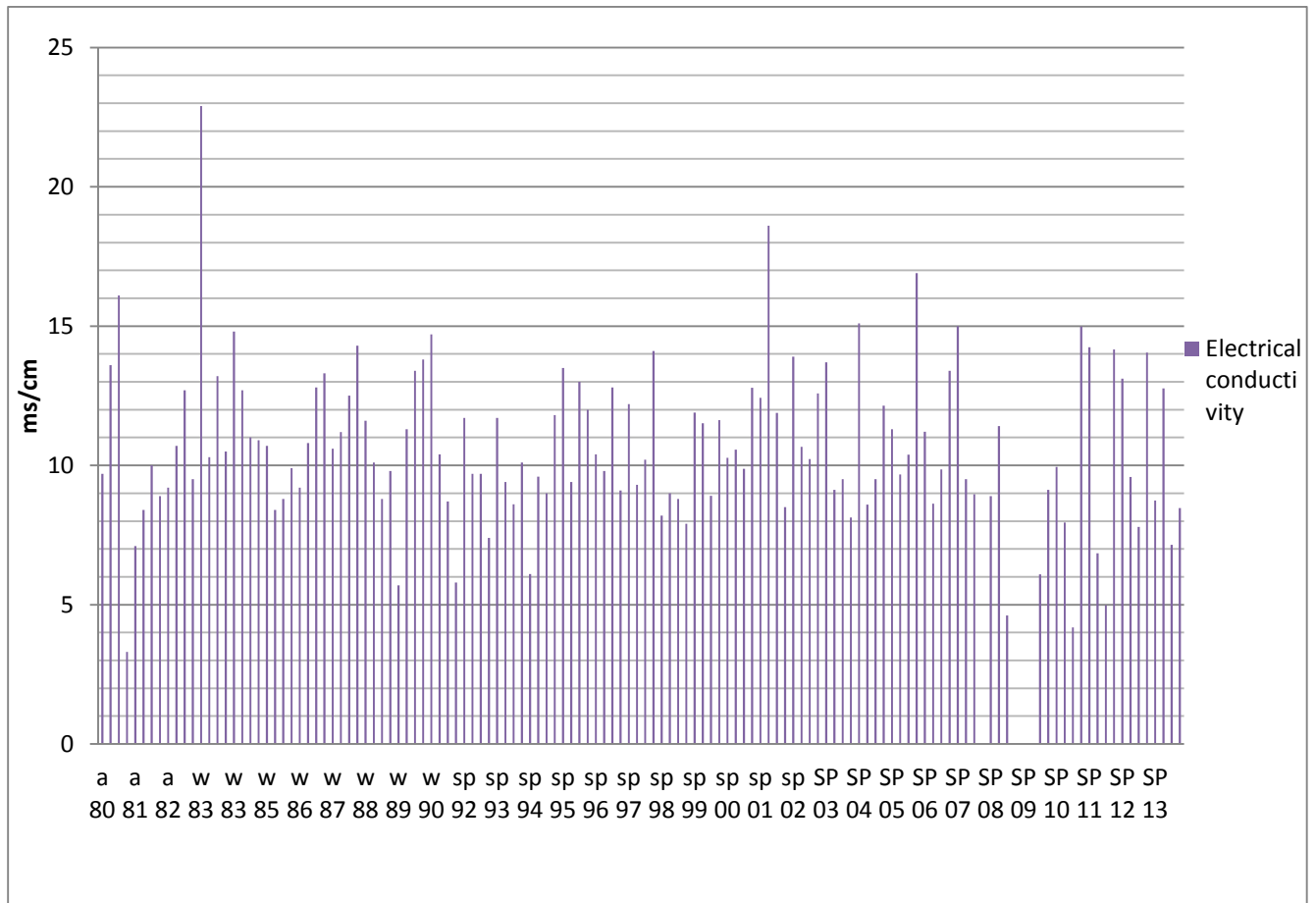


Figure 10: : 20 Year average seasonal water quality record: Electrical Conductivity EC.

The EC values shown in figure 10, show a massive fluctuation in the actual EC values from the EC average. The fluctuation gives a strong indication of the poor water quality conditions associated with the sample site as fluctuations were well above what would be expected from a site with good ecological health.

## Diatoms

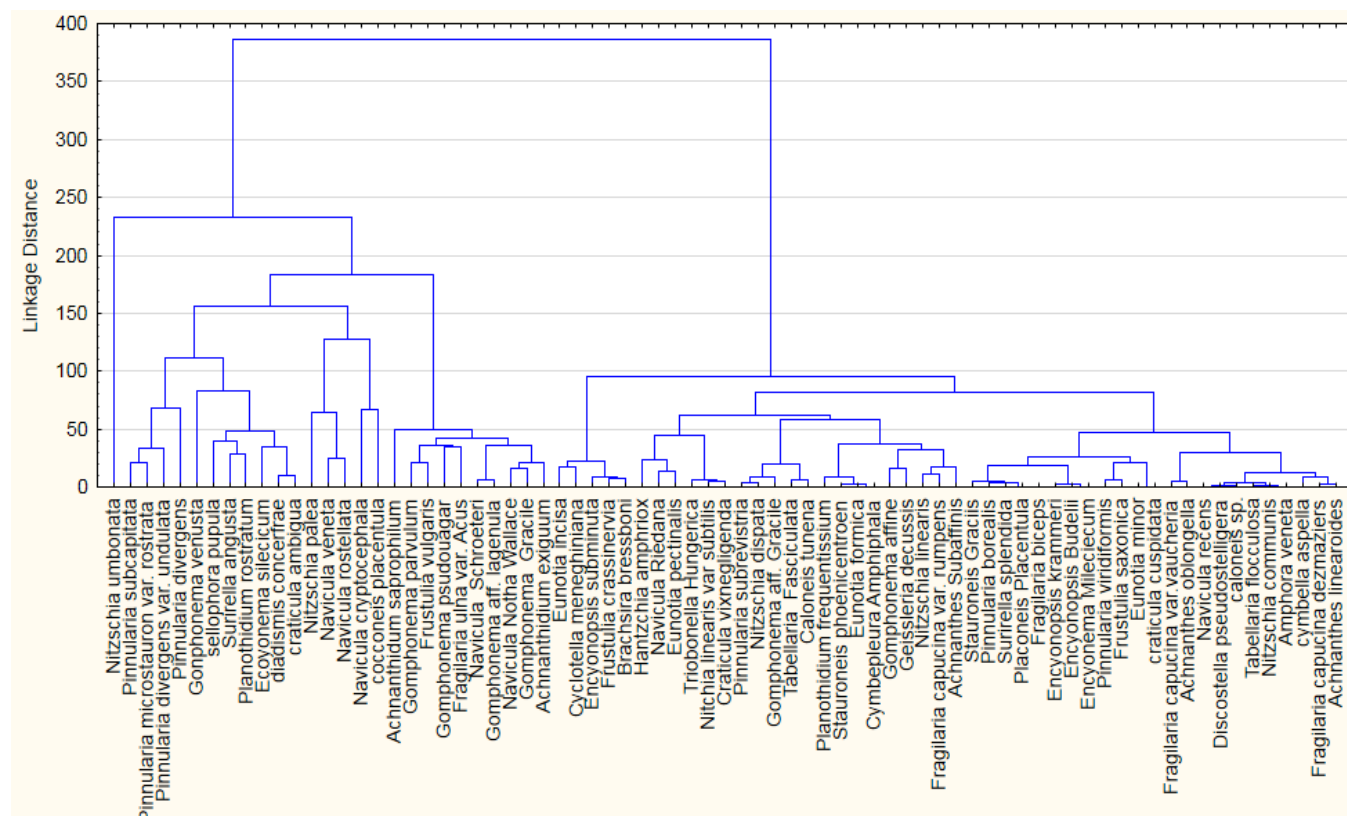


Figure 11: Tree Diagram for 75 variables, Ward's Method, Euclidean distances: Winter Dendrogram.

Winter is split into six clusters when taking the nodal points connecting each branch. Cluster 1 has *Nitzschia umbonata* as the main cluster species. Diatoms present during the winter months are dominated by the *Navicula* as shown by diatom abundances (Addendum D). High numbers of *Navicula cryptocephala* Kützing, *Navicula veneta*, *Nitzschia umbonata*, *Gomphonema venusta* and *Cocconeis placentula* contribute to the water quality during the winter. Species that feature but not as significantly include *Sellaphora pupula* (Kützing) *Mereschkowsky sensu lato*, *Pinnularia divergens* W Smith, *Navicula rostellata* Kützing, *Fragilaria ulna* var. *acus* (Kützing) Lange-Bertalot and *Nitzschia palea*, all contributing over 100 taxon. The high number of pollution tolerant species present during the winter suggest that the water quality was poor. However the presence of non-pollution tolerant species such *Gomphonema venusta* and

*Cocconeis Placentula* suggests that the water quality was at an optimum for those species to survive.

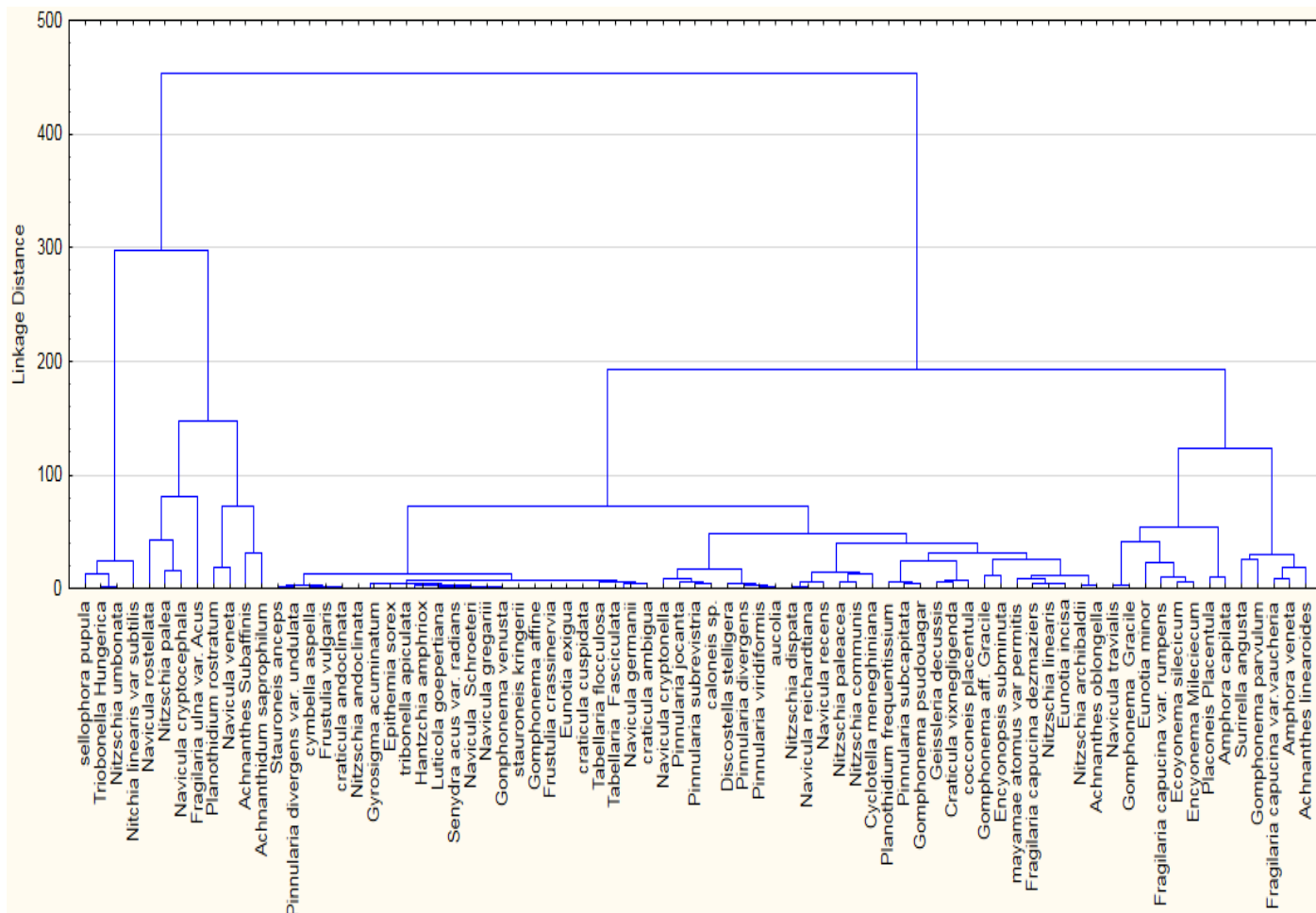


Figure 12: Tree Diagram for 77 variables, Ward's Method, Euclidean distances: Spring Dendrogram.

Spring has four major clusters each making contributions to the population. Clusters are counted by nodal points formed by the first branch split. Pollution tolerant dominant species are favoured during this season as shown in Figure 12. This is made evident by multiple species contributing over 150 taxa to the season and is further dominated by the *Navicula* Genus. Clusters were drawn from the first nodes of each branches. Cluster 1 consists of highly pollution tolerant species. Cluster 2 consists of species associated with eutrophic to highly polluted conditions. Clusters 3 and 4 show no such groupings. The species which contributed the most taxon include *Nitzschia palea*, *Nitzschia umbonata*, *Navicula cryptocephala*, *Navicula roseolata*, *Navicula*

*veneta*, *Fragilaria ulna* var. *arcus* and *Planothidium rostratum* (Oestrup) Round & Bukhtiyarova. Species that still contribute to the water quality and are found in high quantities include *Achnanthes subaffinis* Chohnoky, *Achnanthidium saprophilum* (Kobayasi & Mayama) Round & Bukhtiyarova, *Craticula halophila* and *Surirella splendida*. A high concentration of pollution tolerant species hints at poor water quality with eutrophic water conditions.

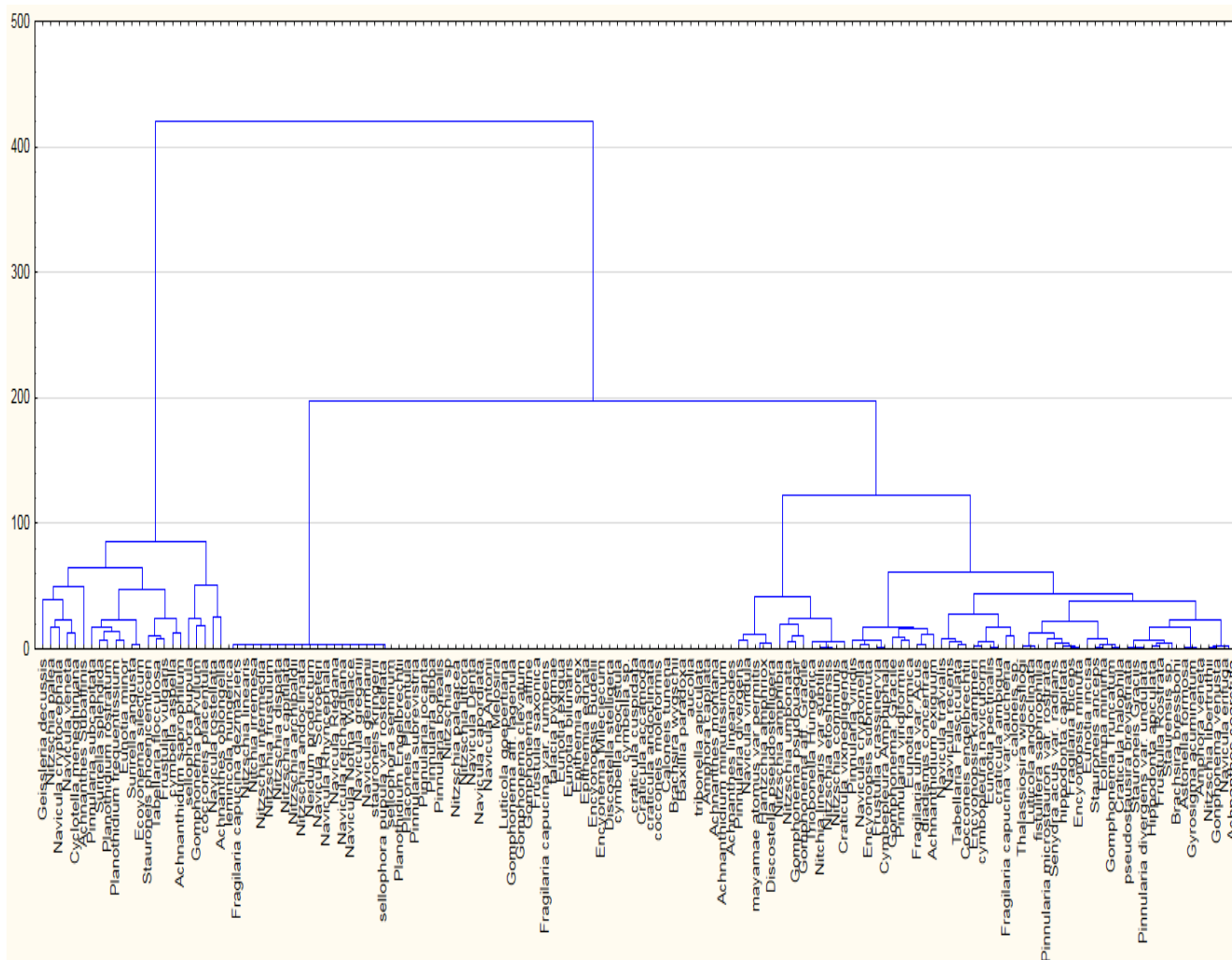


Figure 13: Tree Diagram for 148 variables, Wards Method, Euclidean distances: Summer Dendrogram.

