Diatom, Macroinvertebrate and Riparian Vegetation Community Structure Responses in Agriculturally Impacted Rivers.

By

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O sweet spontaneous
earth how often have
the
doting

          fingers of
purient philosophers pinched
and
poked

thee
,has the naughty thumb
of science prodded
thy

      beauty   .how
often have religions taken
thee upon their scraggy knees
squeezing and

buffeting thee that thou mightest conceive
gods
        (but
true

to the incomparable
couch of death thy
rhythmic
lover

          thou answerest

them only with

                        spring)

~ e.e cummings (1894-1962)

Summary

Pesticides and fertilizers, while allowing increases in food production, also have the ability to find their way into aquatic systems. Irrespective of their route of entry into an aquatic ecosystem, they may affect aquatic biota by influencing survival, growth and reproduction. Secondary effects may occur in which populations of organisms are impacted due to a reduction or elimination of pollution-susceptible species which results in a disturbance of biological processes and interactions due to water quality impacts associated with agricultural practices. Biomonitoring techniques are used to assess the integrity of aquatic ecosystems and provide information on the environmental conditions that have prevailed within a river. Because aquatic organisms are exposed to their environment and all pollutants and toxicants thereof, they will cumulatively reflect the conditions which they are exposed to. This study aims to assess community structure, biotic integrity and feeding traits of aquatic communities at river sites that have varying adjacent land uses.

The chosen study area falls within the Crocodile (West) Marico Water Management Area (WMA). Study sites were selected on the Magalies and Crocodile rivers which form Hartbeespoort Dam at their confluence. Agricultural intensity in North West Province is high and irrigation farming tends to be located on the large floodplains associated with the middle Crocodile River. The main economic activity of the WMA occurs in Gauteng, and is generated by the intense urban and industrial activities of northern Johannesburg. These practices ensure that water pollution from agriculture and urban land use is a major problem along segments of the rivers under investigation.

Study sites were chosen based on their adjacent land use and consisted of sites related to agricultural, urban and natural activities. Environmental driver components that were assessed included water quality and habitat integrity (IHAS). Biotic response indices were implemented to assess the integrity of diatom (GDI, SPI, BDI, EPI and %PTV), macroinvertebrate (SASS5 and MIRAI) and riparian vegetation (VEGRAI) communities. Principal Component Analysis (PCA) analyses were undertaken on water quality data using Primer version 6 to determine patterns in water quality between sites. Multivariate (CLUSTER, NMDS and RDA) and univariate (Margalef's index, Shannon-Wiener diversity index and Pielou's evenness) analyses were performed on macroinvertebrate family data, macroinvertebrate FFG data, diatom species data and riparian vegetation data using Primer version 6 and Canoco version 4.5 in order to elucidate differences in community structure per land use.

Results indicated that particular water quality and habitat impacts were present for each land use. Comparison of community structure of diatoms taken from sites with varying land uses showed differences from one another. Relative reference diatom communities comprised of diatom species that had preferences for clean water, whilst community structures of diatoms were modified and showed specific change in relation to agricultural and urban water quality impacts. An increased diversity in air breathing macroinvertebrates was shown at sites with agricultural practices at high flow, where urban sites were differentiated from agricultural sites due to the presence of the Hydropsychidae and Hirudinea families. At low flow macroinvertebrate families making up communities overlapped between land uses. A difference noted at low flow was that the contribution of the Chironomidae was higher at urban sites in comparison to agricultural sites, indicating organic water pollution.

The statistical comparison of macroinvertebrate communities, FFGs and riparian vegetation showed that differences between sites with different land uses were not significant. Nonetheless, some differences in refined data were noted for the varying land uses. Considering the macroinvertebrate community make up of the relative reference site, which was comprised of macroinvertebrate families that were more sensitive and showed preferences for higher water quality, community structures of macroinvertebrates were modified and showed change in relation to land use.

Macroinvertebrate FFGs indicated that a change in the input of UPOM at agricultural sites, and a change in the presence of FPOM at urban sites were responsible for a shift in the FFG dominance. A difference in riparian integrity was noted between relative reference and test sites, but could not be easily distinguished between test sites with different land uses. Riparian integrity was more predictive of macroinvertebrate FFG structure than actual macroinvertebrate community structure. This indicates that riparian integrity and comparison with biological traits such as FFGs were useful in showing impacts due to organic matter inputs.

Overall biotic indices were less useful in distinguishing between urban and agricultural land uses. It appeared that biotic indices masked the changes in the actual taxonomic components, erroneously suggesting that sites with different land uses are similar in terms of ecosystem integrity. It must be noted that integrity indices certainly have an important place in management of aquatic systems, but it appears to be more useful to utilise taxonomic make up and biological traits (in this case of FFGs) to show specific impacts, as these are factors which can be compared across a relatively broad spatial scale.

It can be concluded that sites could be separated according to land use based on community structure of diatoms and macroinvertebrates, and biological trait analysis of feeding groups. It was noted that diatom communities were more defined in their response to land use practices in comparison to macroinvertebrate communities.

Key words: Land use, macroinvertebrates, diatoms, riparian vegetation, FFG, water quality

Opsomming

Akwatiese sisteme word dikwels blootgestel aan insekdoders and kunsmatige voedingstowwe, wat alledaags gebruik word om voedselproduksie te verhoog. Akwatiese biota se oorlewing, groei en voortplanting potensiaal word beïnvloed deur die teenwoordigheid van die stowwe in die akwatiese omgewing, ongeag van hoe dit die sisteem binnedring. Sekondêre effekte op populasie vlak is dikwels tot gevolg, deurdat daar 'n reduksie of eliminasie van meer sensitiewe spesies is. Dit weer, veroorsaak 'n versteuring in biologiese prosesse en interaksie tussen organismes. Biomoniterings tegnieke word gebruik om die ekologiese integriteit van akwatiese sisteme te kwantifiseer. Hierdie praktyk verskaf waardevolle inligting ten opsigte van stres faktore waaraan akwatiese biota moontlik blootgestel word. Akwatiese biota ervaar 'n meer intense blootstelling ten opsigte van die akwatiese omgewing (respirasie, voortplanting en voeding). Dus sal 'n kumulatiewe stres respons verwag word in die teenwoordigheid van afval, en besoedel stowwe. Hierdie studie poog om die gemeenskap samestelling, biologiese integriteit, asook voeding tendense van organismes te bepaal, wie se akwatiese omgewing blootgestel is aan verskeie impakte.

Die studie area onder bespreking kom binne die krokodil (Wes) marico water bestuur area (WBA) voor. Verskeie liggings van opname is gekies in die Magalies en Krokodil riviere hierdie riviere sluit saam aan by Hartebeespoort Dam. Die Noordwestelike provinsie word geken aan landbou aktiwiteite, deel hiervan is besproeiing aktiwiteite. Besproeiing spilpunt kom hoofsaaklik op die groot vloedvlaktes, geassosieer met die middellope van die Krokodil Rivier, voor. Die oorgrote meerderheid van ekonomiese aktiwiteit wat in die WBA voorkom is geleë in Gauteng. Intensiewe stedelike en industriële aktiwiteite wat in die noordelike gedeeltes van Johannesburg plaas vind , dien as voorbeeld hiervan. Gesamentlik dra hierdie aktiwiteite by tot die probleem van water besoedeling in die rivier sisteme onder bespreking.

Studieliggings is gekies op grond van die omliggende grondgebuike, en sluit liggings in wat geassosieer word met, landbou, stedelike en natuurlike aktiwiteite. Omgewings drywende komponente wat geassesseer was sluit in, water kwaliteit en habitat integriteit (IHAS). Biologiese respons indekse was geïmplementeer om die integriteit van diatome (GDI, SPI, BDI, EPI asook % PTV), makro invertebrate (SASS5 en MIRAI) en rivier-bank plantegroei (VEGRAI) gemeenskapsamestelling te assesseer. Onderliggende tendense in water kwaliteit, tussen onderskeie studie liggings, is geanaliseer deur van Beginsel Komponent Analise (BGA) gebruik te maak. Primer weergawe 6 is vir die doeleindes gebruik. Enkelvoudige (CLUSTER, NMDS en RDA) asook veelvoudige (Margalef's indeks, Shannon Wiener diversiteit indeks en Pielous evenness) statistiese berekeninge was gedoen op makro invertebraat familie data, makro invertebraat FFG data, diatoom data asook rivier-

bank plantegroei data, deur middel van Primer weergawe 6 en Conoco weergawe 4.5, ten einde ooreenkomste in gemeenskap samestellings en land grondgebruike te bepaal.

Resultate toon dat spesifieke water kwaliteit en habitat impakte teenwoordig was vir onderskeie grondgebruike. Gemeenskap samestelling van diatome in vergelyking met verskillende grondgebruike toon verskille ten opsigte van mekaar. Relatiewe diatoom gemeeskapsameltellings bestaan uit diatoom spesies wat ‘n voorkeur vir skoon water het, terwyl diatoom gemeenskapsamestellings wat blootgestel was aan landbou en stedelike aktiwiteite afwykings getoon het. Studieliggings wat geassosieer is met landbou aktiwiteite toon ‘n hoër diversiteit van invertebrate wat atmosferiese kontak nodig het vir respirasie. Terwyl liggings wat geassosieer is met stedelike aktiwiteite ‘n voorkeur vir Hydropsychidae and Hirudinea families toon. Makro invertebraat gemeenskapsamestellings tussen onderskeie grondgebruike het oorvleuel tydens laag vloei. Die teenwoordigheid van Chironomidae by stedelike liggings was hoër as by landbou liggings, tydens laag vloei. Hierdie verskynsel dui waarskynlik op organiese besoedeling teenwoordig in liggings geassosieer met stedelike aktiwiteite.

‘n Statistiese vergelyking tussen makro invertebraat gemeenskappe, FFG en rivier-bank plantegroei toon nie ‘n beduidende verskil tussen liggings met verskillende grondgebruike is nie. Nie te min was daar egter sekere verskille sigbaar vir die onderskeie grondgebruike. Verwysings gemeenskapsamestellings van invertebrate, bestaan uit meer sensitiewe families wat ‘n voorkeur vir skoon water toon. ‘n Vergelyking van invertebraat gemeenskapsamestelling van stedelike asook landelike grondgebruike toon afwykings ten opsigte van mekaar asook verwysing gemeenskapsamestelling.

Makro invertebraat FFGs toon ‘n verandering met die inset van UPOM by landbou liggings, en ‘n verandering in die teenwoordigheid van FPOM by stedelike liggings, wat verantwoordelik is vir ‘n verskrywing in die FFG dominansie. Daar is ‘n beduidende verskil tussen die integriteit van die verwysings rivier-bank plantegroei en die rivier-bank plantegroei met verskillende grondgebruike. Die verskil in rivier-bank plantegroei tussen verskillende grondgebruike is egter nie so ooglopend nie. Die rivier-bank plantegroei was meer beduidend van makro invertebraat FFG samestelling as werklike makro invertebraat gemeenskapsamestelling. Rivier-bank plantegroei integriteit in vergelyking met biologiese kenmerke soos FFGs, toon dus hulpvaardig te wees om impakte veroorsaak deur organiese besoedeling aan te dui.

Algehele biotiese indekse was nie hulpvaardig om verskille tussen landbou en stedelike grondgebruike te toon nie. Dit wil voorkom of biotiese indekse die werklike taksonomiese komponente versteek, wat foutiewelik voorstel dat liggings met verskillende land gebruike soortgelyk is ten opsigte van ekosisteem integriteit. Integriteit indekse speel ‘n belangrike rol in die bestuur van akwatiese hulpbronne, maar dit blyk asof die gebruik van taksonomiese samestellings and biotiese kenmerke (in die geval FFGs) ‘n beter gereedskapstuk is om meer spesifieke impakte aan te dui.

Ter samevatting: studieliggings kon verdeel word tussen land gebruike op grond van gemeenskapsamestelling van diatome, makro invertebrate en biotiese kenmerk analise van voedingsgroepe. Diatoom gemeenskappe is meer reaktief as die van makro invertebraat gemeenskappe, ten opsigte van land gebuik.

Sleutel woorde: Land gebruike, makroinvertebrate, diatome, rivier-bank plantegroei, FFG, water kwaliteit.

Chapter 1 : Introduction

1.1 Motivation for the Study

The mid-year population estimates for 2007 released by Statistics South Africa suggest a population growth estimate of approximately 6.87% on 2001 census figures (Stats SA, 2007). This increase in population has lead to an increase in food requirements and augmented the need for more productive agricultural practices, which in turn has increased the use of pesticides and fertilizers in the agricultural industry (Ongley, 1996).

Agrochemicals and fertilizers, while allowing increases in production to meet food demand, also have the ability to find their way into aquatic systems; for example by spray drift, leaching, run-off and accidental spills. Irrespective of their route of entry into an aquatic ecosystem, they may affect aquatic biota (both target and non-target) by affecting survival, growth and reproduction (Brock & Budde, 1994). Secondary effects may occur in which populations of organisms are impacted in an indirect way due to a reduction or elimination of pollution-susceptible species which results in a disturbance of biological processes and interactions.

The motivation for this study is twofold; aquatic biota, in particular macroinvertebrates, are integral in keeping riverine ecosystems in a healthy, functioning condition in which the maximum amount of goods and services may be delivered to the users of these water resources. They are vital in the processing of transported organic matter in rivers, serve an essential function in the purification of the water in a river, contribute to the recycling of minerals and nutrients and also provide a valuable food source for larger animals, both aquatic and terrestrial (Maltby, 1996).

Secondly, quoting section 24(a) and section 27.1 (b) of The Bill of Rights (1996), everyone has the right to have access to:

- an environment that is not harmful to their health or well-being.
- sufficient food and water."

Section 24(b) goes on to say that everyone has the right to, "have the environment protected, for the benefit of present and future generations, through reasonable legislative and other measures that:

i. prevent pollution and ecological degradation;

ii. promote conservation; and

iii. secure ecologically sustainable development and use of natural resources while promoting justifiable economic and social development."