Summer contains multiple clusters (five) with a diverse range of species in the first cluster. *Geissleria decussis* (Hustedt) Lange-Bertalot is the main cluster species. A mixture of highly pollution tolerant and eutrophilic species are favoured during this season (Figure 13). The most

abundant taxon based off table 10 shows a large number of *Geissleria decussis*, *Navicula palea* and *Sellaphora pupula*, all contributing over 150 taxon to the overall water quality. Other less populous species that contribute to the water quality are *Cyclotella meneghiniana* Kützing, *Nitzschia umbonata*, *Navicula veneta*, *Navicula rostellata* and *Cocconeis placentula* all contributing over 100 taxon. The large amounts of pollution tolerant species present during the summer suggest that the water quality during the summer is poor.

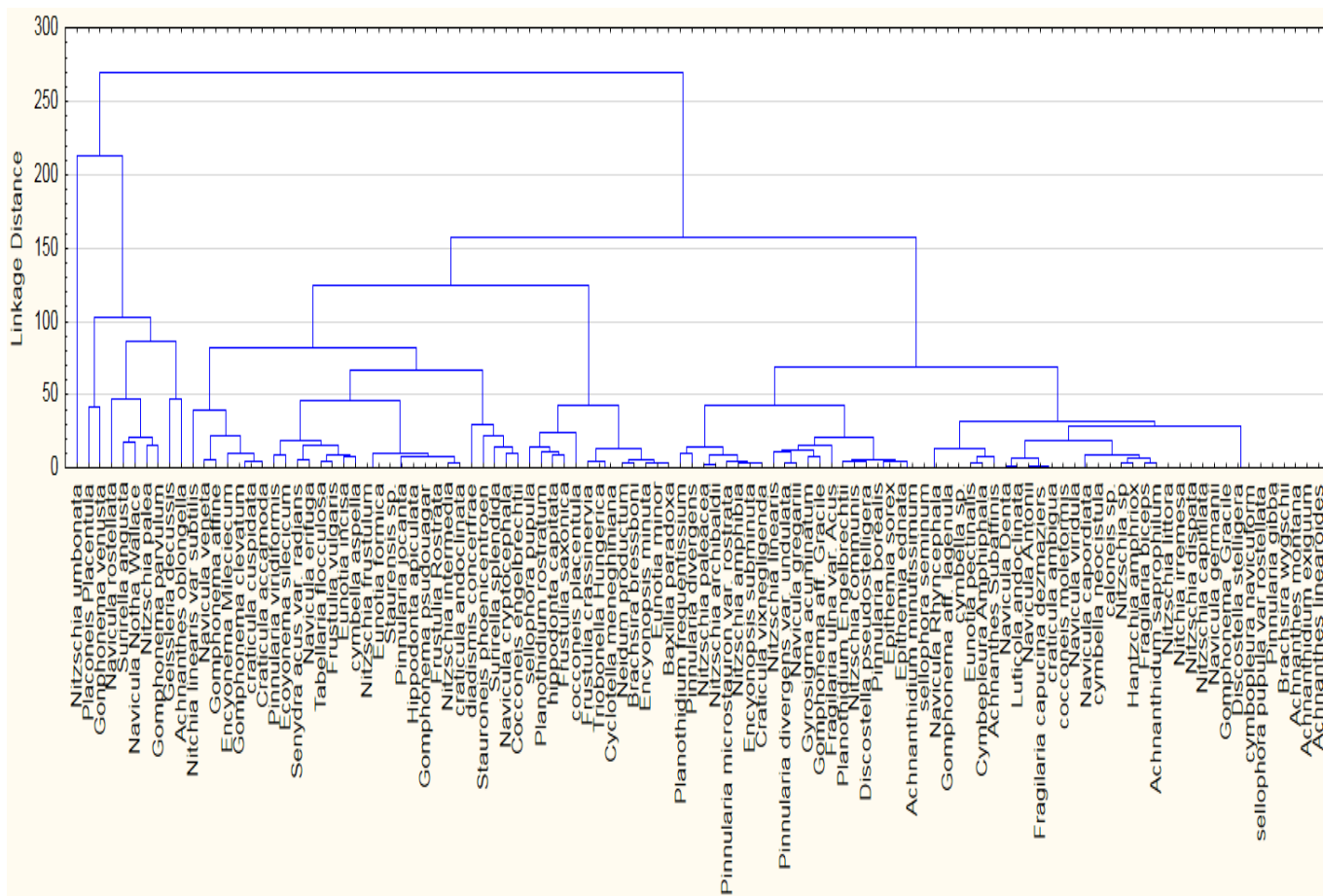


Figure 14: Tree Diagram for 108 variables, Wards Method, Euclidean distances: Autumn Dendrogram

Autumn is split into six major clusters. *Nitzschia umbonata* is the main cluster species, with a mixture of pollution tolerant and moderate pollution species being favoured (Figure 14). According to diatom abundances, *Nitzschia umbonata* is the most common species, accounting

for over 500 taxon to the season with *Geissleria decussis* and *Gomphonema venusta* contributing over 200 taxon each. Further taxonomies that contribute highly to the water quality include *Achnanthes oblongella*, *Cocconeis placentula*, *Navicula rostellata*, *Nitzschia palea* and *Sellaphora pupula*, which are all form a similarity cluster.

### Are the study sites affected by seasonality?

For this project sites 1 (Franschhoek Ref) and site 4 (Paarl Ref) were used to compare how the other 10 sites compared over a 4 season period. Site 1 was used to compare the agricultural zones of 2 and 3, and site 4 was used to compare sites 5 through 12.

Table 2: Winter ANOVA

Sig to #1	R	multiple R <sup>2</sup>	adjusted R	SS MODEL	df	df residual	ms residual	f	p
2	0.486038	0.236233	-0.069274	132.7000	4	10	42.90333	0.773250	0.566882
3	0.661753	0.437918	0.213085	471.9000	4	10	60.57000	1.947746	0.178951
Sig to #4	R	multiple R <sup>2</sup>	adjusted R	SS MODEL	df	df residual	ms residual	f	p
5	0.887800	0.788189	0.576378	1869.90	7	7	71.7857	3.721194	0.052136
6	0.498372	0.248375	-0.503251	1421.10	7	7	614.3571	0.330450	0.916369
7	0.940119	0.883824	0.767648	13034.40	7	7	244.7619	7.607626	0.007863
8	0.766492	0.587510	0.175019	914.40	7	7	91.7143	1.424299	0.326215
9	0.803894	0.646245	0.292490	4946.10	7	7	386.7857	1.826814	0.222503
10	0.926461	0.858330	0.716659	1057.23	7	7	24.9286	6.058644	0.014910
11	0.949202	0.900984	0.801969	3410.77	7	7	53.5476	9.099422	0.004660
12	0.936873	0.877731	0.755462	1783.90	7	7	35.5000	7.178672	0.009283

During the winter, sites 7, 10, 11 and 12 showed significant differences to the reference sites. Site 7 represents a conduit stream representative of Industrial/informal zone and sites 10, 11 and 12 represent an informal residential zone. The significant sites show  $R^2$  values lying between 87 and 90%. This means that at each significant site, 87-90% of the variation can be explained data, which leaves very little uncertainty. The data is suggesting a significant shift in species means possibly linked to increased rainfall. This shift could also be attributed to increased pollution entering the river during the wetter winter months. Diatoms present during the winter months are dominated by the Genus *Gomphonema* accounting for a third of the species available and the Genus *Achnantheidium* contributing about a third of the taxon available at site 1. The most abundant species at site 1 was *Gomphonema gracile Ehrenberg sensu stricto* and *Achnantheidium saprophilum*. The high abundance of these two species suggests water conditions not greater than moderately polluted, with possible electrolyte rich to eutrophic conditions present. Site 4 seemed to have a more even distribution of species with the same species being present. A different species, *Cocconeis placentula* was also present in high numbers. A sound assumption can be made then that site 1 and 4 have similar water quality conditions during the winter.

The biggest changes during the winter were noted at site 7, 10, 11 and 12. Site 7 had a high abundance of then *Nitzschia umbonata* taxon suggesting a heavily polluted environment. Site 10 showed a high abundance of *Gomphonema venusta* and *Nitzschia umbonata*, suggesting possibly a heavy pollution content with shifting oxygen concentrations with a possibly shift towards alkaline pH. *Gomphonema venusta* is a known species to indicate agricultural pollution influences. Site 11 and 12 show a very even distribution of Diatoms, with an abundance of *Cocconeis placentula* and possibly a spike in *Nitzschia umbonata* and *Navicula notha Wallace*. This suggests a possible similarity between site 1, 11 and 12.

Table 3: Spring ANOVA

Sig to #1	R	multiple R <sup>2</sup>	adjusted R	SS MODEL	df	df residual	ms residual	f	p
2	0.704435	0.496229	0.227551	1506.530	8	15	101.9619	1.846927	0.145556
3	0.800672	0.641076	0.449650	1075.619	8	15	40.1476	3.348950	0.020919
Sig to #4	R	multiple R <sup>2</sup>	adjusted R	SS MODEL	df	df residual	ms residual	f	p
5	0.911768	0.831322	0.676700	3997.792	11	12	67.5972	5.37649	0.003619
6	0.966908	0.934911	0.875246	5154.944	11	12	29.9074	15.66941	0.000019
7	0.397433	0.157953	-0.613923	946.667	11	12	420.5556	0.20464	0.993392
8	0.423265	0.179153	-0.573290	432.833	11	12	165.2639	0.23809	0.988078
9	0.446923	0.199740	-0.533832	793.333	11	12	264.8750	0.27228	0.980404
10	0.987662	0.975477	0.952997	2625.333	11	12	5.5000	43.39394	0.000000
11	0.709410	0.503262	0.047919	3793.403	11	12	312.0185	1.10524	0.430698
12	0.786452	0.618506	0.268804	2313.111	11	12	118.8935	1.76867	0.170677

During the spring, sites 3, 5, 6 and 10 showed significant differences to the reference sites. These sites represent agricultural at 3 (mainly vineyards and pastures), residential at 5, industrial at 6 and informal residential at 10. This could be possibly be from increased pollution entering the river at the industrial or residential site. Site 3 shows a R<sup>2</sup> value of 64.1 %, which shows that 64.1% of the variance is explained by the data. This still leaves a high percentage of variance unexplained. From site 4, the R<sup>2</sup> values range from 83-97%, which means that at least 83% of the variance between site 4 and the other sites are explained by the data.

Table 4: Summer ANOVA

Sig to #1	R	multiple R <sup>2</sup>	adjusted R	SS MODEL	df	df residual	ms residual	f	p
2	0.815125	0.664429	0.424736	1507.644	15	21	36.25893	2.771995	0.015993
3	0.778586	0.606197	0.324909	1701.644	15	21	52.63988	2.155075	0.052069
Sig to #4	R	multiple R <sup>2</sup>	adjusted R	SS MODEL	df	df residual	ms residual	f	p
5	0.867926	0.753296	0.613854	903.71	13	23	12.8681	5.402232	0.000226
6	0.781041	0.610025	0.389605	694.67	13	23	19.3081	2.767552	0.015950
7	0.866337	0.750540	0.609540	12651.18	13	23	182.8231	5.323000	0.000252
8	0.824468	0.679748	0.498735	1729.83	13	23	35.4340	3.755258	0.002764
9	0.799027	0.638444	0.434087	1833.16	13	23	45.1362	3.124156	0.008276
10	0.630300	0.397278	0.056610	690.90	13	23	45.5731	1.166172	0.360673
11	0.815936	0.665751	0.476827	631.60	13	23	13.7871	3.523920	0.004092
12	0.781356	0.610517	0.390375	652.53	13	23	18.0993	2.773283	0.015780

The most significant changes when compared to sites 1 and 4, were noted during the summer months where 8 out the 10 sites fell within the critical p value range. This means the sites showed significant differences when compared to the reference sites. When compared to site 1, site showed an R<sup>2</sup> value of 66%, which shows that 66% of the variance in means between site 1 and 2 is represented by the data and 34% is not. From site 4, The R<sup>2</sup> value ranges between

61-76%, explaining the variance between the sites and the reference (site 4). Although the p values are sufficiently small, a large percentage of the variance is left unexplained by the data.

Table 5: Autumn ANOVA

Sig to #1	R	multiple R <sup>2</sup>	adjusted R	SS MODEL	df	df residual	ms residual	f	p
2	0.837215	0.700928	0.581300	2459.727	8	20	52.47571	5.859203	0.000634
3	0.839123	0.704128	0.585779	2066.444	8	20	43.41571	5.949586	0.000576
Sig to #4	R	multiple R <sup>2</sup>	adjusted R	SS MODEL	df	df residual	ms residual	f	p
5	0.872078	0.760520	0.664728	545.476	8	20	8.5883	7.939274	0.000085
6	0.288363	0.083153	-0.283586	385.079	8	20	212.2943	0.226737	0.981457
7	0.351003	0.123203	-0.227515	2683.213	8	20	954.7773	0.351288	0.934021
8	0.213688	0.045662	-0.336073	235.612	8	20	246.2125	0.119618	0.997771
9	0.385090	0.148294	-0.192388	2764.918	8	20	793.9955	0.435286	0.885780
10	0.343347	0.117887	-0.234958	2054.948	8	20	768.8250	0.334105	0.942403
11	0.604973	0.365992	0.112389	705.355	8	20	61.0943	1.443168	0.239399
12	0.810511	0.656928	0.519699	1370.850	8	20	35.7955	4.787094	0.002116

During the Autumn, sites 2, 3, 5 and 12 showed significant differences to the reference site. Site 2 and 3 represent agricultural sites, 5 residential and 12 represents Informal residential. Significance values seemed to show that significant sites are shifting upstream. This could possibly be attributed to an increase farm runoff due to irrigation in over the drier periods. From site 1, sites 2 and 3 have R<sup>2</sup> values ranging from 70.1- 70.5%, explaining the variance between the means of the sites. Around 29% of the variance is left unexplained. From site 4, sites 5 and

12 showed  $R^2$  values between 65 -75%, explaining the variance between the means at each site, although there is some variance that is left unexplained.

Areas that showed the most significant change to the respective reference sites were site 5 (3 out of 4 seasons) and site 12 (3 out of 4) with a further 6 sites affected by 2 seasons. Numerous factors can influence this. At site 5 possible influences may include urban runoff, upstream influences, stormwater. At site 12, possible influences may include upstream influences, social issues surround informal settlements, poor ablution facilities, increased runoff and nutrient contents.

### **How are diatom populations affected by land-use ?**

During a period of winter, spring and summer, a period stretching from about July 2013 to January 2014, site 1 (Franschhoek Reference) has a p value that is less than the pCrit value and falls in the significance range. The result of this test is that the null hypothesis of  $H_0: S1=S4$  is rejected and the premise that  $S4 \diamond S1$  is accepted. This result is permissible as S1 is situated on the outskirts of the town of Franschhoek and S4 is situated on the rural/urban fringe of Paarl. It is expected that the agricultural zone between S3 and S4 will have a changing effect on diatom populations. This test shows that S1 can be considered to be a reference site and S4 can be used to reflect agricultural influence on the rural urban fringe, as  $S1 \diamond S4$ .

The 12 sites reflect varying stages of land-use change. Site 1 is a reference upstream of Franschhoek. Site 2 and 3 reflect an agricultural zone of vines and pastures. Site 4 can now be considered to be the rural/urban fringe of Paarl. Site 5 represents a change into a residential area and site 6 represents an industrial zone. Sites 7 , 8 and 9 represent industrial/informal housing and sites 10 , 11 and 12 represent informal housing.

Table 6: Species Diversity to Reference site 1 as shown by ANOVA for Winter.

Sig to #1	R	multiple R <sup>2</sup>	adjusted R	SS MODEL	df	df residual	ms residual	f	p
2	0.486038	0.236233	-0.069274	132.700	4	10	42.9033	0.7732	0.566882
3	0.661753	0.437918	0.213085	471.900	4	10	60.5700	1.9477	0.178951
4	0.995482	0.990985	0.987379	1725.900	4	10	1.5700	274.8248	0.000000
5	0.576035	0.331816	0.064542	787.200	4	10	158.5200	1.2415	0.354170
6	0.284897	0.081166	-0.286367	464.400	4	10	525.7200	0.2208	0.920700
7	0.599011	0.358814	0.102340	5291.700	4	10	945.6033	1.3990	0.302800
8	0.321727	0.103508	-0.255089	161.100	4	10	139.5300	0.2886	0.878764
9	0.505225	0.255252	-0.042647	1953.600	4	10	570.0000	0.8568	0.521516
10	0.302261	0.091362	-0.272094	112.533	4	10	111.9200	0.2514	0.902314
11	0.862413	0.743757	0.641260	2815.567	4	10	97.0033	7.2564	0.005213
12	0.695071	0.483123	0.276373	981.900	4	10	105.0500	2.3367	0.126010

During the winter, critical values when compared to Site 1, are present at sites 4 and 11. Applying hypothesis testing we are able to reject the null hypothesis that the population means at site 1 are equal at sites 4 and 12, and accept the alternate hypothesis that they are not equal. Thus site 1 showed significant differences between site 4 (urban/agricultural fringe) and site 11 (informal residential).

Table 7: Species Diversity to Reference site 1 as shown by ANOVA for Spring.

Sig to #1	R	multiple R <sup>2</sup>	adjusted R	SS MODEL	df	df residual	ms residual	f	p
2	0.704435	0.496229	0.227551	1506.530	8	15	101.9619	1.84693	0.145556
3	0.800672	0.641076	0.449650	1075.619	8	15	40.1476	3.34895	0.020919
4	0.456632	0.208513	-0.213614	1845.744	8	15	467.0810	0.49396	0.842152
5	0.711251	0.505878	0.242346	2432.744	8	15	158.4143	1.91961	0.131536
6	0.353342	0.124850	-0.341896	688.405	8	15	321.6952	0.26749	0.967205
7	0.977947	0.956380	0.933116	5731.905	8	15	17.4286	41.10997	0.000000
8	0.628573	0.395104	0.072493	954.571	8	15	97.4286	1.22471	0.349497
9	0.732501	0.536558	0.289389	2131.119	8	15	122.7143	2.17081	0.093127
10	0.239578	0.057398	-0.445324	154.476	8	15	169.1238	0.11417	0.997878
11	0.690587	0.476910	0.197928	3594.768	8	15	262.8571	1.70947	0.176517
12	0.386904	0.149695	-0.303801	559.833	8	15	212.0000	0.33009	0.941095

During the spring, critical values when compared to site 1, are present at sites 3 and 7. When compared from site 4, sites 1, 3, 5, 6 and 10 are shown to have critical values with p values less than the stipulated 0.05 alpha. Applying hypothesis testing, considerable differences were noted between the sites when compared to site 4 as opposed to site 1. Site 1 showed significant differences between site 3 (agricultural) and site 7 (Informal residential/industrial). Site 4 showed significant differences between sites 1 (reference), site 3 (agricultural), site 5 (residential), site 6 (industrial) and site 10 (informal residential).

Table 8: Species Diversity to Reference site 1 as shown by ANOVA for Summer.

Sig to #1	R	multiple R <sup>2</sup>	adjusted R	SS MODEL	df	df residual	ms residual	f	p
2	0.815125	0.664429	0.424736	1507.644	15	21	36.2589	2.771995	0.015993
3	0.778586	0.606197	0.324909	1701.644	15	21	52.6399	2.155075	0.052069
4	0.616160	0.379653	-0.063452	1480.277	15	21	115.1786	0.856801	0.614275
5	0.686676	0.471524	0.094041	565.676	15	21	30.1905	1.249126	0.312801
6	0.705516	0.497753	0.139005	566.819	15	21	27.2351	1.387471	0.239825
7	0.704822	0.496774	0.137326	8373.671	15	21	403.9256	1.382048	0.242371
8	0.647180	0.418842	0.003729	1065.873	15	21	70.4256	1.008983	0.481967
9	0.681451	0.464375	0.081786	1333.360	15	21	73.2351	1.213771	0.334261
10	0.613540	0.376431	-0.068976	654.644	15	21	51.6399	0.845140	0.624929
11	0.677688	0.459262	0.073020	435.703	15	21	24.4286	1.189052	0.349972
12	0.762437	0.581310	0.282246	621.311	15	21	21.3095	1.943766	0.079195

During the summer, critical values when compared to site 1, are present at site 2. When compared from site 4, sites 1, 2, 5, 6, 7, 8, 9, 11 and 12 are shown to have critical values with p values less than the stipulated 0.05 alpha. Applying hypothesis testing, results show differences between the sites when compared to site 4 as opposed to site 1. Site 1 showed significant differences between site 2 (agricultural site). Site 4 showed significant differences between 8 out of the 11 sites including site 2.

Table 9: Species Diversity to Reference site 1 as shown by ANOVA for Autumn.

Sig to #1	R	multiple R <sup>2</sup>	adjusted R	SS MODEL	df	df residual	ms residual	f	p
2	0.837215	0.700928	0.581300	2459.727	8	20	52.476	5.85920	0.000634
3	0.839123	0.704128	0.585779	2066.444	8	20	43.416	5.94959	0.000576
4	0.828440	0.686312	0.560837	1246.438	8	20	28.485	5.46971	0.000968
5	0.815174	0.664508	0.530312	476.613	8	20	12.031	4.95175	0.001743
6	0.949354	0.901273	0.861783	4173.766	8	20	22.860	22.82243	0.000000
7	0.275045	0.075650	-0.294090	1647.559	8	20	1006.560	0.20460	0.986563
8	0.779326	0.607348	0.450288	3133.833	8	20	101.301	3.86697	0.006677
9	0.257286	0.066196	-0.307326	1234.213	8	20	870.531	0.17722	0.991546
10	0.319062	0.101801	-0.257479	1774.534	8	20	782.846	0.28335	0.963808
11	0.507367	0.257421	-0.039610	496.113	8	20	71.556	0.86665	0.559366
12	0.769054	0.591444	0.428022	1234.201	8	20	42.628	3.61912	0.009286

During the autumn, critical values when compared to Site 1, are present at Sites 2, 3, 4, 5, 6, 8 and 12. When compared from site 4, sites 2, 5 and 12 are shown to have critical values with p values less than the stipulated 0.05 alpha. Applying hypothesis testing, differences were noted between the sites when compared to site 1 as opposed to site 4. Site 1 showed significant differences between 7 out of 11 sites. These sites included site 2 & 3 (agricultural), site 4 (urban/agricultural fringe), site 5 (residential), site 6 (industrial), 8 (informal residential/industrial) and 12 (informal residential). Site 4 showed significant differences between sites 2 (agricultural), site 5 (residential) and site 12 (informal residential).

### Do diatom indices indicate any seasonality in the data?

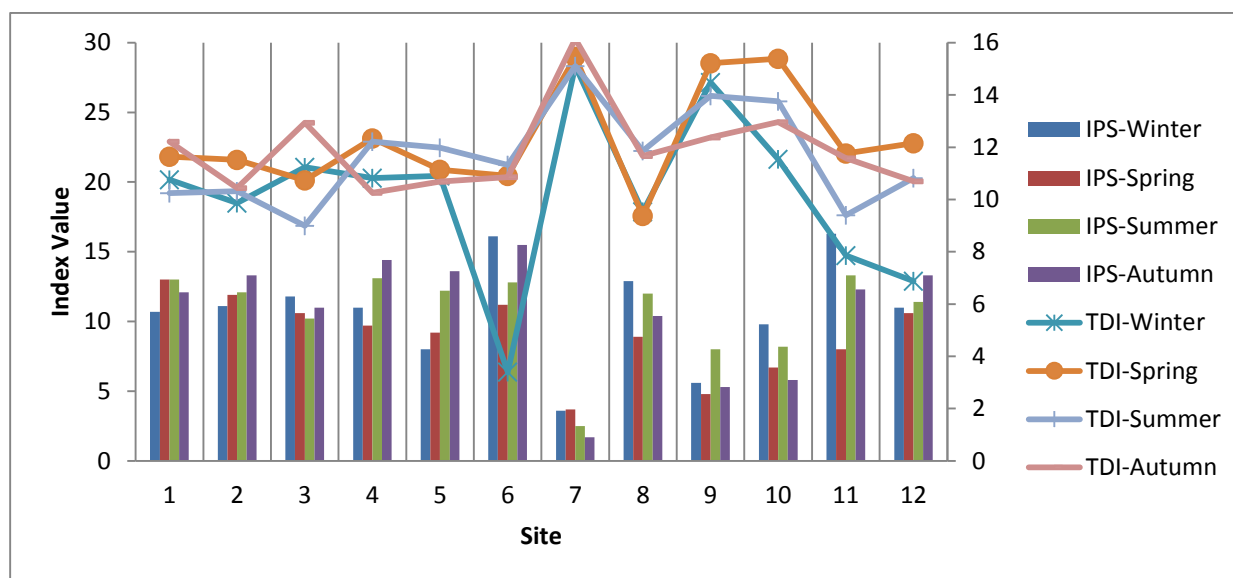


Figure 15: Index seasonality: IPS vs TDI.

Seasonal fluctuations are very apparent from this graph (Figure 15). Figure 15 shows that some sites show a similar seasonal response to changing seasons. Specific Polluosensitivity Index (IPS) shows similar indices changes at sites 4, 6 and 12, with another site similarity at 8 and 11. Sites 2 and 5 too seem to link well with each other showing similar changes. The remaining sites all showed random indices changes, which cannot be associated with another site. The Trophic Diatom Index (TDI) graph (Figure 15), allows for a site specific seasonal comparison. It is apparent then that seasonality does play an important role as seasonal diatom indexes appear to shift. Site similarity is less visible looking at TDI. However TDI does give an indication on how pollution affects the river seasonally. It appears then that the river water quality improves downstream during the winter, with particular emphasis at site 6. Site 7 appears to be a particularly polluted site, showing increased indices values each season. Linked to site 7 is site 9, showing the same seasonal pollution spikes that are so apparent at site 7. The TDI graph shows strong similarities between fluctuations in the spring and summer periods. Autumn seems to reflect a period of constant water quality as much less pollution spike are noted. Considering pollution levels over the stretch of the river, it is noted that an improved water quality is noted

between sites 1 and 12 in the winter, and the water quality remains mostly the same (when compared to its respective reference) if not slightly poorer during the rest of the year.

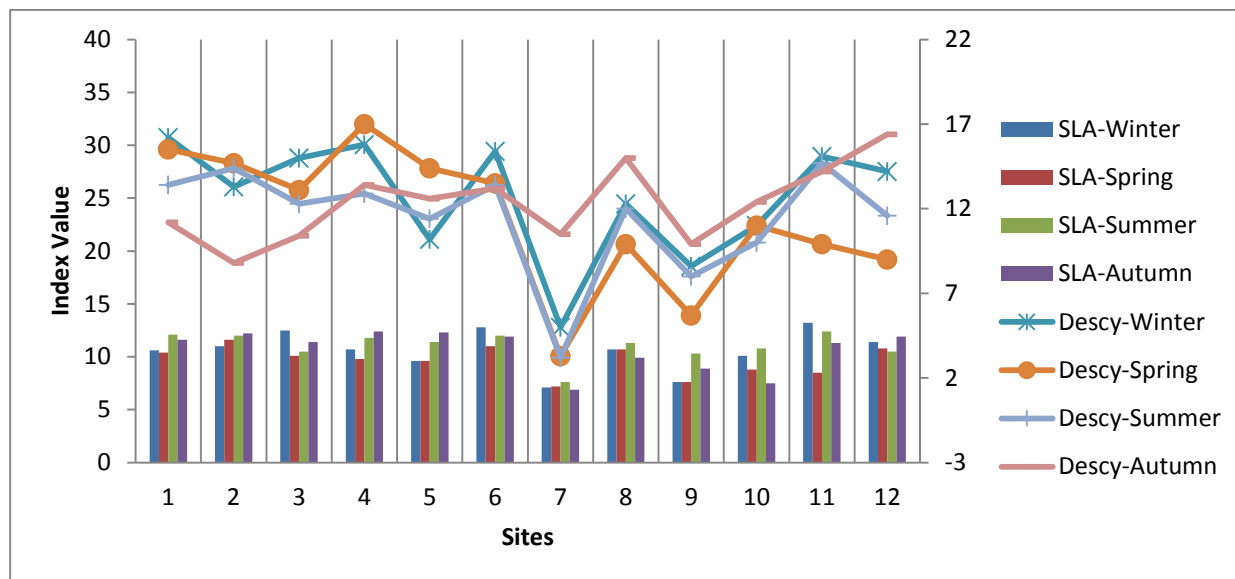


Figure 16: Index seasonality: SLA vs Descy.

Seasonal fluctuations are apparent from Figure 16. Sites 4, 6 and 12 still show similar index changes when compared to SLA. Some other sites such as site 5 and 8 show similar index trends, differ slightly over some seasons. The rest of the sites show random seasonal changes, although sites 1 to 6 represent minute fluctuations in the indices.

The Descy Index graph (Figure 16), shows in particular water quality of the stretch of the river. Descy and TDI show seasonal fluctuations at site 1-6 but these do not necessarily have drastic effects on the overall water quality. This means these sites lie within a similar water quality range. In winter, water quality diminishes as it leaves the reference and gradually returns to a similar index value at site 12. Spring's water quality diminishes to such an extent that it is unable to return to the quality seen in the reference. A similar graph is noted during the summer, although the water quality does improve significantly during this period. The autumn sees the water quality exceeding that of the reference at site 1, however the reference value is not particularly

high to begin with. Sites 7 to 12 show a steadily increasing water quality, with pollutions spikes noted at 7, 9 and 10. Sites 11 and 12 do reflect somewhat similar qualities to the upstream sites.

Comparing both SLA and Descy, it is apparent then that water quality/pollution levels improves during the winter and remains constant if not gets slightly more polluted during the rest of the year. Autumn has the least variation amongst the index graphs, suggesting less variable changes over this period.

Table 10: Diatom Index Scores.