In the preamble of the National Water Act (NWA, No. 36 of 1998) it identifies and recognises the need for protection of the quality of water in South Africa. It also recognises that basic human and environmental reserves are to be identified and met so that utilisation is equitable and sustainable in terms of quality, quantity and reliability of supply. Aquatic ecosystems thus must be protected and managed to ensure that the water resource in question will remain fit for use in agriculture, recreation, industry and human consumption on a long term basis.

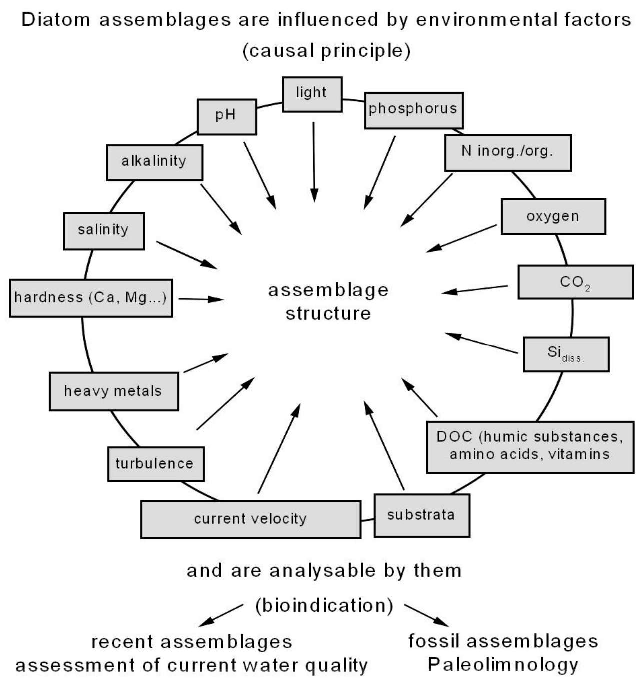
1.2. Aquatic Biota as Indicators

Biomonitoring techniques assess the integrity of aquatic ecosystems and provide information on the environmental conditions that have prevailed within a river (Davies & Day, 1998). Because aquatic organisms are exposed to their environment and all pollutants and toxicants thereof, they will cumulatively reflect the conditions which they are exposed to. Different indicator groups and advantages for using these groups for biomonitoring are discussed briefly below.

1.2.1 Diatoms

Diatoms have recently been used in the State of the Rivers Report for the Crocodile (West) Marico Water Management Area as an indicator of water quality (RHP, 2005). In various studies diatoms have also proved to be useful in indicating specific water quality problems such as organic pollution, eutrophication and heavy metal pollution (Taylor, de la Ray & van Rensburg, 2005a). The reasons why diatoms are considered to be useful tools for biomonitoring include the fact that they are cosmopolitan, their cell cycle is rapid and they react quickly to disturbances. Unlike other aquatic biota, diatoms do not have specific food requirements or specialised habitat niches and are not predominantly governed by stream flow.

Because diatoms make up a large proportion of algal communities, they provide a representative group of species that are indicative of the effects of specific water quality problems. Changes in water chemistry will inhibit the multiplication of some species, while supporting that of others, thus the percentage composition of certain species within a community will be changed (Cholnoky, 1960 cited in Harding, Archibald and Taylor, 2005). Changes in species composition can then be used to reflect changes in water quality in a more integrated manner than traditional chemical sampling (Figure 1.1).



1.1: The causal principle (Schonfelder, 2000).

1.2.2 Macroinvertebrates

Of particular interest and suitability to the present study are riverine macroinvertebrates. The appropriateness of macroinvertebrates for bioassessments is outlined by Rosenberg and Resh (1993) and Barbour, Gerritsen, Snyder and Stribling (1999);

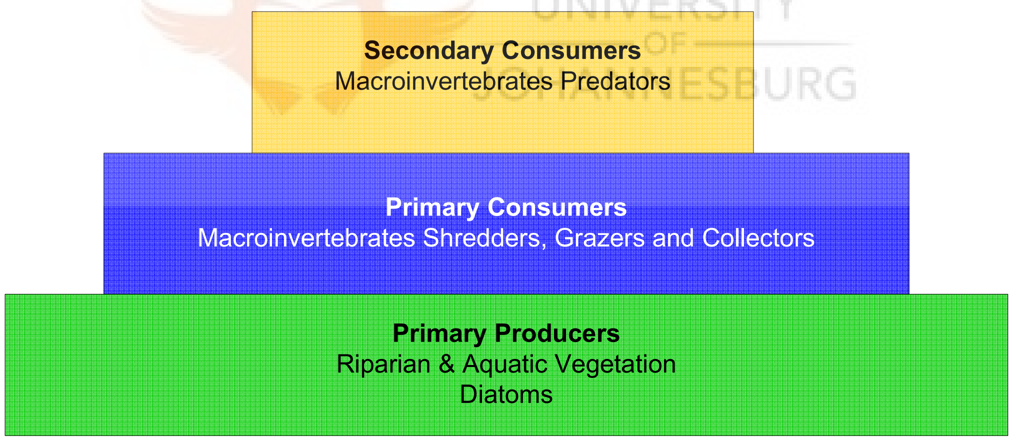
- they compose the bulk of riverine biotic diversity
- they have wide sensitivity ranges to toxicants and pollutants in their ambient environment and show effects of short term variations, and
- macroinvertebrates have short life cycles thus exposing them to potential pollution for a large part of their life cycle which makes them useful for interpreting cumulative effects.

Barbour *et al*. (1999) note that macroinvertebrates are well-suited for assessing site specific impacts as they are confined to certain habitat reaches and have limited migratory pattern or are sessile. This makes them useful in pin-pointing areas that are exposed to pollutants and changes in water quality (Dickens & Graham, 2002).

1.2.3 Riparian Vegetation

Riparian vegetation integrity plays an integral role in providing food and habitat to fish and macroinvertebrates, and is the main link between terrestrial health and instream integrity (Kleynhans, Mackenzie & Louw, 2007). Riparian zones shield streams from adjacent lands by trapping sediment, nutrients and contaminants (Rios & Bailey, 2006). Riparian vegetation provides shade necessary to prevent an increase in solar radiation reaching the water and protects the health of stream ecosystems (Bunn, Davies & Mosisch, 1999). Because the riparian belt is at the border of the terrestrial and aquatic zones, riparian areas are powerful indicators of river quality (Richards, Host & Arthur, 1993).

Taking into consideration the advantages of utilising each of the above biotic components of river ecosystems as indicators of ecosystem health and integrity, incorporation of the above river fauna and flora will give an integrated and holistic view of what is unfolding ecologically at each site (Figure 1.2). The combination of insights offered by using aquatic biota from varying trophic levels as indicators of pollution are invaluable, and ensures a reflection of integrated ecosystem health (Barbour *et al*., 1999)



1.2: A trophic pyramid indicating the integration of biotic indicators with varying trophic status that are used in the present study.

1.3 Research Area

The chosen study area falls within the Crocodile (West) Marico Water Management Area (WMA 3) (RHP, 2005). The rivers that are incorporated in the study are the Magalies and Crocodile rivers which form Hartbeespoort Dam at their confluence. The upper portion of the catchment, south east of Hartbeespoort Dam, is located in the Gauteng Province, whereas the central or western sections fall within the North West Province.