Season	Site	SLA	DESCY	IDSE/5	TDI	IPS	EPI-D	Key	bad	good
<b>Winter</b>	1	1.98	4.2	2.96	53.8	3.05	2.09	SLA	0	4
	2	1.9	3.6	3.06	49.3	3.12	1.84	DESCY	0	5
	3	1.58	3.94	3.06	56.2	3.28	1.97	IDSE	0	5
	4	1.96	4.12	2.91	54.1	3.11	2.07	TDI	100	0
	5	2.19	2.93	2.53	54.5	2.47	2.02	IPS	0	5
	6	1.51	4.04	3.64	17	4.18	1.03	EPI-D	5	0
	7	2.71	1.84	1.79	75.5	1.55	3.23			
	8	1.97	3.38	3.3	47.7	3.52	1.58			
	9	2.61	2.59	2.31	72.4	1.96	2.74			
	10	2.08	3.1	2.69	57.7	2.86	2.06			
	11	1.42	3.96	3.73	39.3	4.23	1.42			
	12	1.82	3.79	3.15	34.4	3.1	1.74			
<b>Spring</b>	1	2.01	4.05	3.23	58.2	3.53	1.76			
	2	1.77	3.89	3.01	57.6	3.29	2.07			
	3	2.09	3.54	2.94	53.7	3.02	2.07			
	4	2.15	4.37	2.9	61.7	2.84	1.95			
	5	2.18	3.83	2.84	55.7	2.73	1.9			
	6	1.9	3.62	3.04	54.5	3.15	1.72			
	7	2.69	1.48	1.86	77.3	1.57	2.98			
	8	1.96	2.87	2.69	46.9	2.66	1.96			
	9	2.61	2	2.12	76.1	1.79	2.87			
	10	2.37	3.11	2.34	76.9	2.19	2.96			
	11	2.42	2.86	2.6	58.8	2.46	2.13			
	12	1.94	2.68	3.01	60.8	3.02	1.86			
<b>Summer</b>	1	1.66	3.62	3.64	51.2	3.53	1.61			
	2	1.69	3.82	3.31	51.6	3.34	1.74			
	3	1.99	3.39	3.08	45	2.94	1.8			
	4	1.72	3.5	3.13	61.1	3.54	1.84			
	5	1.82	3.18	3.26	59.9	3.37	1.67			
	6	1.69	3.6	3.41	56.6	3.49	1.68			
	7	2.61	1.47	2.09	75.5	1.32	2.95			
	8	1.83	3.32	3.08	59.3	3.31	1.83			
	9	2.05	2.47	2.65	69.8	2.48	2.32			
	10	1.94	2.9	2.7	68.8	2.52	2.28			
	11	1.59	3.88	3.57	47	3.58	1.29			
	12	1.99	3.24	3.16	54.1	3.19	1.92			
<b>Autumn</b>	1	1.77	3.14	3.27	61.1	3.33	1.68			
	2	1.65	2.64	3.52	52.2	3.58	1.66			
	3	1.81	2.99	2.92	64.7	3.11	1.9			
	4	1.6	3.62	3.62	51.3	3.82	1.28			
	5	1.62	3.44	3.38	53.4	3.65	1.34			
	6	1.7	3.56	3.76	54.3	4.06	1.29			
	7	2.76	3	1.59	80.7	1.14	3.54			
	8	2.12	3.95	2.87	58.3	2.99	1.88			
	9	2.33	2.87	2.04	61.9	1.91	2.69			
	10	2.63	3.39	1.88	64.8	2	2.77			
	11	1.84	3.77	3.32	57.9	3.39	1.58			
	12	1.71	4.25	3.58	53.5	3.59	1.55			

Table 11: IPS Index values.

IPS Index Values	Condition
3.5-4	Weak pollution
4-4.5	Moderate pollution
4.5<	No pollution

Table 12: EPI-D index values.

EPI-D Index Values	Water quality conditions
0-1	Excelent water quality
1-1.5	Good Water quality
1.5-1.8	Satisfactory water quality
1.8-2	Lightly pollluted
2-2.2	moderately polluted
2.2-2.5	Heavily polluted
2.5<	Very heavily polluted

## Site summary

### Site 1-3

Specific Pollution Sensitivity Index (IPS) at site 1, shows some change of the four seasons with heavily polluted category (2.95) in the winter and autumn (3.32) and moderately polluted category (3.52) in the spring and summer. Eutrophication-Pollution Diatom Index (EPI-D) values shows moderately polluted conditions during the winter (2.09) and then satisfactory conditions (1.64-1.70) during the rest of the year. IPS values at site 2, The water conditions remain in the heavily polluted category between winter and summer (3.1-3.32) and shift to moderately polluted in autumn (3.57). EPI-D values shows lightly polluted conditions during the winter (1.86) and moderately polluted conditions in the spring (2.08) and then satisfactory conditions (1.66-1.75). All IPS indice values for site 3 obtained lie within the heavily polluted range between winter and autumn, with the lowest value obtained during the summer. EPI-D values shows moderately polluted conditions during the winter (2.13) and spring (2.07) and lightly polluted conditions in the summer (1.82) and autumn (1.92). Van Dam shows that all three site remain eutrophic during each season.

### Site 4-5

All IPS indices at site 4 show heavily polluted conditions in the winter and spring, with moderate conditions achieved during the summer and autumn. EPI-D values show moderately polluted conditions during the winter (2.07), light pollution conditions in the spring (1.95) and summer (1.86) and good water quality in the autumn (1.3). A very low IPS value in the winter (2.94) and (2.84) in the spring suggests a possible upstream pollution problem, also highlighted by the EPI-D value (2.33). Through all seasons the site remains eutrophic. IPS indices for site 5 show heavily polluted conditions in the winter (2.38), spring (2.73) and summer (3.35), but improving to moderate pollution in the autumn (3.64). EPI-D values show moderate polluted conditions during the winter (2.08), light pollution conditions in the spring (1.95), satisfactory conditions in the summer (1.69) and good water quality in the autumn (1.36). During the winter, Van Dam shows that site 5 become hyper-eutrophic and eutrophic in the spring and autumn. Values achieved at site 5 seem strongly linked to values shown at site 4.

## Site 6

IPS shows fairly good quality water at this site. The winter (4.18) and autumn (4.06) sees weak pollution content with Spring (3.15) showing heavily polluted conditions and summer (3.5) showing moderate pollution conditions. The IPS pollution spike can possibly be attributed in the spring to upstream pollution incidences, which is made more evident by sites 4 and 5. EPI-D shows good water condition during the winter (1.03), satisfactory conditions in the spring (1.72) and summer (1.68) and good water quality in the autumn (1.29). During the winter, site 6 becomes oligotrophic and eutrophic in the spring and remains like that until autumn.

## Site 7-9

IPS shows extremely poor quality water at this site 7. EPI-D values concur with evaluation with indices results ranging only in the very heavily polluted category. IPS at site 8 shows that in winter (3.5) the site shows moderately polluted waters. The water then becomes increasingly more polluted from spring to autumn. IPS values suggest a possible pollution spike during the spring (as denoted by a change from 3.5 to 2.6). EPI-D values point at a possible pollution spike as spring's water quality variable is poorer than the one for winter. EPI-D values show satisfactory to almost good water condition during the winter (1.59) and then moderately polluted in the spring (2.01) and then back to satisfactory conditions in the summer (1.86) and autumn (1.9). During the winter, site 6 becomes oligotrophic to eutrophic in the spring and remains like that until autumn. IPS values at site 9 shows poor quality water at this site. All seasons show a high pollution content, with summer reflecting the best indices readings. EPI-D values show a variation between very highly polluted and conditions and in one instance a shift to highly polluted during the summer. Site 8 has slightly better water quality than sites 7 and 9. Furthermore Van Dam shows that Sites 7 and 9 compare very well together, with common hyper-eutrophic conditions noted over three quarters of the year.

## Site 10-12

IPS at 10 shows poor quality water at this site. All seasons show a high pollution content. EPI-D values at 10 shows moderate pollution in the winter (2.14) and then very heavily polluted from spring to autumn. Winter sees a moderate concentration of pollution tolerant species per sample, with spring, summer and autumn receiving a high percentage contribution. IPS values at site 11 shows varying water quality at this site, with a potential pollution spike noted during the spring. The winter (4.27) sees weak pollution content with spring (2.46), well under the critical value for IPS values of 3.5 indicating a shift from heavily polluted to moderately polluted, summer (3.61) and autumn (3.39), although reflecting moderate and heavily polluted conditions respectively, do not represent a massive differences when looking at the indices values. EPI-D values at 11 showed good water condition during the winter (1.47) and summer (1.30), moderate pollution conditions in the Spring (2.13) and satisfactory conditions in the autumn (1.58). The pollution spike was very evident here as the water quality shifts from good quality to moderately polluted (over 2 categories of change). IPS values for site 12, shows varying water quality. The winter (3.02), spring (3.01) and summer (3.19) reflect heavily polluted conditions with autumn (3.58), reflecting a shift to moderate pollution conditions. EPI-D values at 12 shows lightly polluted conditions during summer (1.96) and spring (1.87), and moderate pollution and satisfactory conditions in the winter (1.8) and autumn (1.56). Van Dam seem to suggest a relation between site 10 and 12, as they both reflect a similar trophic status

## Chapter 5

### Discussion

#### Nutrients and Ionic compounds

Seasonal fluctuations in nutrients and rainfall seem to play a large role in pollution levels in the Berg River system. The 20 year nutrient average taken from Franschhoek to Mbekwini points towards a largely varying nutrient content, with more particular variation noted between seasons (see Figures 5-10). Ionic variations of  $\text{NO}_3$  &  $\text{NO}_4$ , Na,  $\text{SO}_4$ , and Cl show cyclical variations with peaks noted during different seasons. However other nutrients show little seasonal change with small seasonal spikes and others remain relatively constant through the seasons. To effectively use diatoms as an environmental management analysis tool, it is required to effectively deal with this variability. Electro-conductivity (EC) shows large degrees variation, well above the recommended means for good ecosystem health.

Table 13: Diatom pH conditions.

PH changes with Diatoms (Van Dam et al., 1994)	Winter	Spring	Summer	Autumn
1	neutral	alkaline	alkaline	alkaline
2	Alkaline	alkaline	alkaline	neutral
3	Alkaline	Neutral	Neutral	alkaline
4	Alkaline	alkaline	alkaline	neutral
5	Alkaline	Neutral	alkaline	neutral
6	acidic	alkaline	alkaline	alkaline
7	neutral	alkaline	alkaline	neutral
8	neutral	alkaline	alkaline	neutral
9	Alkaline	alkaline	alkaline	neutral
10	Alkaline	alkaline	alkaline	alkaline
11	Alkaline	alkaline	alkaline	neutral
12	Alkaline	alkaline	alkaline	alkaline

As was noted in the results, pH changes along the Berg were common with alkaline conditions being favoured over the last 10 years. From Table 13, the diatom analysis supports a continuation of a neutral to alkaline trend along the river. Site 3 during the winter suggests then that pH levels are not uniformly distributed on the Berg River and it is a distinct possibility to receive differing pH's based on the sites location. As site 3 is situated in an agricultural zone, the changing pH could be due to agricultural pollution, however this is unlikely as a similar trend would be expected during the autumn as this was another period where *Gomphonema venusta* was present.

Data for these sites were particularly hard to come by as seasonal samples were not always available, which only serves to strengthen the argument for a new monitoring method. It could be noted that a possible reason for the chlorine spikes noted upstream could be due to external influences at the WWTW in Franschoek. Furthermore, DWA water quality results have shown us that seasonal variation does play a role in assessing water quality particularly when considering individual nutrients.

### Diatoms

The dendograms (Figures 11 to 14) suggest that the pollution tolerant species respond best to changing seasons. Both the *Nitzschia* and *Navicula* Genus were found all year round. *Gomphonema venusta* was dominant during the winter and autumn period, which is a probable symptom of agricultural pollution. *Geisleria descussis* and *Fragilaria ulna var. acus* seem to favour two seasons out of the year. When considering seasonality between genus, *Sellaphora pupula* was abundant during the winter, summer and autumn period, while *Surirella splendida* was abundant during the spring months. *Cocconeis placentula* showed favourable conditions during the winter, summer and autumn with less favourable conditions during the spring. The summer months have high concentrations of pollution tolerant species. Some species like *Craticula halophila*, *Achnanthes oblongella* and *Surirella. splendida* found optimum conditions during only one season.

## Diatom indices

Table 14: Summary of significant Species Diversity changes as shown by ANOVA.

Summary of Significant Diversity changes (Table 6 to 9)	Seasons	ANOVA	DIATOM INDICE
	Winter	4, 11	4, 7, 9, 10, 12
	Spring	3, 7	6, 8, 11
	Summer	2	7, 9, 10
	Autumn	2, 4, 5, 6, 12	2, 3, 4, 5, 6, 8, 12

Significant sites (sites showing a p value < 0.05), show a statistical difference between the reference site and the site its being compared to. Site 2 and 4 show the most difference seasonally to the reference as they occur on the significance table twice. Winter and autumn are both periods where *Gomphonema venusta* thrived. This diatom species is typical of an increase in agricultural pollution. The presence of this species could explain the increase in significant sites during the autumn. Diatom indices showing significant changes reflect pollution changes that are greater than or less than that of the reference but not equal to it. There is little overlap between the diatom indices sites and the ANOVA significant sites, except at site 4 during the winter. However autumn shows a strong overlap between the variables showing significant changes between the sites and the reference.

In its current form, the statistics link to significance changes in species biodiversity, which is known to have a strong relation to water quality (De la Rey et al., 2008). Based on results obtained, it is expected that sites with similar species abundance to have a similar water quality. Significant differences in species available suggest that there is a difference between each site, not necessarily only in water quality but in other variables such as pH and dissolved nutrients.

Diatoms that are more abundant at the time of sampling with thus give an indication of the water quality at that particular time.

Figure 15 shows seasonality using diatom indices. It is apparent that diatom indices and by inference water quality, does not remain constant along the river stretch. The river is in constant flux and its state is dependent on seasonal conditions affecting the river. There is no seasonal pattern within the diatom index data except that it is constantly changing. Figure 17 shows that autumn shows the least variability between sites as the sites mean lie closer together. Conversely winter shows the greatest variability as it has the greatest pollution ranging from 'good water quality' to 'heavily polluted'. Both Figures 15 and 16 hint at the rivers ability to improve its water quality. This is made evident by the decreasing water quality as you go away from site 1 and the steadily increasing water quality after site 11.

Site 1 (Figure 17 to 20 in Addendum A) shows largely satisfactory water conditions during the year with moderate pollution conditions noted during the winter. Site 2 (seems to show a seasonal increase in water quality from winter to autumn, but more samples are required to prove this result. Site 3 shows very little water quality variation between winter and spring with a decrease in water quality in the autumn. Site 4 shows fluctuations of poor water quality from winter to spring possibly suggesting an external pollution influence further upstream. Evidence for this is shown by a drastic increase in water quality in the autumn. Like site 4, site 5 shows a increase in water quality possibly starting in Summer and at its maximum in the autumn, with winter and spring showing similar moderately polluted conditions. Site 6 shows fairly good water quality each season except for the spring. The water quality results for the latter site suggests that there was a potential external influence influencing this site during this season. Site 7 was shown to be heavily polluted and shows little seasonal variation. This was also the case for two other sites namely site 9 and 10, although site 10 does show very slight improvement during the winter. Site 8 is shows a decrease in pollution during the winter and a decrease in water quality from spring. The results from spring remain constant until autumn. Site 11 shows a cyclical trend in its water quality. Winter and summer show increased water quality results with spring and autumn showing poorer water quality results. More testing is required to prove this. Site 12 shows good water conditions in relationship with DWA guidelines in the winter, with average conditions in the summer and autumn. A pollution spike is noted in the spring. This

pollution spike could be from upstream influences as site 11 too depicted a decrease in water quality. It could also be as a result of increased river flow from 10 causing spillage into site 11 and 12.

Diatoms respond well to changes in water quality. Seasonal variation may cause changes in nutrient concentrations as well as pollution levels. Diatoms are very useful in monitoring changes in pollution when using diatom indices. Diatom samples can be taken seasonally and indices can be compared to assess fluctuations in pollution. There may be issues with compatibility of species which are found in a South African, Mediterranean climate due to the fact that most diatom databases are drawn from European rivers. This is however a minor problem as most species found in this study were available in the Omnidia database. That being said, diatom analysis is difficult without Omnidia, but not impossible. Diatom analysis provides sufficient evidence for possible implementation as part of an effective environmental water quality management scheme. Water quality results from the DWA suggest that nutrient levels within the river system are constantly fluctuating. Diatoms have been shown to change in response to stimuli, seasonally showing that the data results varied sufficiently to notice distinct changes in both species available and water quality. This was further backed up by the continued species diversity shown by table 14.

The River Health Programme (2004) noted that water quality through the urban areas of both Franschhoek and Paarl consisted of moderately to heavily polluted waters. These results are reflected by the diatom results of this study. Seasonality does play a role at some sites that are less affected by pollution. The sites that are more polluted show less variation to the change in season.

It was predicted that during the wet winter months, the results would show the best water quality conditions and summer being the most polluted period, due to it being the season with the least rainfall. Due to the acidic soil type found in the area, it was largely expected that the general river pH would be acidic. At the upstream sites, it was expected to see influences from the stormwater runoff pipe leading from a WWTW. Further downstream, the river was expected to show possible pollution spikes surrounding the factory site with possible metal tolerant diatom species present and strong linkages between sites 7 and 9 due to the visually polluted nature of site 7.

Winter was shown to have the least polluted sites, but also the least variation (ANOVA) between the reference site and the others. The pH of the river was alkaline with only one instance of it showing an acid pH. The DWS results do show cycling pH conditions in previous years. The upstream sites showed some effect of the WWTW's with an fluctuation of pollution tolerant species noted. Minor numbers of metal tolerant species were found at the factory site, which is insufficient evidence to conclude that there is metal dumping. There were no obvious signs of diatom deformity at this site, but it is possible that some deformities were missed during sampling.