Agricultural intensity in North West Province (after Hartbeespoort Dam) is high and constitutes one of the main land uses (Lehohla, 2002). Irrigation farming tends to be located on the large floodplains associated with the middle and lower Crocodile River. The mean annual gross irrigation requirement, which is based on rainfall and evaporation, ranges from 1400mm in the south east to approximately 2000mm in the drier north western parts. All in all, about 650km^2 of irrigation has been recorded for these sub-management areas (DWAF, 2002).

The ecological state of the Upper Crocodile sub-management area is "poor" according to data gathered from the River Health Programme (2005). The extent to which agriculture contributes to this poor ecological state is as of yet not quantified. In a provincial profile by Statistics South Africa (Lehohla, 1999) the outcomes showed that an average of 1.7% of the urban and non-urban population living in North West obtained daily drinking water from rivers or dams. This figure translates into approximately 12 515 people per day that are being exposed on a long term basis to possible agricultural pollutants which find their way into surface water.

The main economic activity of the WMA occurs in Gauteng, and is generated by the intense urban and industrial activities of northern Johannesburg and Pretoria (situated in the South of the WMA) together with extensive platinum mining (near Rustenburg). These urban areas associated with WMA3 are responsible for the fact that the Crocodile (West) Marico WMA is the second most populated water management area in South Africa (DWAF, 2002). This ensures that water pollution from urban practices is a major problem along segments of the Crocodile River.

1.4 Water Quality Impacts Related to Agriculture

As mentioned above, the increase in food requirements has augmented the need for more productive agricultural practices, which in turn has increased the use of pesticides and fertilizers, and caused expansion of irrigation. Tilman (1996) reports that this increase in food production has been associated with a 6.87-fold increase in nitrogen fertilization, a 3.48-fold increase in phosphorus fertilization, a 1.68-fold increase in the amount of irrigated cropland and a 1.1-fold increase in land in cultivation.

The impacts and consequences of irrigation, pesticide use, fertilizer application and land preparation have the potential to affect water quality in the receiving aquatic ecosystems. In a natural aquatic ecosystem biota achieve a long term balance with the natural variation in abiotic factors, if conditions are disturbed, the abundance and composition of species in the ecosystem may change irreversibly. Sensitive aquatic biota will respond to unfavourable changes in water quality (Rosenberg & Resh, 1993). A few main water quality impacts related to agricultural practices, namely salinisation, soil disturbance (sedimentation), nutrient enrichment and pesticide pollution are discussed below.

1.4.1 Salinisation

The clearance of natural vegetation and irrigation are the two most important measures resulting in salinity problems (Williams, 2001). Catchments (particularly in low rainfall areas) where clearing has taken place for agriculture have river salinities varying from marginal to saline (Schofield & Ruprecht, 1989). Removal of natural, deep-rooted vegetation from catchments and its replacement by shallow-rooted agricultural plant species, together with the discharge of saline agricultural wastewater, causes the salinity of many rivers to rise. The salinities of rivers also increase as water is diverted from inflows for irrigation and other purposes (Williams, 1987).

A study on the Blackwood River (south-western Australia) showed salinisation impacts due to land use practices (Williams, 2001). The catchment had been largely cleared of natural vegetation for agricultural purposes. Before 1910, the salinity of the river water was <0.5 g/l, but salinity gradually rose throughout the century as agriculture started to predominate so that now the salinity is around >3 g/l throughout the river and a reversed longitudinal salinity zonation is present.

According to Lemly (1994), irrigation of crops in semi-arid regions occurs at several times the rate of precipitation. Surface run-off is a product of irrigation, and occurs due to spillage of irrigation water and over watering of crops. As is common with irrigation farming, much more water is applied to the crops than is necessary which leads to flushing of salts that have accumulated in the root zones after evaporation (Moore, Winckel, Detwiler, Klasing, Gaul, Kanim, Kesser, Debevac, Beardsley, & Puckett, 1990 cited by Lemly, 1994).

Sub-surface drainage is produced due to soil conditions where subsurface clay hinders the vertical and lateral movement of irrigation water as it infiltrates downward. This results in water logging of the crop root zone and build-up of salts as excess water evaporates from the soil surface (Moore *et al*., 1990). The accumulated subsurface water must then be removed in order for crop production to continue.

The ecological effects of the salinisation of rivers and streams are noted by Williams (2001) as;

(1) overall, biodiversity decreases, and

(2) the opportunistic inhabitation of salt-tolerant (halophilous) biota.

1.4.2 Soil Disturbance

Agricultural activities have important hydrological, geomorphological and ecological implications in terms of sedimentation. Typical agricultural practices that cause soil disturbance and that are associated with water pollution are tillage, non-contour ploughing, crop clearing, riparian zone clearing and burning. These practices generally allow for sediment loss via erosion, and inevitably, these sediments land up in rivers and streams (Dallas & Day, 2004). Practices, such as the ones mentioned above, contribute to the synergy of impacts of sedimentation not only by affecting the volume and timing of sediment delivery to a river, but also by increasing runoff to the aquatic environment (Wood & Armitage, 1997).

Pollution by sediments has physical and chemical water quality impacts. Physical pollution due to land degradation by erosion leads to increased levels of turbidity and sedimentation, while the silt and clay fraction have adsorption properties which have implications in terms of chemical pollution (Ongley, 1996). The silt and clay fraction are charged and are carriers of phosphorous, chlorinated pesticides and trace metals. Because particles that are adsorbed to the clay and silt fraction of the suspended sediment are not bioavailable to aquatic organisms, it is advantageous in terms of toxic trace metals and pesticides, but is unfavourable in the case of nutrients (DWAF, 1996).

Elevated turbidity influences primary production by limiting the amount of light entering the aquatic ecosystem, and decreasing water temperature by reflecting heat from the surface. The effects of these impacts are felt from diatom and algae communities (primary producers), right through to invertebrate and fish communities (tertiary and secondary consumers) (Wood & Armitage, 1997).

Wood & Armitage (1997) report that primary producers are affected in terms of a:

(1) decrease in biomass and productivity,

(2) decrease in species diversity, and

(3) decrease in abundance when sediment transport and deposition are increased by anthropogenic activities.

A study on the influence of land use on stream integrity showed that sediment loads were higher in areas of greater agriculture (Allan, Erickson & Fay, 1997). Sediment yields noted in the study were up to a 1000% higher in the area with increased agricultural land use.

1.4.3 Nutrient Enrichment

Wastes from livestock, agricultural land clearing, land preparation and fertilizer application may increase nitrogen and phosphorous concentration in aquatic ecosystems (Dallas & Day, 2004). Because poor land preparation practices eventuate in erosion, degraded agricultural soils that are stripped of their nutrients need to be supplemented with fertilizers to maintain productivity (Joly, 1993).

Nutrient enrichment from agricultural runoff of fertilizers initially impact river systems by increasing the productivity, thus different species of algae may become more competitive and species composition may change (Kelly, 1998). Invertebrate communities may then experience an increase in invertebrate abundance and altered community structure (Bourassa & Cattaneo, 1998). Miltner and Rankin (1998) showed that aquatic community structure could be correlated directly with the phosphate concentration.