## Chapter 6

### Conclusion

Are diatoms a reliable indicator for water quality along the Berg River? Diatoms have shown that they fluctuate seasonally, having seen changing species abundances as shown by indicator results (Table 10 & Figures 15 and 16) as well as in abundance counts (Addendum D). Areas that were heavily polluted, namely site 7, showed little taxon variation (Duong et al., 2006) seasonally and the other sites with less pollution showed more taxon variability, which was expected. Diatom abundance on its own can give an indication of the water quality at a specific site (as shown by Kriel, 2008), but it works best with an applied index in freshwater systems (De la Rey et al., 2008). Omnidia works well in dealing with Mediterranean climate diatoms in the Western Cape although a minute number of species were not available on the package. Diatom monitoring could be implemented as a visual representation of water quality along a river stretch using GIS. This is particularly useful in assessing and reporting daily, weekly and seasonal changes along the river.

Cluster analysis showed varied results linking diatom abundances with each other. More distinct clusters were noted during the winter showing a particular favouring towards the winter conditions to around 7 species. *Nitzschia umbonata* was the most common species and contributed the most taxon over the year. In both the winter and autumn months, *Nitzschia umbonata* reflected a singular arm (branch) of the dendrogram suggesting a tolerance to water conditions present at the time. The other seasons showed less distinct clustering, with individual diatom taxa not distinctly being favoured. The effect of the winter rains could be a possible reason for dendrogram showing more favourable relationships during the spring. This randomised clustering seems to suggest more favourable water quality conditions as it is more favourable to greater species diversity, which contributes to the total abundance count. Some seasonal clustering (Winter & Spring) has shown no distinct link to species preferring a certain trophic status, while other seasons (Summer & Autumn) show a real strong link in similarity between species favouring highly polluted conditions.

Do diatoms respond well to seasonal changes? Yes. However, seasonality is not simply linked to increased rainfall during the winter or dry periods during the summer. As shown by tables 2 to 5, in fact seasonality affects multiple factors including nutrient concentrations, rainfall, pollution levels and many others (Sebastia et al., 2013). This means that active patterns within the data may be much hard to find. A better summation would be that diatoms respond well to the symptoms associated with seasonal changes. This, among other factors, makes diatoms good indicators of changes linked to seasonality. As the Western Cape receives winter rainfall, we expect to see results reflecting this during the winter. Winter showed three of the lowest diatom indices values which represented improving water quality. Conversely it was expected that during the summer and autumn months, a higher concentration of pollution tolerant species would be present. Summer's top diatom taxa were all pollution tolerant species and the autumn sample represented the site with the most polluted conditions. The diatom abundance counts and index scores all supported the assumed view that the best conditions are found in winter and worst in summer. Furthermore with the Western Cape's Mediterranean climate, it was expected that the river's pH levels would be largely acidic. It was found though (through diatom analysis and DWAS records) that the river's pH is largely alkaline. This could be due to numerous factors including upstream influences, lack of natural fynbos, agricultural effects and possibly nutrient loading from the WWTW in Franschhoek.

Diatoms reported seasonal changes particularly well. Diatoms show strong variations during the winter and autumn period, but remained fairly similar through the summer autumn months. There was no indication of industrial waste affecting the Berg at site 6 as spikes in *Fragilaria Tenera* (WM Smith) Lange-Bertalot would indicate this. The universal presence of *Nitzschia umbonata* suggests a sustained pollution gradient along the river. Autumn and winter showed strong a strong agricultural pollution influence based off the presence of *Gomphonema venusta*. Furthermore the seasonal appearance of *Achnantheidium exiguum* (Grunow) Czarnecki shows seasonal improvement in water quality along the river. All seasons contained a high level of pollution tolerant taxa of the *Nitzschia* and *Navicula* Genus. This means that in the context of a polluted river, pollution tolerant species will be less affect by seasonality. However other species are differentially affected by seasonality. *Achnanthes oblongella* and *Craticula halophila* are species that favour only one season. *Surirella angusta* Kützing is dominant over three seasons and *Surirella splendida* is more common only in one season. Species like *Surirella splendida* are

not found at certain times of the year too. Having prior knowledge of seasonal diatoms will give an indication of changes at that particular site. If a sample is taken and a greater number of pollution tolerant species are present and the seasonal diatoms are omitted, this suggests that the site's water quality is decreasing. Conversely if a greater abundance of seasonal diatoms are present, with less pollution tolerant species and newer uncommon species are present, then this could be an indication of improving water quality.

Areas of concern pertain to storm water and conduit systems entering the river. These systems transport effluence from various zones and pollute the river in patches, which are clearly indicated by the diatom index scores. It is imperative that an intervention takes place in an attempt at remediating these systems before it enters the river. The results show that the Berg River attempts to return to similar state as the reference sites (Figures 15 and 16). These figures clearly show the affect that the conduit system has on a site that is situated directly downstream from it. Improving the quality of the water coming in will go a long way in helping to restore the water quality of the river as a whole.

The ability of diatoms to adapt and respond to changing to water quality shows its robustness. The results of this study have shown the seasonal variation in diatoms and, with the relative ease with which diatoms are collected, which makes them a particularly reliable indicator, more especially in a South African context where access to private laboratories are not always available. The integration of diatom monitoring techniques will be slow. It will require the training of individuals and equipping them with the necessary resources to conduct regular sampling which can then be sent off for analysis. The sampler could also be trained to analyse and report on findings. The latter idea may be a problem though as laboratory equipment is not easily accessible for the public. Diatom monitoring should be conducted with the aid of a GIS map as it allows for easy translation between sectors of the public. Diatom analysis combined with GIS modelling can serve to educate the public on sampling zones and relay water quality data that is easy to understand.

Diatoms have been shown to be robust indicators of water quality (Beyene et al., 2014; Blanco et al., 2012; Bate & Smailes, 2013; Harding & Taylor, 2011.; Van Vuuren, 2007; Harding & Archibald, 2005; De la Rey et al., 2004). They respond well to changes in water quality associated with seasonal change. Diatoms and diatom indices are a useful management tools

when assessing changes at different site, as new data can be acquired on an ad hoc basis and diatom abundances translate well onto visual information whether it be GIS or in the form of a graph.

## Chapter 7

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<http://web.sbe.hw.ac.uk/staffprofiles/bdgsa/temp/12th%20ICUD/PDF/PAP005118.pdf>