Dodds and Welch (2000) reported that in severe nutrient pollution cases, carbon may build up and cause low dissolved oxygen (DO) concentrations and high pH levels. Macroinvertebrate and fish growth in a system with low DO and high pH is reduced and mortality may occur in these communities.

1.4.4 Pesticide utilisation

South Africa is the foremost agricultural power in sub-Saharan Africa and forms approximately 60 % of the pesticide market in this region (Buckley & Naidoo, 2001). Therefore, the potential for environmental and ecological hazards to occur in South Africa due to pesticide use is high. The agricultural sector is a major user of pesticides in South Africa and accounts for a large percentage of the sales (Buckley & Naidoo, 2001).

In agricultural areas, rivers may be subjected to non-point inputs of pesticides via irrigation returns, spray drift and surface runoff (Schriever, Hansler Ball, Holmes, Maund & Liess, 2007). Surface runoff from irrigation contains high concentrations of pesticides and herbicides if aerial spraying is undertaken. If land-based application of these materials has occurred then agricultural runoff becomes the main route of pesticide entry into rivers (Moore *et al.,* 1990 cited by Lemly, 1994; Schulz, Peall, Dabrowski & Reinecke, 2001), however, spray drift is considered the most important and well researched of the above mentioned modes of entry into aquatic ecosystems.

There are five main properties that dictate a pesticide's impact on water quality (Ongley, 1996);

(1) The active ingredient of the formulation,
(2) impurities in the active ingredient,
(3) additives mixed with the active ingredient such as wetting agents, solvents and emulsifiers,
(4) the persistence of the pesticide, and
(5) the degradate of the active compound that is formed after degradation.

According to Ansara-Ross, Wepener & van den Brink (2008), spraying season for North West Province is in March/April and August/October each year and is conducted according to planting of winter and summer crops. The main pesticides used in this province, listed from most commonly used to least common are deltamethrin and cypermethrin (pyrethroids), parathion (organophosphate), endosulfan (organochloride) and aldicarb (carbamate) (Ansara-Ross *et al.,* 2008).

Each of the classes of pesticides used in the North West Province has their own characteristic modes of action that cause changes in aquatic communities. Pyrethroids require lower application rates in comparison to other pesticides so the chances of them escaping into the general aquatic environment are lessened. However, they are 'highly toxic' to macroinvertebrates, and 'extremely' toxic to fish if they do enter rivers (Laws, 2000). Because the general toxic action of organophosphates is the inhibition of acetylcholinesterase, the potential for organophosphates to cause mortalities in non-target species of insects is of major concern (Dallas & Day, 2004). Organochlorines tend to be extremely persistent in the environment, are associated with biomagnification and are thus extremely toxic to fish (Laws, 2000).

Information on the impact of levels of current-use insecticides on in-stream communities in South African surface waters is sparse due to the difficulty with interpretation of cause-effect relationships and environmental variables (Davies & Day, 1998; Schulz & Liess, 1999).

1.5 Main Research Question

The main research question is, "are there any specific associated impacts on community structures of aquatic macroinvertebrates and diatoms due to agriculture and its associated practices?"

1.6 Hypotheses

Hypothesis One

'*Agriculture and its associated impacts have a specific influence on the taxonomic make up of communities of macroinvertebrates and diatoms in comparison to communities found at sites associated with urban and natural land use activities.* '

Hypothesis Two

'*The macroinvertebrate functional feeding group structure of agricultural communities will differ in comparison to communities found at sites associated with urban and natural land use activities.*

1.7 Aims

Aim 1

The first aim will be to determine whether the composition of the instream community structure of macroinvertebrates and diatoms adjacent to agricultural land is significantly different taxonomically to the aquatic communities associated with urban and natural land use.

Aim 2

The second aim will be to elucidate whether indicator species or taxa are present in instream communities that are specific to each adjacent land use.

Aim 3

Finally, the third aim will be to determine whether aquatic macroinvertebrates at agriculturally impacted sites will show different FFG traits in comparison to sites with urban and natural land use activities.

1.8 Specific Research Objectives

1. To collect community structure data for macroinvertebrates, diatoms and riparian vegetation from seven sites in the Crocodile (West) Marico Water Management Area for two seasons (high flow and low flow).
2. To compare community structure data (taxonomic and abundance) for the above stated aquatic biota taken from agriculturally impacted sites, to sites impacted by urban inputs and natural/reference sites.
3. To compare feeding traits of macroinvertebrates taken from agriculturally impacted sites and sites impacted by urban inputs and natural/reference sites.
4. To elucidate in what way community structures of macroinvertebrates and diatoms are impacted or modified (if any) due to agricultural inputs into the aquatic system.
5. Determine riparian vegetation and physico-chemical factors which may interact with agricultural inputs that may increase change in community structure.
6. To identify suitable end-point/s for indication of change due to agricultural pollution and impacts in these communities.

1.9 Brief Dissertation Outline

Chapter 2 serves as an introduction to the biomonitoring sites selected for monitoring of water quality, riparian vegetation, macroinvertebrates and diatom components the of the study.

Chapter 3 will focus on water quality and diatom communities in relation to adjacent land use. Related field, laboratory and statistical methodology will be addressed in this chapter.

Aquatic macroinvertebrate community structure, community integrity, habitat and feeding traits are statistically compared and presented in Chapter 4. Relevant field and laboratory methodology will also be addressed.

Chapter 5 deals with the relationship between riparian vegetation integrity and macroinvertebrate structures.

Chapter 6 contains a summary of all the work presented in chapter 2, 3, 4 and 5 and includes a section on conclusions drawn from the present study and recommendations for further studies.

Chapter 7 serves as a reference chapter for works referenced in Chapters 1 to 6. Finally, appendices contain site photos and detailed macroinvertebrate, diatom, riparian vegetation, habitat and water quality data sheets.

Chapter 2 : Site Selection and Site Description

2.1 Introduction

Study sites were chosen in three sub-management areas according to the main land use as ascertained by the Internal Strategic Perspective for WMA3. The Magalies and Crocodile rivers were the focus of this study (Figure 2.1).

2.1.1 Upper Crocodile River Sub-management Area

The southern segment of this sub-catchment is highly developed with the large industrial and urban developments of northern Johannesburg. The rest of this area, north of the Magaliesberg Mountain Range, includes significant irrigation (approximately 270 km^2) and mining activities. Irrigated cash crop agriculture takes place below the Hartbeespoort Dam extending towards the town of Brits. Smallholding and commercial farming activities, with restricted formal irrigation taking place in the area to the north west of Johannesburg (DWAF, 2004).

2.1.2 Elands River Sub-management Area

This area forms the western drier portion of the catchment. There are many platinum mining activities here with potential for new mines to develop. There is a significant amount of irrigation in this region (approximately 50 km^2). The area between Rustenburg and Brits on the northern side of the Magaliesberg range is characterised by its large citrus farming activities (DWAF, 2004).

2.1.3 Lower Crocodile River Sub-management Area

This area is typified by large-scale irrigation activities along the main stem of the Crocodile River (approximately 134 km^2) while in the rest of the sub-area the main activity is noted as cattle and game farming (DWAF, 2004).