Addendum A

EPI-D: GIS maps

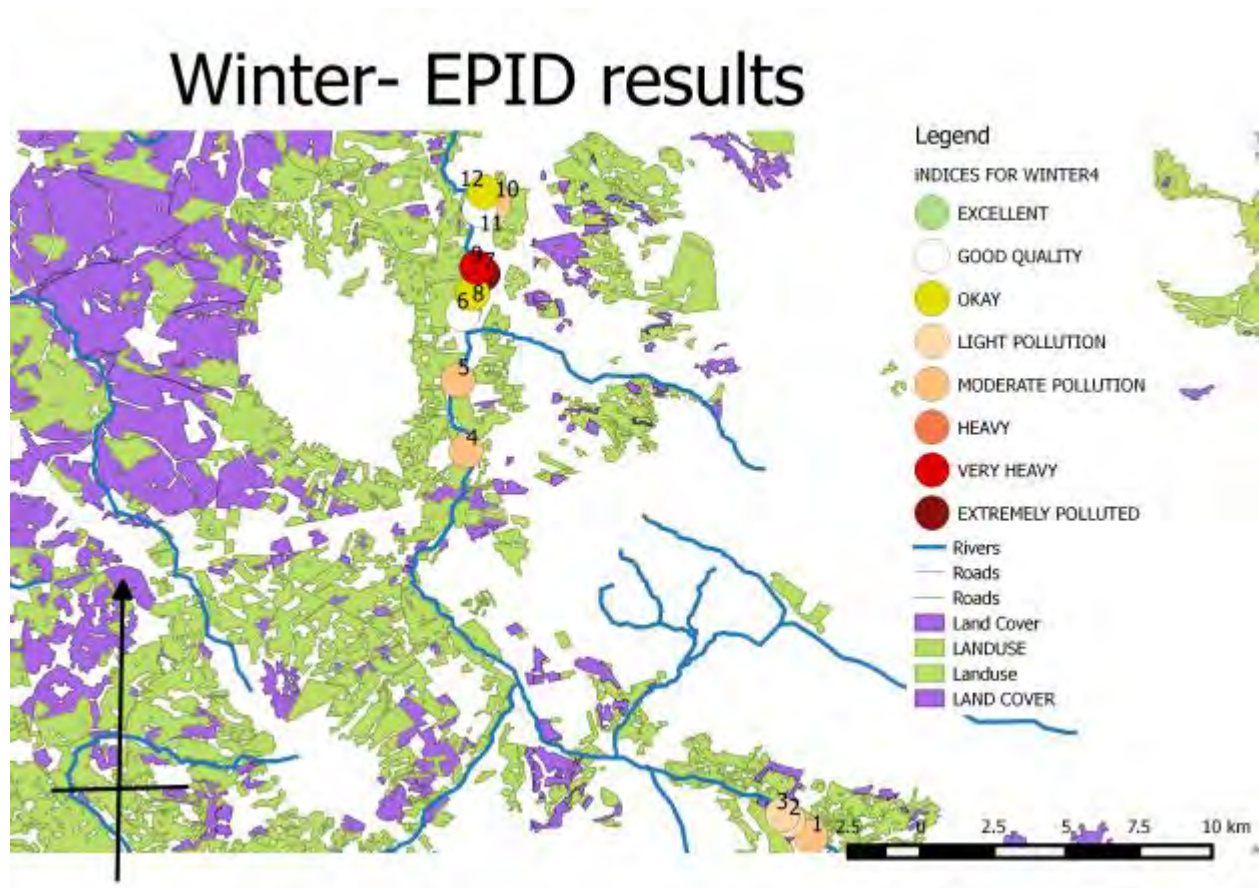


Figure 17 : Winter EPI-D pollution GIS

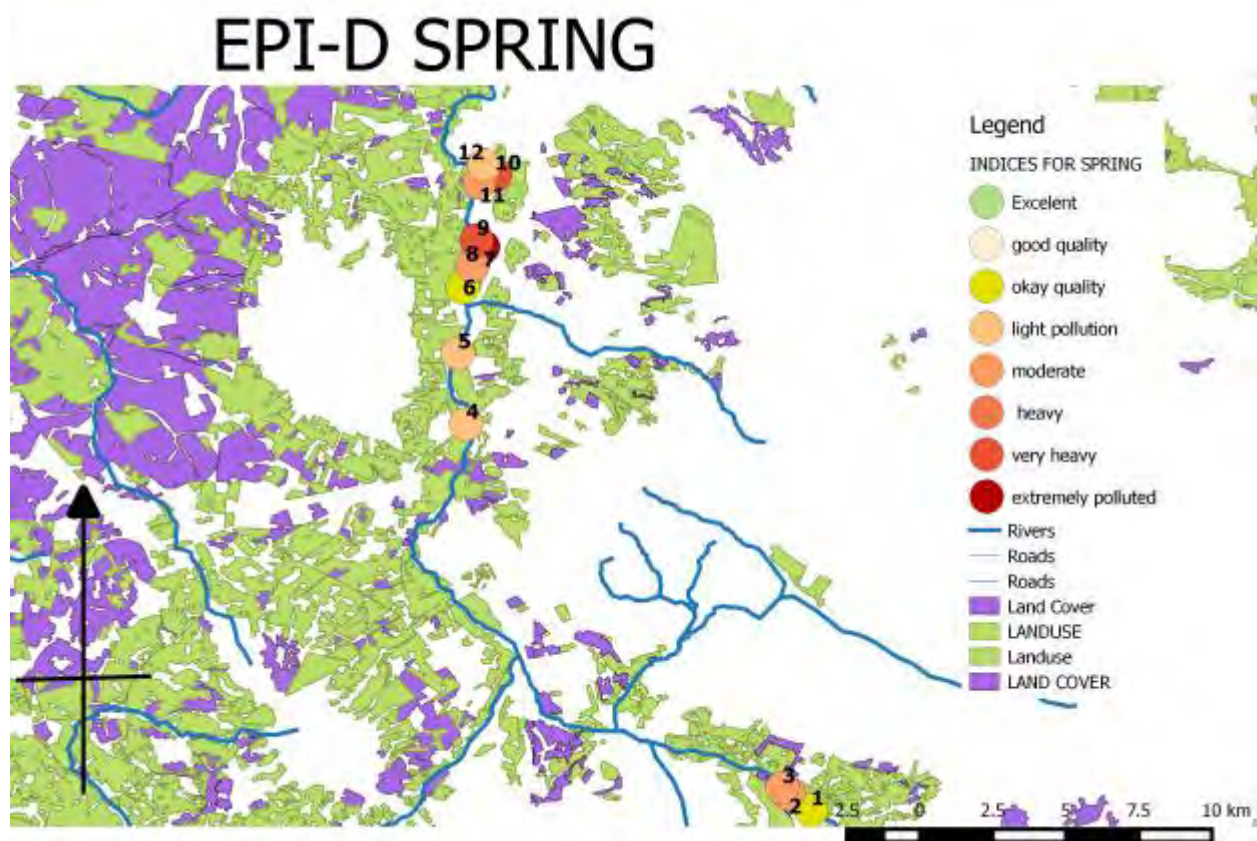


Figure 18: Spring EPI-D pollution GIS

# EPI-D Summer

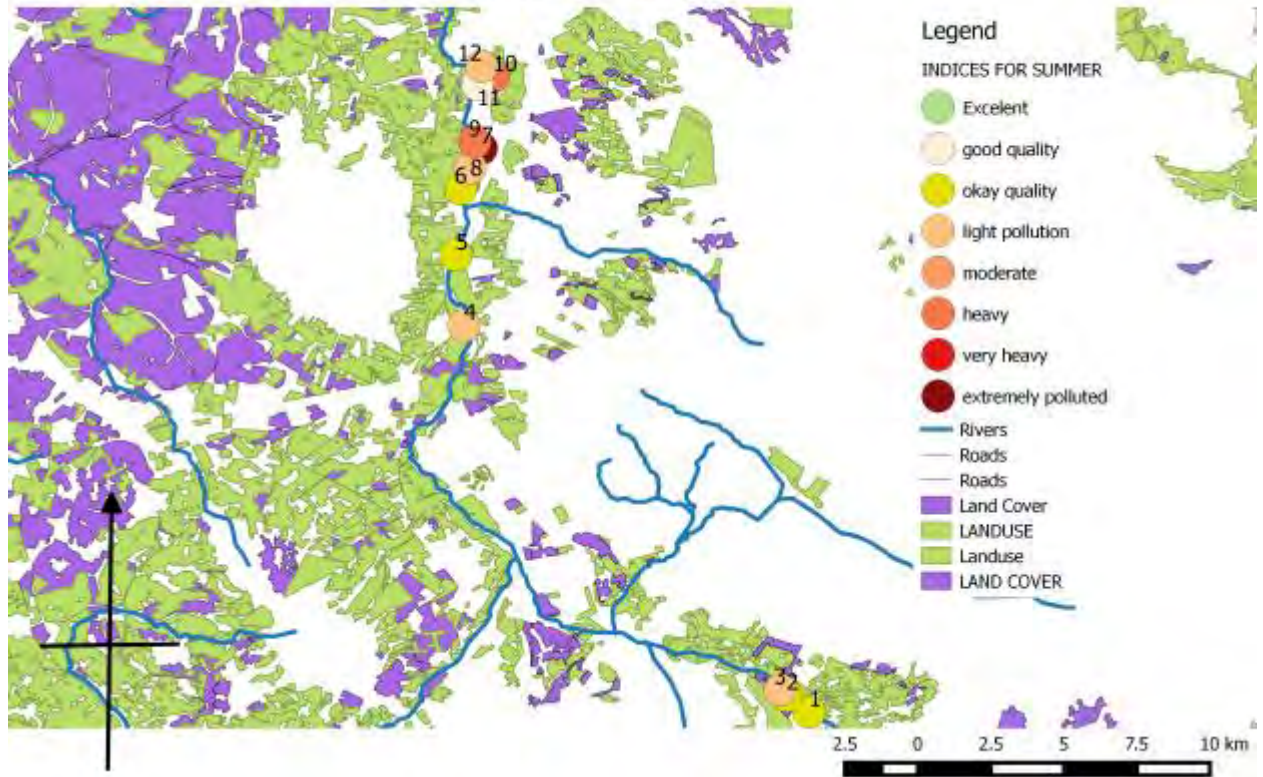


Figure 19: Summer EPI-D pollution GIS

## EPI-D Autumn

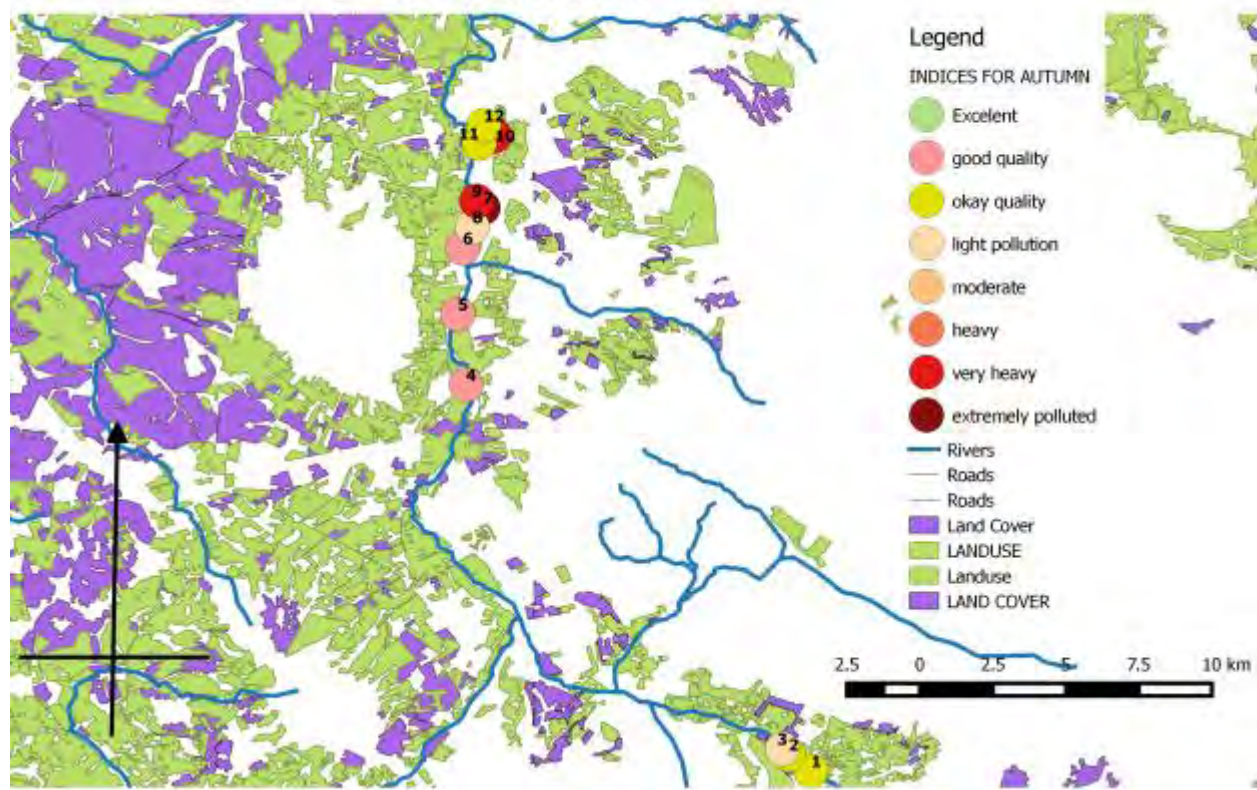


Figure 20: Autumn EPI-D pollution GIS

## Addendum B

### Diatom preparation

#### Sample collection

- Label 3 to 4 glass test tubes per site.
- Choose 3 to 4 boulders with algae present
- Use a toothbrush to scrub algae into test tube, making sure to rinse after each use.

#### Sample preparation:

- Allow samples to settle for 24 hours.
- The clear supernatant liquid was decanted from the vials without pouring out any of the sample.
- Fill vials with distilled water and allow to settle.
- Samples were shaken well in order to re-suspend diatoms.
- The distribution of the diatoms on the cover slip should be not be significantly clumped, but be evenly dense, with no clustering of the diatoms along the edges of the slide or drops of the sample. With 1 to 15 diatoms present per field of view at 1000x magnification.
- Use clean slides and cover slips.
- Use a pipette to extract diatom sample and drop sample on to a glass slide.
- Four drops of sample were placed linearly across each cover slip.
- Place slide on a heating tray to evaporate supernatant liquid..
- A large drop of mountant was placed in the centre of each slide. The dry cover slips were then inverted and lowered onto the slide with the drop of mountant in the middle of the cover slip.
- The slide was then placed on the hot plate momentarily to allow the mountant to seal the edges of the slide.

- The slides were then allowed to set over night.

Identification of diatoms:

- Two slides from each sample site were selected in order to give a representative sample of the site. Slides were selected based on even distribution and adequate presence of Diatoms. This is defined by Taylor et al, (2007b) as at least 15 diatoms per field of view at 1000x magnification at across the whole slide.
- Diatoms were identified using An Illustrated Guide to Some Common Diatom Species from South Africa by Taylor et al, (2007b).
- One linear path across the slide was made over the slide using a light microscope at 1000x magnification.
- Diatoms were identified and noted in a Microsoft excel spreadsheet.
- Another non-linear path was taken across the slide in the opposite direction at 400x magnification to pick up any species that were not identified in the first pass

Sample points for the Franschhoek River were taken from Frick (2010)

Data analysis will be done using Statistica 9 and Omnidia (Lecointre,1993). Omnidia is a software package that is in the form of a diatom database that calculates river conditions based on input values and indices. Diatom viewing will be done using the Zeiss Axiostar microscope and all analysis will be done in the Water analysis Laboratory at the University of Cape Town.

## Addendum C

## Diatom Reference list

UUCT - Oliver Slingers CODE DENOMINATION	
ACAP * <i>Amphora capitellata</i> Frenguelli	NETO <i>Nitzschia etoshensis</i> Cholnoky
ACEC * <i>Achnantheidium exiguum</i> var. <i>constrictum</i> (Grunow) Andresen. Stoerme	NFTE * <i>Nitzschia frustulum</i> (Kützing) Grunow f. <i>anomale</i>
ACOA * <i>Achnanthes coarctata</i> (Brebisson) Grunow in Cl. & Grun.	NGER * <i>Navicula germanii</i> Wallace
ADEG * <i>Achnantheidium exiguum</i> (Grunow) Czamecki	NGRE * <i>Navicula gregaria</i> Donkin
ADMI * <i>Achnantheidium minutissimum</i> (Kützing) Czamecki	NIAR * <i>Nitzschia archibaldii</i> Lange-Bertalot
ADSA * <i>Achnantheidium saphrophilum</i> (Kobayasi et Mayama) Round & Bukhtiyar	NINT * <i>Nitzschia intermedia</i> Hantzsch ex Cleve & Grunow
AFOR * <i>Asterionella formosa</i> Hassall	NIRM * <i>Nitzschia irremissa</i> Cholnoky
ALIO * <i>Achnanthes linearoides</i> Lange-Bertalot	NLBT * <i>Nitzschia liebethuthii</i> Rabenhorst var. <i>liebethuthii</i>
AOBG * <i>Achnanthes oblongella</i> Oestrup	NLIN * <i>Nitzschia linearis</i> (Agardh) W.M. Smith var. <i>linearis</i>
AUGR * <i>Aulacoseira granulata</i> (Ehr.) Simonsen	NLSU * <i>Nitzschia linearis</i> (Agardh) W.M. Smith var. <i>subtilis</i> (Grunow) Husted
AVCA * <i>Amphora veneta</i> Kützing var. <i>capitata</i> Haworth	NLTT * <i>Nitzschia littorea</i> Grunow in Van Heurck
BBRE * <i>Brachysira brebissonii</i> Ross in Hartley ssp. <i>brebissonii</i>	NNOT * <i>Navicula notha</i> Wallace
BPAR * <i>Bacillaria paradoxa</i> Gmelin in Linnaeus	NPAE * <i>Nitzschia paleacea</i> (Grunow) Grunow in van Heurck
BWYG <i>Brachysira wygaschii</i> Lange-Bertalot	NPAL * <i>Nitzschia palea</i> (Kützing) W. Smith var. <i>palea</i>
CACD <i>Craticula acidoclinata</i> Lange-Bertalot & Metzeltin	NPMI <i>Neidium productum</i> (W.M. Smith) Cleve var. <i>minor</i> Cleve-Euler
CAMB * <i>Craticula ambigua</i> (Ehrenberg) Mann	NRCF * <i>Navicula reichardtiana</i> Lange-Bertalot f. <i>anomale</i>
CASP * <i>Cymbella aspera</i> (Ehrenberg) Peragallo	NRCS * <i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot
CATE * <i>Caloneis tenuis</i> (Gregory) Krammer	NRHY * <i>Navicula rhychocephala</i> Kützing
CBAM * <i>Cymbopleura amphicephala</i> Krammer	NRIE <i>Navicula riediana</i> Lange-Bertalot & Rumrigh
CBNA * <i>Cymbopleura naviculiformis</i> (Auerswald) Krammer var. <i>naviculiform</i>	NROS * <i>Navicula rostellata</i> Kützing
CHAL * <i>Craticula halophila</i> (Grunow ex Van Heurck) Mann	NSHG * <i>Navicula schroeteri</i> Meister var. <i>schroeteri</i> f. <i>anomale</i>
CMEN * <i>Cyclotella meneghiniana</i> Kützing	NTRV * <i>Navicula trivialis</i> Lange-Bertalot var. <i>trivialis</i>
CNOI * <i>Cymbella neocistula</i> Krammer var. <i>neocistula</i> Krammer	NUMB * <i>Nitzschia umbonata</i> (Ehrenberg) Lange-Bertalot
COPR <i>Cocconeis placentula</i> Ehrenberg var. <i>rouxii</i> (Herb. & Brun in Herb)	NVEN * <i>Navicula veneta</i> Kützing
CRAC * <i>Craticula accomoda</i> (Hustedt) Mann	NVIR * <i>Navicula viridula</i> (Kützing) Ehrenberg
CRCM <i>Craticula cuspidata</i> Kützing var. <i>media</i> (Meister) Aboal	PBOR * <i>Pinnularia borealis</i> Ehrenberg var. <i>borealis</i>
CVIX <i>Craticula vixnegligenda</i> Lange-Bertalot	PDIV * <i>Pinnularia divergens</i> W.M. Smith var. <i>divergens</i>
DCOF * <i>Diadesmis confervacea</i> Kützing var. <i>confervacea</i>	PDUN <i>Pinnularia divergens</i> W.M. Sm. var. <i>undulata</i> (M. Perag. & Herib.) Hu
DPST * <i>Discostella pseudostelligera</i> (Hustedt) Houk & Klee	PGIB * <i>Pinnularia gibba</i> Ehrenberg
DSTE * <i>Discostella stelligera</i> (Cleve et Grun.) Houk & Klee	PJOC <i>Pinnularia jocolata</i> (Manguin) Krammer
EADN * <i>Epithemia adnata</i> (Kützing) Brebisson	
EBIL * <i>Eunotia bilunaris</i> (Ehr.) Mills var. <i>bilunaris</i>	PDUN <i>Pinnularia divergens</i> W.M. Sm. var. <i>undulata</i> (M. Perag. & Herib.) Hu
ECBU <i>Encyonopsis buedeli</i> Krammer	PGIB * <i>Pinnularia gibba</i> Ehrenberg
ESLE * <i>Encyonema silesiacum</i> (Bleisch in Rabh.) D.G. Mann	PJOC <i>Pinnularia jocolata</i> (Manguin) Krammer
ESOR * <i>Epithemia sores</i> Kützing	
ESUM * <i>Encyonopsis subminuta</i> Krammer & Reichardt	PDUN <i>Pinnularia divergens</i> W.M. Sm. var. <i>undulata</i> (M. Perag. & Herib.) Hu
FBCP * <i>Fragilaria biceps</i> (Kützing) Lange-Bertalot	PGIB * <i>Pinnularia gibba</i> Ehrenberg
FCAP * <i>Fragilaria capucina</i> Desmazieres var. <i>capucina</i>	PJOC <i>Pinnularia jocolata</i> (Manguin) Krammer
FCRS * <i>Frustulia crassineria</i> (Breb.) Lange-Bertalot et Krammer	PLEN * <i>Planolithidium engelbrechtii</i> (Choln.) Round & Bukhtiyarova
FCRU * <i>Fragilaria capucina</i> Desmazieres var. <i>rumpens</i> (Kützing) Lange-Ber	PLFR * <i>Planolithidium frequentissimum</i> (Lange-Bertalot) Lange-Bertalot
FCVA * <i>Fragilaria capucina</i> Desmazieres var. <i>vaucheriae</i> (Kützing) Lange-B	PMRO <i>Pinnularia microstauron</i> (Ehr.) Cleve var. <i>rostrata</i> Krammer
FPYG * <i>Fallacia pygmaea</i> (Kützing) Stickle & Mann ssp. <i>pygmaea</i> in Lange-B	PPLC * <i>Placconeis placentula</i> (Ehr.) Heizerling
FROA <i>Frustulia rostrata</i> Hustedt fo. <i>angustior</i> Maillard	PRST * <i>Planolithidium rostratum</i> (Oestrup) Lange-Bertalot
FSAP * <i>Fistulifera saphrophila</i> (Lange-Bertalot & Bonik) Lange-Bertalot	PSBE <i>Pseudostaurosira brevistriata</i> var. <i>elliptica</i> (Heribaud) Kingston
FSAX * <i>Frustulia saxonica</i> Rabenhorst	PSBV <i>Pinnularia subbrevistriata</i> Krammer
FUAC * <i>Fragilaria ulna</i> (Nitzsch.) Lange-Bertalot var. <i>acus</i> (Kütz.) Lange-	PSCA * <i>Pinnularia subcapitata</i> Gregory var. <i>subcapitata</i>
FVUL * <i>Frustulia vulgaris</i> (Thwaites) De Toni	PVFI <i>Pinnularia viridiformis</i> Krammer var. <i>viridiformis</i> morphotype 5
GAFF * <i>Gomphonema affine</i> Kützing	PVID <i>Pinnularia viridis</i> (Nitzsch) Ehrenberg var. <i>viridis</i> morphotype 2
GCLA * <i>Gomphonema clavatum</i> Ehr.	SANG * <i>Surirella angusta</i> Kützing
GDEC * <i>Geissleria decussis</i> (Ostrup) Lange-Bertalot & Metzeltin	SARA <i>Synedra acus</i> Kützing var. <i>radians</i> (Kützing) Hustedt
GGRG * <i>Gomphonema gracile</i> Ehrenberg	SGRC <i>Stauroneis graeilis</i> Ehrenberg
GPAR * <i>Gomphonema parvulum</i> (Kützing) Kützing var. <i>parvulum</i> f. <i>parvulum</i>	SPHO * <i>Stauroneis phoenicenteron</i> (Nitzsch) Ehrenberg
GPSA * <i>Gomphonema pseudoaugur</i> Lange-Bertalot	SPRS <i>Sellaphora pupula</i> var. <i>rostrata</i> (Hustedt) Aboal
GTRU * <i>Gomphonema truncatum</i> Ehr.	SPUP * <i>Sellaphora pupula</i> (Kützing) Mereschkowsky
GYNJ <i>Gomphonema venusta</i> Passy, Kociolek & Lowe	SSEM * <i>Sellaphora seminulum</i> (Grunow) D.G. Mann
GYAC * <i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst	SSPL * <i>Surirella splendida</i> (Ehrenberg) Kützing
HAAM <i>Hantzschia amphioxys</i> (Ehr.) Grunow var. <i>amphilepta</i> Grunow	STAN * <i>Stauroneis anceps</i> Ehrenberg
HCAP * <i>Hippodonta capitata</i> (Ehr.) Lange-Bert Metzeltin & Witkowski	STKR * <i>Stauroneis kriegeri</i> Patrick
LACD * <i>Luticola acidoclinata</i> Lange-Bertalot	TAPI * <i>Tryblionella apiculata</i> Gregory
LGOE * <i>Luticola goeppertiana</i> (Bleisch in Rabenhorst) D.G. Mann in Round	TFAS * <i>Tabularia fasciculata</i> (Agardh) Williams et Round
LHUN * <i>Lemnicola hungarica</i> (Grunow) Round & Basson	TFLO * <i>Tabularia flocculosa</i> (Roth) Kützing
MAPE * <i>Mayamaea atomus</i> var. <i>permittis</i> (Hustedt) Lange-Bertalot	THUN * <i>Tryblionella hungarica</i> (Grunow) D.G. Mann
MVAR * <i>Melosira varians</i> Agardh	TWEI * <i>Thalassiosira weissflogii</i> (Grunow) Fryxell & Hasle
NAMP * <i>Nitzschia amphibia</i> Grunow f. <i>amphibia</i>	XXXX <i>DIATOMEE NON IDENTIFIEE</i> (indéterminée)
NANT * <i>Navicula antonii</i> Lange-Bertalot	ZZZZ <i>GENRE NON IDENTIFIE</i>
NCOM * <i>Nitzschia communis</i> Rabenhorst	
NOPL * <i>Nitzschia capitellata</i> Hustedt in A. Schmidt & al.	
NCPR * <i>Navicula capitatoradiata</i> Germain	
NCRY * <i>Navicula cryptocephalata</i> Kützing	
NCTE * <i>Navicula cryptotenella</i> Lange-Bertalot	
NDIS * <i>Nitzschia dissipata</i> (Kützing) Grunow ssp. <i>dissipata</i>	
NDNS <i>Navicula densa</i> Hustedt	
NERI * <i>Navicula erifuga</i> Lange-Bertalot in Krammer & Lange-Bertalot	