Two different types of study sites were selected, namely “relative reference” sites which were reference sites relative to the impacts being assessed in this study; and monitoring sites that have site specific impacts relating to land use (Eekhout, Brown & King, 1996). “Relative reference” sites were compared to monitoring sites to elucidate the change in community structure composition from sites that had agriculture and urban as adjacent land use as their main activities.

A desktop study was done using 1:50 000 topographic maps and aerial photos to select potential reference and monitoring sites that fell in line with the objectives of the project (Appendix A). A reconnaissance survey was undertaken in February 2006 for ground-truthing purposes to finalize the study sites.

A total of seven sites were selected above and below Hartbeespoort Dam wall on the Crocodile and Magalies rivers to collect diatom, aquatic macroinvertebrate, fish, water quality and riparian vegetation data based on the land use adjacent to the Rivers. Two sites were selected on the Magalies River and five sites on the Crocodile River. These study sites are indicated in Table 2.1 and Figure 2.1 and range from perceived least impacted to highly impacted in terms of agricultural practices. Field sampling took place at high (April 2006) and low flow periods (August 2006).

Table 2.1: Coordinates and locations of study sites selected and sampled at high and low flow scenarios in the North West Province (NWP) and Gauteng indicating level 1 Ecoregions.

	Site	**Coordinates**		**Position of Site**	**Ecoregion Level 1**
Crocodile River	*Downstream of Hartbeespoort Dam*				
	Crocodile 1	25 12'21.6"S	27 33'28.0"E	Assen (NWP)	Western Bankenveld
	Crocodile 2	25 32'50.9"S	27 42'24.6"E	Bapong (NWP)	Bushveld Basin
	Crocodile 3	25 43'00.4"S	27 50'36.2"E	Brits (NWP)	Western Bankenveld
	Upstream of Hartbeespoort Dam				
	Crocodile 4	25 49'19.4"S	27 54'40.4"E	Broederstroom (NWP)	Western Bankenveld
	Crocodile Reference	26 05'11.1"S	27 50'36.6"E	Roodepoort (Gauteng)	Highveld
Magalies River	*Upstream of Hartbeespoort Dam*				
	Magalies Reference	26 00'57.8"S	27 33'55.5"E	Randfontein (Gauteng)	Western Bankenveld
	Magalies 2	25 47'32.3"S	27 44'10.0"E	Hekpoort (NWP)	Western Bankenveld

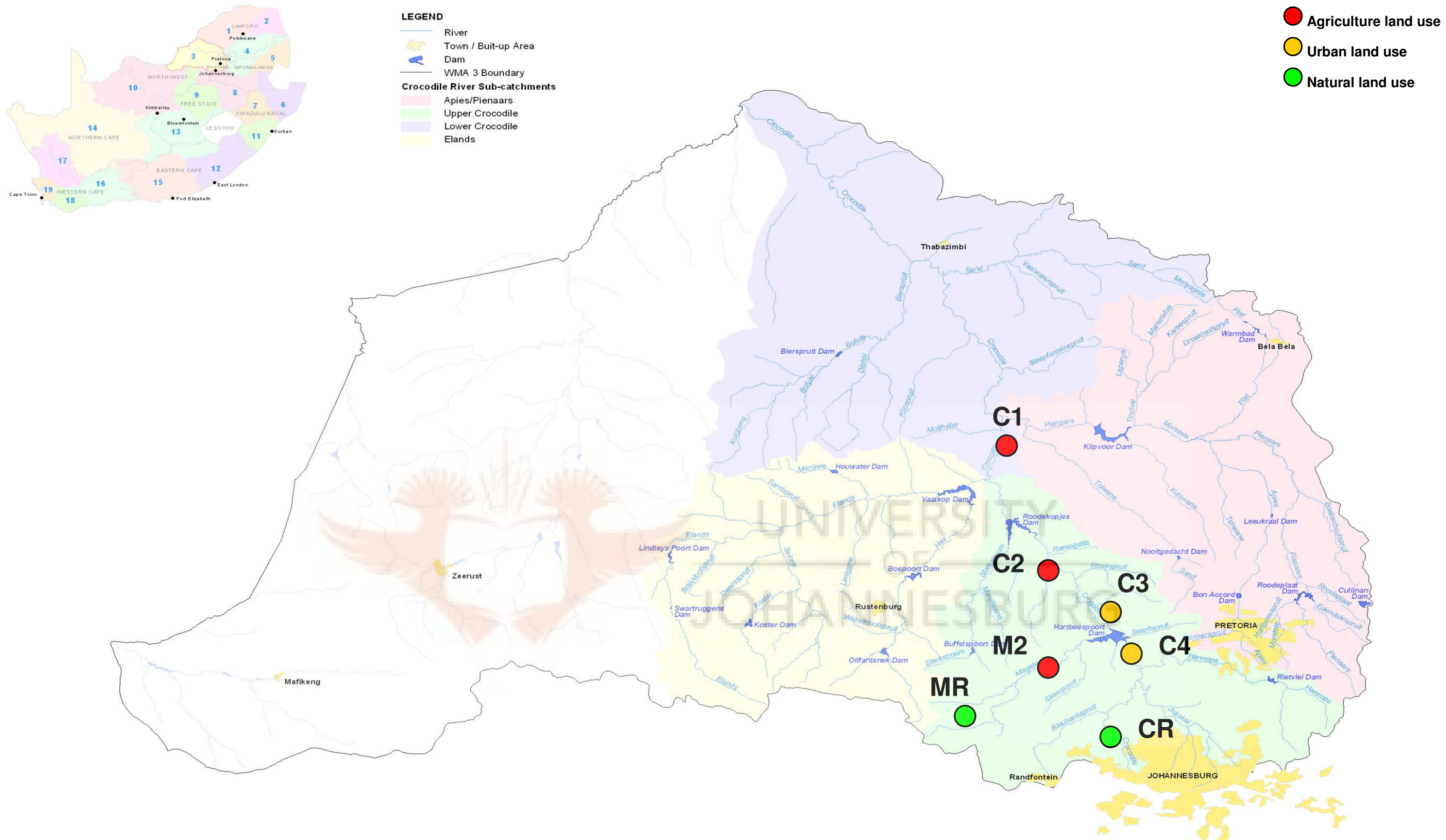


Figure 2.1: The Crocodile (West) Marico Water Management study area indicating the seven biomonitoring sites that were selected along the Crocodile and Magalies rivers for the present study. The Water Management Area is divided into sub-management areas, as indicated in the legend [Red= agricultural monitoring sites; Orange= urban monitoring sites; Green= relative reference sites].

2.2 Study Sites

2.2.1 The Crocodile River

Ecologically, the Crocodile River is one of the most significant rivers in the country. The river is typified by a variety of riverine habitats, which makes it one of the most biologically diverse systems in South Africa (DWAF, 2004). Figure 2.1 indicates the position of the sites in WMA3, and Appendix A contains topographical maps and aerial photos for each site.