Figure 21: Diatom Reference list with Omidia codes

**Addendum D****Table 15 : Diatom Counts**

(See attached – Microsoft Excel spreadsheet: *Diatoms Final Count 2015-1 final thesis.xlsx*)





Brachysira brebissonii Ross in Hartley ssp.*	BWYG	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachysira wygaschii Lange- Bertalot	BBRE	0	0	0	0	0	0	0	0	0	0	11	10	21
Caloneis sp.	XXXX	0	3	0	0	0	0	0	0	0	0	0	0	3
Caloneis tenuis (Gregory) Krammer *	CATE	0	0	18	0	0	0	0	0	0	0	0	0	18
Cocconeis engelbrechtii Cholnoky	XXXX	0	0	0	0	0	0	0	0	0	0	0	0	0
cocconeis moleformis	XXXX	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis placentula Ehrenberg var. placentula	CPLA	12	9	27	37	30	0	0	15	0	13	60	40	243
Craticula accomoda (Hustedt) Mann *	CAMB	0	0	0	0	0	0	0	0	21	0	0	0	21
Craticula acidoclinata Lange-Bertalot & Met	CACD	0	0	0	0	0	0	0	0	0	0	0	0	0
Craticula ambigua (Ehrenberg) Mann *	CRAC	0	0	0	0	0	0	0	0	0	0	0	0	0
Craticula cuspidata Kützing var.media (Meis)	CRCM	0	0	0	0	0	0	0	0	0	0	8	0	8
Craticula halophila (Grunow ex Van Heurck) *	CVIX	0	4	0	0	0	0	0	0	0	17	0	0	21
Craticula vixnegligenda Lange-Bertalot	CHAL	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana Kützing *	CMEN	0	8	0	0	0	12	0	0	0	0	22	4	46
Cymbella aspera	CASP	0	6	0	0	0	3	0	0	0	0	5	0	14

(Ehrenberg) Peragallo *														
Cymbella sp.	XXXX	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella neocistula Krammer var.neocistula *	CBAM	0	0	0	0	0	0	0	9	0	0	0	0	9
Cymbopleura amphicephala Krammer *	CNCI	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbopleura naviculiformis (Auerswald) Kram*	CBNA	0	0	0	0	0	0	0	0	0	0	0	0	0
Diadesmis confervacea Kützing var. conferva*	DCOF	0	0	0	0	0	0	0	9	18	0	0	0	27
Discostella pseudostelligera (Hustedt) Houk*	DPST	0	4	0	0	0	0	0	0	0	0	0	0	4
Discostella stelligera (Cleve et Grun.) Hou*	DSTE	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonema mesianum (Cholnoky) D.G. Mann in *	ENME	0	0	0	0	0	0	0	0	0	6	0	0	6
Encyonema silesiacum (Bleisch in Rabh.) D.G*	ELSE	0	0	0	0	0	12	0	30	18	0	0	0	60
Encyonopsis buedelii Krammer	ECBU	0	0	0	0	0	0	0	0	0	6	0	0	6
Encyonopsis krammeri Reichardt *	ECKR	0	0	0	0	0	0	0	0	0	4	0	0	4
Encyonopsis minuta Krammer & Reichardt *	ECPM	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonopsis subminuta Krammer & Reichardt *	ESUM	0	7	0	0	0	0	0	0	0	3	15	8	33



Desmazieres var.capucin*														
Fragilaria capucina Desmazieres var.vaucher*	FCVA	0	15	0	0	0	0	0	0	0	0	0	0	15
Fragilaria ulna (Nitzsch.)Lange- Bertalot va*	FUAC	18	0	0	16	15	12	0	9	0	8	17	11	106
Frustulia crassinervia (Breb.) Lange- Bertal*	FCRS	0	0	0	0	0	0	0	0	0	0	19	11	30
Frustulia rostrata Hustedt fo.angustior Mai	FSAX	0	0	0	0	0	12	0	0	0	0	0	0	12
Frustulia saxonica Rabenhorst *	FROA	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia vulgaris (Thwaites) De Toni *	FVUL	15	5	15	15	9	9	0	0	0	0	0	0	68
Geissleria decussis(Ostrup) Lange-Bertalot *	GDEC	0	12	18	0	6	0	0	12	0	6	6	0	60
Gomphonema affine Kützing *	GAFF	0	5	6	0	0	0	0	12	0	6	12	0	41
Gomphonema clavatum Ehr. *	GCLA	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema aff. Gracile	XXXX	0	8	9	0	0	0	0	0	0	0	0	0	17
Gomphonema gracile Ehrenberg *	GGRA	30	0	0	25	0	0	0	0	0	0	0	0	55
Gomphonema aff. lagenula	XXXX	15	0	6	12	0	0	0	0	0	0	0	0	33
Gomphonema parvulum (Kützing) Kützing var. *	GPAR	18	16	18	15	18	0	0	0	0	11	0	0	96
Gomphonema pseudoaugur	GPASA	18	0	15	18	0	0	0	24	0	12	0	0	87











Mann *														
Stauroneis anceps Ehrenberg *	SARA	0	0	0	0	0	0	0	0	0	0	0	0	0
Stauroneis gracilis Ehrenberg	STAN	0	0	0	0	0	0	0	0	0	0	0	0	0
Stauroneis kriegeri Patrick *	SGRC	0	0	0	0	0	0	0	0	0	0	2	0	2
Stauroneis phoenicenteron (Nitzsch) Ehrenbe*	SPHO	0	0	0	0	0	0	0	9	0	0	2	0	11
Surirella angusta Kützing *	STKR	0	0	0	0	0	0	0	0	0	0	0	0	0
Stauroneis sp.	XXXX	0	0	0	0	0	0	0	0	0	0	0	0	0
Surirella splendida (Ehrenberg) Kützing *	SANG	0	15	0	0	0	0	0	0	18	18	0	0	51
Synedra acus Kützing var. radians (Kützing) Hu	SSPL	0	0	0	0	0	0	0	0	0	0	0	3	3
Tabellaria flocculosa (Roth) Kützing *	TFAS	0	0	24	0	0	0	0	0	0	0	0	0	24
Tabularia fasciculata (Agardh) Williams et R*	TFLO	0	6	0	0	0	0	0	0	0	0	0	0	6
Thalassiosira weissflogii (Grunow) Fryxell *	TWEI	0	0	0	0	0	0	0	0	0	0	0	0	0
Tryblionella apiculata Gregory *	TAPI	0	0	0	0	0	0	0	0	0	0	0	0	0
Tryblionella hungarica (Grunow) D.G. Mann *	THUN	0	0	0	0	0	0	0	0	0	13	0	0	13



in Linnaeus *														
Brachysira brebissonii Ross in Hartley ssp.*	BWYG	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachysira wygaschii Lange- Bertalot	BBRE	0	0	0	0	0	0	0	0	0	0	0	0	0
Caloneis sp.	XXXX	0	3	5	0	0	0	0	21	0	0	4	12	45
Caloneis tenuis (Gregory) Krammer *	CATE	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis engelbrechtii Cholnoky	XXXX	0	0	0	0	0	0	0	0	0	0	0	0	0
cocconeis moleformis	XXXX	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis placentula Ehrenberg var. placentula	CPLA	8	3	1	0	0	0	0	21	0	0	0	5	38
Craticula accomoda (Hustedt) Mann *	CAMB	3	0	0	0	0	0	4	0	4	0	0	0	11
Craticula acidoclinata Lange-Bertalot & Met	CACD	0	0	0	0	0	0	0	0	0	0	0	4	4
Craticula ambigua (Ehrenberg) Mann *	CRAC	0	0	0	0	0	0	0	0	0	0	0	0	0
Craticula cuspidata Kützing var.media (Meis)	CRCM	0	0	0	0	0	0	11	1	0	0	0	0	12
Craticula halophila (Grunow ex Van Heurck) *	CVIX	6	1	3	0	0	4	63	13	15	0	0	0	105
Craticula vixnegligenda Lange-Bertalot	CHAL	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyclotella meneghiniana	CMEN	0	0	0	0	3	0	4	4	0	8	7	6	32

Kützing *														
Cymbella aspera (Ehrenberg) Peragallo *	CASP	0	0	0	0	1	0	0	0	0	0	0	3	4
Cymbella sp.	XXXX	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella neocistula Krammer var.neocistula *	CBAM	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbopleura amphicephala Krammer *	CNCI	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbopleura naviculiformis (Auerswald) Kram*	CBNA	0	0	0	0	0	0	0	0	0	0	0	0	0
Diademesis confervacea Kützing var. conferva*	DCOF	0	0	0	0	0	0	0	0	0	0	0	0	0
Discostella pseudostelligera (Hustedt) Houk*	DPST	0	0	0	0	0	0	0	0	0	0	0	0	0
Discostella stelligera (Cleve et Grun.) Hou*	DSTE	0	0	0	0	0	0	0	0	0	2	3	6	11
Encyonema mesianum (Cholnoky) D.G. Mann in *	ENME	21	0	1	0	0	0	0	6	0	0	0	0	28
Encyonema silesiacum (Bleisch in Rabh.) D.G*	ELSE	21	0	0	0	0	2	0	0	0	0	4	3	30
Encyonopsis buedelii Krammer	ECBU	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonopsis krammeri Reichardt *	ECKR	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonopsis minuta Krammer & Reichardt *	ECPM	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonopsis subminuta	ESUM	0	6	15	0	0	15	0	0	0	0	10	0	46

Krammer & Reichardt *														
Eolimna minima(Grunow) Lange-Bertalot *	EOMI	0	0	0	0	0	0	0	0	0	0	0	0	0
Epithemia adnata (Kützing) Brébisson *	EADN	0	0	0	0	0	0	0	0	0	0	0	0	0
Epithemia sorex Kützing *	ESOR	0	0	0	0	0	0	0	0	0	0	1	0	1
Eunotia bilunaris (Ehr.) Mills var. bilunar*	EBIL	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia exigua (Brebisson ex Kützing) Raben*	EEXI	0	0	4	0	0	0	0	0	0	0	0	0	4
Eunotia formica Ehrenberg sensu stricto *	EFOR	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia incisa Gregory var.incisa *	EINC	0	0	0	0	0	3	0	0	0	0	0	4	7
Eunotia minor (Kützing) Grunow in Van Heurc*	EMIN	16	7	5	4	5	2	0	0	0	0	16	0	55
Eunotia pectinalis(Kütz.)R abenhorst var.und*	EPUN	0	0	0	0	0	0	0	0	0	0	0	0	0
Fallacia pygmaea (Kützing) Stickle & Mann s*	FPYG	0	0	0	0	0	0	0	0	0	0	0	0	0
Fistulifera saprophila (Lange-Bertalot & Bo*)	FSAP	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria biceps (Kützing) Lange-Bertalot *	FBCP	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria capucina Desmazieres var.	FCAP	0	6	5	0	0	2	0	0	2	0	2	0	17

rumpen*														
Fragilaria capucina Desmazieres var.capucin*	FCRU	15	0	19	11	1	7	0	3	0	0	0	0	56
Fragilaria capucina Desmazieres var.vaucher*	FCVA	0	24	6	13	5	6	0	5	2	0	0	0	61
Fragilaria ulna (Nitzsch.)Lange-Bertalot va*	FUAC	0	0	4	80	46	71	2	4	2	0	4	25	238
Frustulia crassinervia (Breb.) Lange-Bertal*	FCRS	0	0	3	0	0	0	0	0	0	0	0	0	3
Frustulia rostrata Hustedt fo.angustior Mai	FSAX	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia saxonica Rabenhorst *	FROA	0	0	0	0	0	0	0	0	0	0	0	0	0
Frustulia vulgaris (Thwaites) De Toni *	FVUL	0	0	0	0	0	0	0	6	0	0	0	3	9
Geissleria decussis(Ostrup) Lange-Bertalot *	GDEC	10	0	4	0	0	0	0	5	0	0	0	0	19
Gomphonema affine Kützing *	GAFF	0	0	10	0	0	0	0	0	0	0	0	0	10
Gomphonema clavatum Ehr. *	GCLA	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema aff. Gracile	XXXX	0	5	7	0	0	11	0	0	0	0	0	0	23
Gomphonema gracile Ehrenberg *	GGRA	10	0	0	15	17	0	0	0	0	0	0	0	42
Gomphonema aff. lagenula	XXXX	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphonema parvulum (Kützing) Kützing	GPAR	0	26	12	11	12	0	0	0	0	0	13	13	87



*														
Navicula capitatoradiata Germain *	NCPR	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula cryptocephala Kützing *	NCRY	0	6	18	11	6	16	7	28	15	0	8	31	146
Navicula cryptotenella Lange-Bertalot *	NCTE	0	6	0	0	0	5	0	2	0	3	0	10	26
Navicula densa Hustedt	NDNS	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula erifuga Lange-Bertalot in Krammer *	NERI	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula germainii Wallace *	NGER	0	2	0	0	0	0	0	0	0	0	0	0	2
Navicula gregaria Donkin *	NGRE	0	0	14	0	0	0	0	0	0	0	0	0	14
Navicula notha Wallace *	NNOT	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula recens (Lange-Bertalot) Lange-Bert*	NRCS	0	0	8	3	1	4	10	18	27	6	0	0	77
Navicula reichardtiana Lange-Bertalot f. an*	NRCF	0	0	0	4	0	0	4	9	0	6	0	0	23
Navicula rhynchocephala Kützing *	NRIE	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula riediana Lange-Bertalot & Rumrich	NRHY	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula rostellata Kützing *	NROS	0	7	0	10	28	31	6	9	16	3	0	50	160
Navicula schroeteri Meister var.	NSHR	0	0	0	0	0	0	10	0	0	0	0	0	10







Sellaphora seminulum (Grunow) D.G. Mann *	SPRS	0	0	0	0	0	0	0	0	0	0	0	0	0
Stauroneis anceps Ehrenberg *	SARA	0	0	1	0	0	0	0	0	0	0	0	0	1
Stauroneis gracilis Ehrenberg	STAN	0	0	0	0	0	0	0	2	0	0	0	1	3
Stauroneis kriegeri Patrick *	SGRC	0	0	0	0	0	0	0	0	0	0	0	0	0
Stauroneis phoenicenteron (Nitzsch) Ehrenbe*	SPHO	0	0	0	0	0	0	0	0	0	0	0	0	0
Surirella angusta Kützing *	STKR	0	0	0	2	0	0	0	0	0	0	0	0	2
Staurensis sp.	XXXX	0	0	0	0	0	0	0	0	0	0	0	0	0
Surirella splendida (Ehrenberg) Kützing *	SANG	14	33	19	3	0	16	0	0	16	2	10	8	121
Synedra acus Kützing var. radians(Kützing) Hu	SSPL	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabellaria flocculosa (Roth) Kützing *	TFAS	0	2	4	0	0	0	0	0	0	0	0	0	6
Tabularia fasciculata (Agardh)Williams et R*	TFLO	0	0	0	5	0	4	0	0	0	0	0	0	9
Thalassiosira weissflogii (Grunow) Fryxell *	TWEI	0	0	0	0	0	0	0	0	0	0	0	0	0
Tryblionella apiculata Gregory *	TAPI	0	0	0	0	0	0	0	0	0	1	0	0	1
Tryblionella hungarica (Grunow) D.G.	THUN	0	0	0	0	0	0	20	0	44	56	0	0	120