2.2.1.1 Crocodile River Site 1 (C1)

The first site on the Crocodile River (C1) is situated in Assen in the Northwest Province and the majority of adjacent land use is agricultural/cultivation (Figure 2.2). The Crocodile River runs through the Roodekopjes Dam before reaching this site, after which it meets with the Elands River. A main irrigation canal leaves Roodekopjes Dam and re-enters the Crocodile River before this site, indicating that there is a point source of agricultural pollutants entering the river upstream. Diffuse source contamination by agrochemicals is likely to occur due to spray drift from spraying the adjacent crops. Because of the land use adjacent to the river, it is expected that land run off will contribute to water quality problems that are related to agricultural practices. Site C1 is selected as a monitoring site for agriculturally impacted water quality and habitat.

Figure 2.2: Various views of the Crocodile River site 1 (Assen, North West Province).

2.2.1.2 Crocodile River Site 2 (C2)

Crocodile River site 2 (C2) is near Brits in the Northwest Province and is positioned on the Crocodile River downstream of an irrigation canal which enters the river (Figure 2.3). The adjacent land-use is agricultural and recreational with the surrounding area being highly canalised. Site C2 is placed before Roodekopjes Dam and is selected as a monitoring site for agriculturally impacted riverine habitat.

Figure 2.3: Various views of the Crocodile River site 2 (Brits, North West Province).

2.2.1.3 Crocodile River Site 3 (C3)

The third site (C3) is approximately 1 km downstream of Hartbeespoort Dam wall (Figure 2.4). Adjacent land use is recreational and preceding this site, an aqueduct and a furrow flank out of Hartbeespoort Dam on either sides of the dam wall. The impacts due to land use at this site are more to do with the development and maintenance of the recreational/residential areas surrounding the river and impacted by urban run off related disturbances. Site C3 is selected as a monitoring site for urban-related impacts.

Figure 2.4: Various views of the Crocodile River site 3 (Hartbeespoort, North West Province).

2.2.1.4 Crocodile River Site 4 (C4)

The site is positioned approximately 5 km upstream of the Hartbeespoort Dam in a very sparse rural residential area in Lime Hill (Figure 2.5). The adjacent land is open and largely natural with a few very small developments. The site is situated down stream of its confluence with the Hennops and Jukskei rivers, and receives effluent related to the Northern Sewage works which service Alexandra and Diepsloot. Impacts that are related to this site involve nutrient overloads linked to sewage treatment and urban run off. Site C3 is selected as a monitoring site for urban-related impacts.

Figure 2.5: Various views of the Crocodile River site 4 (Lime Hill).

2.2.1.5 Crocodile River relative reference Site (CR)

The site is positioned in the Walter Sisulu Botanical Gardens in Roodepoort and is near the source of the Crocodile River (Figure 2.6). The land use is recreational and the riparian zone appears to be relatively intact. For the purpose of this study, the site is selected as a reference site from which the other monitoring sites on the Crocodile River will be compared. Possible impacts noted were urban run-off and a series of weirs which upset the continuity of the upper reaches of the Crocodile River.

2.2.2 The Magalies River

2.2.2.1 Magalies River Site 2 (M2)

The Magalies site 2 (M2) is situated downstream of the reference site on the Magalies River (Figure 2.7). The surrounding land use is agricultural and recreational with small rural settlements interspersed between these activities. Site M2 is selected as a monitoring site for an agriculturally impacted river reach.

2.2.2.2 Magalies River Relative Reference Site (MR)

The site is within the Happy Acres recreational facility near the source of the Magalies River at Maloney's Eye (Figure 2.8). The land adjacent to the River is in a natural condition, and impacts at this site are minimal, thus site MR is used as a reference site for the Magalies River.

Figure 2.6: Various views of the Crocodile River relative reference site (Roodepoort, Gauteng Province).

Figure 2.7: Various views of the Magalies River site 2 (North West Province).

Figure 2.8: Various views of the Magalies River relative reference site.

2.3 Summary

A summary of selected sites and impacts that are caused by surrounding land use are indicated in Table 2.2.

Table 2.2: Summary of monitoring and reference sites selected for the study indicating the type of site, adjacent land use at each specific site and expected associated impacts in relation to land use.

Site	Type of Study Site	Adjacent Land-Use	Associated Impacts on the Aquatic Environment (Ongley, 1996).
C1	Monitoring	Agriculture	▪ Irrigation return containing salts, nutrients and pesticides. ▪ Leachates from fertilizers such as nitrogen entering ground/surface water. ▪ Enrichment of surface/ground water with salts and nutrients (nitrate). ▪ High levels of turbidity and sedimentation. ▪ Disruption of the hydrologic regime.
C2	Monitoring	Agriculture	
MR	Monitoring	Agriculture	
C3	Monitoring	Recreational/ Urban	▪ Urban run off causing: ○ Heavy metal pollution ○ Organic contaminants ○ Nutrient overloads. ○ Sedimentation. ○ Increased COD[***]
C4	Monitoring	Open/Urban	
CR	Relative Reference	Recreational/ Semi Natural	▪ Possible nutrient overloads ▪ Sedimentation ▪ heavy metal and pesticide
M2	Relative Reference	Natural	▪ Little or no impacts are perceived.

Chapter 3 : Diatoms, Water Quality and Land Use

3.1 Introduction

3.1.1 Role of Diatoms in Biomonitoring.

Diatoms as indicators of water quality were recognized in South Africa by Dr. B.J Cholnoky. Cholnoky's diatom work (1952–1970) endeavoured to provide information on water quality based on the specific pollution tolerances of diatom species (Taylor, Harding, Archibald & van Rensburg, 2005b). The use and relevance of diatoms as biomonitoring tools in riverine environments in South Africa have recently been investigated again (De la Rey, Taylor, Laas, van Rensburg & Vosloo, 2004; Harding, Archibald & Taylor, 2005; Taylor *et al*., 2005b; Taylor, Janse van Vuuren & Pieterse, 2007a). Bate, Smailes and Adams (2004) have also reported on the Water Quality Index values for dominant benthic diatoms located thus far in the rivers and estuaries of South Africa.

Biomonitoring techniques using diatoms as bioindicators have been implemented in national monitoring programmes because of certain limitations in traditional chemical methods. Diatoms are primary producers of the aquatic food web, and thus occupy a vital position at the boundary between biological communities and their physico-chemical environment (Lavoie, Vincent, Pienitz & Painchaud, 2004). Using diatoms as bioindicators can give more integrated and specific answers to water quality questions, as well as allowing the establishment of water quality reference conditions in South Africa (Taylor, *et al*., 2005a; Taylor *et al*., 2005b). Taylor (2004) showed that diatom pollution indices may be suitable water quality bioindicators for South African rivers because they had strong correlations to various water quality variables such as pH, conductivity, phosphorous and nitrogen.

In terms of land use impacts in diatom communities, there is a gap in the knowledge of diatom community responses in agriculturally stressed rivers in South Africa; however, studies have been carried out in some parts of Australia, Europe, Canada and America on this topic.

3.1.2 Study Area Land-Use and Potential for Pollution.

Modern agriculture is responsible for chemical and physical impacts due to increased contaminant and nutrient runoff, increases in suspended solids and changes in discharge and channel morphology (Skinner, Lewis, Bardon, Tucker, Catt & Chambers, 1997). Thus, the potential for water pollution at sites where the adjacent land use is agricultural is high. Chapter 1

(Section 1.4) details the impacts of adjacent agricultural land use on water quality and the potential for pollution due to this practice, where Chapter 1 (Section 1.3) and Chapter 2 discuss the research area and land use associated with each site.