Simonsen *														
Bacillaria paradoxa Gmelin in Linneaeus *	BPAR	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachysira brebissonii Ross in Hartley ssp.*	BWYG	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachysira wygaschii Lange- Bertalot	BBRE	0	0	0	0	0	0	0	0	0	0	4	0	4
Caloneis sp.	XXXX	13	0	29	4	0	0	0	0	7	0	8	0	61
Caloneis tenuis (Gregory) Krammer *	CATE	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis engelbrechtii Cholnoky	XXXX	0	5	0	0	0	0	0	0	0	0	2	0	7
cocconeis moleformis	XXXX	0	0	0	0	0	0	0	0	0	0	0	0	0
Cocconeis placentula Ehrenberg var. placentula	CPLA	18	14	11	12	8	0	0	21	0	12	8	11	115
Craticula accomoda (Hustedt) Mann *	CAMB	8	0	0	0	0	0	0	0	5	0	0	0	13
Craticula acidoclinata Lange-Bertalot & Met	CACD	0	0	0	0	0	0	0	0	0	0	0	0	0
Craticula ambigua (Ehrenberg) Mann *	CRAC	0	0	0	0	0	0	0	0	0	0	0	0	0
Craticula cuspidata Kützing var.media (Meis)	CRCM	0	0	0	0	0	0	0	0	0	0	0	0	0
Craticula halophila (Grunow ex Van Heurck) *	CVIX	0	0	0	1	0	3	0	0	10	12	3	0	29
Craticula vixnegligenda	CHAL	0	0	0	0	0	6	0	0	0	0	0	0	6

Lange-Bertalot														
Cyclotella meneghiniana Kützing *	CMEN	0	11	0	32	8	8	12	18	8	5	0	17	119
Cymbella aspera (Ehrenberg) Peragallo *	CASP	3	8	0	4	8	0	0	9	0	0	13	12	57
Cymbella sp.	XXXX	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbella neocistula Krammer var.neocistula *	CBAM	0	0	0	1	6	3	0	6	3	0	4	8	31
Cymbopleura amphicephala Krammer *	CNCI	0	0	0	0	0	0	0	0	0	0	0	0	0
Cymbopleura naviculiformis (Auerswald) Kram*	CBNA	3	0	0	0	0	0	0	0	0	0	0	0	3
Diadesmis confervacea Kützing var. conferva*	DCOF	0	0	0	0	7	6	0	0	0	0	6	3	22
Discostella pseudostelligera (Hustedt) Houk*	DPST	0	0	0	12	7	0	3	0	0	5	0	0	27
Discostella stelligera (Cleve et Grun.) Hou*	DSTE	0	0	0	0	0	0	0	13	0	0	0	0	13
Encyonema mesianum (Cholnoky) D.G. Mann in *	ENME	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonema silesiacum (Bleisch in Rabh.) D.G*	ELSE	14	0	0	7	4	4	0	10	6	0	7	9	61
Encyonopsis buedelii Krammer	ECBU	0	0	0	0	0	0	0	0	0	0	0	0	0
Encyonopsis krammeri Reichardt *	ECKR	5	0	0	3	0	0	0	0	0	0	0	0	8
Encyonopsis minuta Krammer	ECPM	0	0	0	0	4	4	0	5	0	0	0	0	13

& Reichardt *														
Encyonopsis subminuta Krammer & Reichardt *	ESUM	0	0	11	0	5	0	0	4	0	0	3	5	28
Eolimna minima(Grunow) Lange-Bertalot *	EOMI	0	0	0	0	0	3	0	0	0	0	0	0	3
Epithemia adnata (Kützing) Brébisson *	EADN	0	0	0	0	0	0	0	0	0	0	0	0	0
Epithemia sorex Kützing *	ESOR	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia bilunaris (Ehr.) Mills var. bilunar*	EBIL	0	0	10	0	0	0	0	0	0	0	0	0	10
Eunotia exigua (Brebisson ex Kützing) Raben*	EEXI	0	0	0	0	0	0	0	0	0	0	0	0	0
Eunotia formica Ehrenberg sensu stricto *	EFOR	0	0	0	0	0	0	0	0	0	0	8	7	15
Eunotia incisa Gregory var.incisa *	EINC	0	0			0	10	0	0	0	0	0	0	10
Eunotia minor (Kützing) Grunow in Van Heurc*	EMIN	9	8	0	5	5	6	0	8	10	0	8	4	63
Eunotia pectinalis(Kütz.)R abenhorst var.und*	EPUN	7	0	0	3	0	0	0	0	0	0	0	0	10
Fallacia pygmaea (Kützing) Stickle & Mann s*	FPYG	0	0	0	0	0	0	0	0	8	0	0	0	8
Fistulifera saprophila (Lange-Bertalot & Bo*	FSAP	0	0	0	0	4	0	0	0	0	0	0	0	4
Fragilaria biceps (Kützing) Lange- Bertalot *	FBCP	0	0	0	4	4	3	0	0	3	0	0	0	14



Gomphonema parvulum (Kützing) Kützing var. *	GPAR	6	18	11	0	0	0	0	3	2	15	0	4	59
Gomphonema pseudoaugur Lange-Bertalot *	GPSA	0	0	0	0	0	0	0	0	3	14	7	14	38
Gomphonema truncatum Ehr. *	GTRU	0	0	0	0	0	5	0	0	0	0	0	0	5
Gomphonema venusta Passy. Kociolek & Lowe	GVNU	0	0	0	0	0	0	0	0	0	5	0	0	5
Gyrosigma acuminatum (Kützing) Rabenhorst *	GYAC	0	0	0	0	0	0	0	0	0	0	0	5	5
Hantzschia amphioxys (Ehr.) Grunow var. amph	HAA M	0	0	0	0	4	0	0	0	0	7	0	0	11
Hippodonta apiculata	XXXX	0	0	0	0	0	0	0	0	0	0	6	3	9
Hippodonta capitata (Ehr.) Lange-Bert. Metzger*	HCAP	0	0	0	6	6	4	0	0	0	0	0	0	16
Lemnicola hungarica (Grunow) Round & Basson*	LHUN	0	0	0	0	0	0	0	0	0	0	0	1	1
Luticola acidoclinata Lange-Bertalot *	LACD	0	0	0	0	4	0	0	0	7	0	0	0	11
Luticola goeppertiana (Bleisch in Rabenhors*)	LGOE	0	0	0	0	0	0	0	0	0	0	0	0	0
Mayamaea atomus var. permissis (Hustedt) Lan*	MAPE	0	0	0	0	4	0	0	0	0	7	0	0	11
Melosira varians Agardh	MVAR	0	0	0	6	0	0	0	0	8	0	0	0	14

*														
Navicula antonii Lange-Bertalot *	NANT	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula capitatoradiata Germain *	NCPR	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula cryptocephala Kützing *	NCRY	0	0	12	5	12	18	0	6	8	0	8	12	81
Navicula cryptotenella Lange-Bertalot *	NCTE	0	0	0	1	4	3	0	0	0	0	3	3	14
Navicula densa Hustedt	NDNS	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula erifuga Lange-Bertalot in Krammer *	NERI	0	0	0	0	0	0	0	0	13	4	0	0	17
Navicula germainii Wallace *	NGER	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula gregaria Donkin *	NGRE	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula notha Wallace *	NNOT	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula recens (Lange-Bertalot) Lange-Bert*	NRCS	3	8	12	0	0	4	0	0	3	0	0	0	30
Navicula reichardtiana Lange-Bertalot f. an*	NRCF	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula rhynchocephala Kützing *	NRIE	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula riediana Lange-Bertalot & Rumrich	NRHY	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula rostellata Kützing	NROS	43	21	0	14	12	15	0	8	8	11	7	16	155



Nitzschia intermedia Hantzsch ex Cleve & Gr*	NINT	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia irremissa Cholnoky *	NIRM	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia liebetruthii Rabenhorst var.liebe*	NLBT	0	0	0	0	0	0	0	0	0	6	0	0	6
Nitzschia linearis(Agardh) W.M.Smith var.li*	NLIN	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia linearis(Agardh) W.M.Smith var.su*	NLSU	0	0	0	0	0	0	0	0	0	12	0	0	12
Nitzschia littorea Grunow in Van Heurck	NLTT	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia palea (Kützing) W.Smith var. pale*	NPAL	8	11	22	21	19	16	114	14	36	0	9	19	289
Nitzschia paleacea (Grunow) Grunow in van H*	NPAE	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia sp	XXXX	0	0	0	0	0	0	0	0	8	0	0	0	8
Nitzschia umbonata(Ehrenb erg)Lange- Bertalot*	NUM B	0	0	21	0	0	0	57	9	24	14	5	8	138
Pinnularia borealis Ehrenberg var. borealis*	PBOR	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinnularia divergens W.M.Sm. var.undulata (	PDIV	0	0	13	2	9	0	0	11	0	12	2	0	49
Pinnularia divergens W.M.Smith var.	PDUN	1	0	0	0	0	0	0	0	5	0	2	0	8

diverge*														
Pinnularia gibba Ehrenberg *	PGIB	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinnularia joculata (Manguin) Krammer	PJOC	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinnularia microstauron (Ehr.) Cleve var. r	PMRO	0	0	0	1	9	8	0	0	0	0	0	0	18
Pinnularia subbrevistriata Krammer	PSCA	12	6	17	0	0	16	0	0	3	0	0	0	54
Pinnularia subcapitata Gregory var. subcapi*	PSBV	0	0	13	0	0	0	0	10	0	0	0	0	23
Pinnularia viridiformis Krammer var. viridi	PVID	0	0	0	0	5	0	0	0	0	0	0	9	14
Pinnularia viridis (Nitzsch) Ehrenberg var.	PVFI	0	0	0	5	0	6	0	5	6	0	9	3	34
Placoneis placentula (Ehr.) Heinzerling *	PPLC	0	0	0	4	0	0	0	0	0	0	0	0	4
Planothidium engelbrechtii (Choln.) Round &*	PLEN	0	0	0	0	0	0	0	0	0	0	0	0	0
Planothidium frequentissimum( Lange-Bertalot*	PLFR	12	7	0	0	8	9	0	0	0	0	6	0	42
Planothidium rostratum (Oestrup) Lange- Bert*	PRST	11	8	13	11	7	7	0	9	0	0	0	7	73
Pseudostaurosira brevistriata var. elliptic	PSBE	0	0	0	0	0	0	0	0	0	0	1	0	1
Sellaphora pupula (Kützing) Mereschkowsky	SPUP	14	24	0	2	7	12	29	24	21	28	5	7	173











Fragilaria biceps (Kützing) Lange- Bertalot *	FBCP	3	0	0	0	3	0	0	0	0	0	0	0	6
Fragilaria capucina Desmazieres var. rumpen*	FCAP	0	0	0	0	3	0	0	0	0	0	0	0	3
Fragilaria capucina Desmazieres var.capucin*	FCRU	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria capucina Desmazieres var.vaucher*	FCVA	0	0	0	0	0	0	0	0	0	0	0	0	0
Fragilaria ulna (Nitzsch.)Lange- Bertalot va*	FUAC	3	16	0	0	4	3	0	6	16	6	0	3	57
Frustulia crassinervia (Breb.) Lange- Bertal*	FCRS	0	0	0	9	2	0	0	0	0	0	0	18	29
Frustulia rostrata Hustedt fo.angustior Mai	FSAX	0	0	0	9	6	6	0	6	0	0	0	15	42
Frustulia saxonica Rabenhorst *	FROA	0	0	0	0	0	0	0	0	0	0	6	0	6
Frustulia vulgaris (Thwaites) De Toni *	FVUL	6	0	6	0	6	0	0	6	0	0	9	3	36
Geissleria decussis(Ostrup) Lange-Bertalot *	GDEC	12	40	42	27	24	12	0	13	21	0	24	39	254
Gomphonema affine Kützing *	GAFF	0	3	0	9	6	0	0	0	0	15	6	0	39
Gomphonema clavatum Ehr. *	GCLA	0	0	0	0	0	0	0	0	0	9	0	0	9
Gomphonema aff. Gracile	XXXX	0	5	0	0	0	3	0	3	0	0	0	9	20
Gomphonema gracile Ehrenberg	GGRA	0	0	0	0	0	0	0	6	0	0	0	0	6



Lan*														
Melosira varians Agardh *	MVAR	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula antonii Lange-Bertalot *	NANT	0	0	0	0	2	0	0	0	0	0	0	0	2
Navicula capitatoradiata Germain *	NCPR	3	0	0	0	0	0	0	0	0	0	0	0	3
Navicula cryptocephala Kützing *	NCRY	3	3	0	9	3	0	0	0	0	6	15	6	45
Navicula cryptotenella Lange-Bertalot *	NCTE	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula densa Hustedt	NDNS	0	0	0	0	5	0	0	0	0	0	0	0	5
Navicula erifuga Lange-Bertalot in Krammer *	NERI	12	0	0	0	0	0	0	0	0	0	6	0	18
Navicula germainii Wallace *	NGER	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula gregaria Donkin *	NGRE	0	9	0	0	0	0	0	0	0	0	0	0	9
Navicula notha Wallace *	NNOT	27	9	0	0	10	0	0	0	0	0	0	9	55
Navicula recens (Lange-Bertalot) Lange-Bert*	NRCS	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula reichardtiana Lange-Bertalot f. an*	NRCF	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula rhynchocephala Kützing *	NRIE	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula riediana Lange-Bertalot &	NRHY	0	0	0	3	0	3	0	6	3	0	0	0	15

Rumrich														
Navicula rostellata Kützing *	NROS	51	12	0	6	6	9	0	9	0	12	0	6	111
Navicula schroeteri Meister var. schroeteri*	NSHR	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula trivialis Lange-Bertalot var. triv*	NTRV	0	0	0	0	0	0	0	0	0	0	0	0	0
Navicula veneta Kützing *	NVEN	0	3	15	6	3	0	6	3	0	12	9	0	57
Navicula viridula (Kützing) Ehrenberg *	NVIR	3	0	0	0	0	0	0	0	0	0	0	0	3
Neidium productum (W.M.Smith)Cleve var.mino	NPMI	0	0	0	0	0	0	0	0	0	0	0	9	9
Nitzschia amphibia Grunow f.amphibia *	NAMP	0	6	9	6	6	0	0	9	0	0	0	0	36
Nitzschia andoclinata	XXXX	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia archibaldii Lange- Bertalot *	NIAR	0	0	0	3	9	0	0	0	0	0	0	0	12
Nitzschia capitellata Hustedt in A.Schmidt *	NCOM	3	3	6	0	0	0	0	0	0	0	0	0	12
Nitzschia communis Rabenhorst *	NCPL	0	0	0	0	0	0	60	0	0	0	0	0	60
Nitzschia dissipata (Kützing) Grunow ssp.di*	NDIS	0	0	6	0	0	0	0	0	0	0	0	0	6
Nitzschia etoshensis Cholnoky	NETO	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia	NIFR	0	0	0	0	0	0	0	0	0	0	3	0	3

frustulum (Kützing) Grunow var.frustulum														
Nitzschia intermedia Hantzsch ex Cleve & Gr*	NINT	0	0	0	0	0	0	0	0	0	0	9	0	9
Nitzschia irremissa Cholnoky *	NIRM	0	0	6	0	0	0	0	0	0	0	0	0	6
Nitzschia liebethuthii Rabenhorst var.liebe*	NLBT	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitzschia linearis(Agardh) W.M.Smith var.li*	NLIN	8	6	0	0	0	0	0	0	0	0	0	0	14
Nitzschia linearis(Agardh) W.M.Smith var.su*	NLSU	0	0	0	0	0	0	0	0	0	36	0	0	36
Nitzschia littorea Grunow in Van Heurck	NLTT	0	0	0	0	0	0	9	0	0	0	0	0	9
Nitzschia palea (Kützing) W.Smith var. palea*	NPAL	17	15	30	9	6	0	0	0	24	0	3	0	104
Nitzschia paleacea (Grunow) Grunow in van H*	NPAE	2	0	0	0	10	0	0	0	0	0	0	0	12
Nitzschia sp	XXXX	0	0	0	0	0	0	36	0	0	0	0	3	39
Nitzschia umbonata(Ehrenb erg)Lange- Bertalot*	NUM B	0	2	15	0	6	3	150	48	137	135	9	3	508
Pinnularia borealis Ehrenberg var. borealis*	PBOR	0	4	0	0	0	0	0	0	0	0	0	0	4
Pinnularia divergens W.M.Sm. var.undulata (	PDIV	0	6	9	3	6	3	0	0	0	6	0	3	36