3.1.3 Land use and Diatom Community Structure

A multi-spatial scale assessment of land-use and diatom assemblages using partial canonical correspondence analysis (CCA) showed that the percentage of agricultural land-use at varying spatial scales explained between 3.7%–6.3% of variability in the diatom species dataset (Pan, Herlihy, Kaufmann, Wigington, van Sickle & Moser, 2004). Pan and colleagues (2004) showed that the percent of obligate nitrogen-heterotrophic taxa was the only diatom auto-ecological metric that showed a significant but weak correlation with the percentage agricultural land-use along the river network. Diatom assemblages, however, clearly reflected agriculture related impacts on sampled streams, where 72% of diatoms were salt tolerant taxa which suggest that sampled streams may be affected by salinisation due to irrigation.

Further studies on using diatoms as bioindicators of agricultural pollution (and hence land use) were undertaken by Lavoie *et al.* (2004). This study showed that ordination of agricultural sites were unmistakably separated from natural sites, though no significant separation was noted in relation to the intensity and type of agriculture, which indicated the importance of farming practices on a local scale.

3.1.4 Diatom Responses to Agricultural Land use Patterns.

The effects of specific water quality changes, which are related to agriculture, upon diatom communities are discussed below.

The effects of salinisation on periphyton were examined in 39 streams in Victoria, Australia (Blinn & Bailey, 2001). Non-Metric Multi-Dimensional ordination of diatom communities located in drainages with varied land-use practices were significantly different from one another with strong correlation to land-use practices, such as historic clear cutting, and secondary salinisation. Results reported that streams influenced by heavy irrigation practices and dry-land farming had reduced species diversity and richness compared to systems with low to moderate land use. Conductivity was found to be a useful indicator of agricultural intensity (Munn, Black & Gruber, 2002).

Paleolimnological studies in Dallas, Texas (Bradbury & Van Metre, 1997) linking the start of agriculture to diatom assemblages, found that accelerated soil erosion and increased turbidity from agriculture lowered productivity in diatom communities and changed the species composition. The same trend was noted for diatom community analysis carried out on a stream draining agricultural water in Kintore (Ontario, Canada) by Winter and Duthie (2000). The diatom species community make up of agriculturally impacted sites differed once again from sites that had natural adjacent land use. A decrease in the abundance of diatoms at the agriculturally impacted site in comparison to the relative reference site was also noted. In contrast an outdoor mesocosm experiment on the growth of the diatom *Skeletonema costatum*, it was found that turbidity caused by turbulence did not significantly affect growth of the phytoplankton (Patel, Guganesharajah & Thake, 2004).

Anderson, Renberg and Segerstrom (1995) analysed diatoms in sediment from Kassjon in Northern Sweden to determine the response of the periphyton communities to the start of the agricultural era in the 13th century. Results of the study showed that prior to the 12th century, diatom community species make up differed 'dramatically' to when agriculture became the main land use. When arable agriculture declined in the 1980's and was replaced by pasture and re-forestation, the diatom community changed once more in response to increased nutrient ratios.

In a study involving the effects of nutrients on diatoms, functional groups of diatoms were identified and the relative contribution of each of these guilds to total community diversity was quantified by using manipulated nutrient gradients. One functional group's biomass was significantly altered by nutrient enrichment. The results of this study indicate that nutrient enrichment may limit the conditions that are agreeable to many "nutrient generalists", creating a niche that may be occupied by "nutrient specialists" that are equipped to gain from such conditions (Carrick, Lowe & Rotenberry, 1988).

In a study by Downing, Delorenzo, Fulton, Scott, Madden and Kucklick (2004), microbial assemblages in a highly impacted watershed in terms of pesticide usage is Florida, were colonized onto artificial substrates, transported to the laboratory and exposed to atrazine, chlorothalonil and endosulfan. It was shown that atrazine and chlorothalonil significantly reduced chlorophyll a, phototrophic carbon assimilation and bacterial biomass, but stimulated diatom productivity, while endosulfan reduced diatom abundance. Diatom assemblages did not exhibit increased resistance to subsequent doses of these pesticides.

Differentiation between impacts of urban waste waters and farmland nutrient pollution using diatom community structure is possible. Results from literature (Rott, Duthie & Pipp, 1998) report that subtle differences between eutrophication and biodegradable organic pollution can be observed according to indicator species.

The objective of this chapter is to compare and relate changes in diatom species assemblages to the major types of land use noted at the sites in this study. This will be undertaken by elucidating in which way water chemistry is changed by land use patterns, and subsequently how community structures of diatoms are modified due to agricultural impacts on the aquatic system. The relative importance of the effects of agricultural land use and other environmental variables such as water chemistry on diatom assemblages will be explored. Two seasons of water quality and diatom data were collected to see if the effects of land use on stream water chemistry varied between seasons.

3.2 Materials and Methods

3.2.1 Study Sites

Study sites and the selection there of are described in Chapter 2. Please see Table 2.1, Table 2.2 and Figure 2.1.

3.2.2 Water Quality

Physico-Chemical water variables were measured *in situ* at each site before biotic sampling was carried out and included temperature, pH, dissolved oxygen (DO saturation and concentration), total dissolved solids (TDS) and conductivity. Handheld water quality meters were used for *in situ* analysis (Eutech pH 110 RS232C; Eutech DO6 dissolved oxygen meter and a Eutech CON 110 RS232C conductivity, TDS and temperature meter).

Two litre sub-surface water samples were collected at high (April) and low flow (August) periods in 2006 at each site. Water samples were immediately placed on ice and transported to the laboratory for further analysis.

Samples were allowed to reach room temperature before spectrophotometric analysis was carried out using a Merck Photometer SQ 118. Variables measured in the laboratory using the spectrophotometric method were turbidity, nitrite (NO_2^-), nitrate (NO_3^-), orthophosphate (PO_4), total phosphate (TP), calcium (Ca), soluble chloride (Cl), sulphate (SO_4), ammonium (NH_4), ammonia (NH_3) and chemical oxygen demand (COD). Water quality results were compared to the Target Water Quality figures for aquatic ecosystems as set out by DWAF (1996).

Total suspended solids (TSS), total suspended organic matter (TSOM) and total suspended inorganic matter (TSIM) were measured by filtering 1 litre of water collected from each site, for each sampling season, through a pre-weighed, pre-dried nitrocellulose filter membrane (47mm/0.45 µm pore size) using a Millipore glass vacuum filtration system with a Millipore filter holder attached to a vacuum. Once the samples had been filtered, the filter papers were dried at 60ºC for 48 hours, after which they were weighed again. Dried filter paper was placed inside a pre-dried and pre-weighed crucible and incinerated at 600ºC for 8 hours to obtain the proportions of organic to inorganic suspended matter. TSS concentrations were estimated using the weight of residuals on the membrane filters and was expressed as g/l.

3.2.3 Diatoms

Diatom field and laboratory procedures where carried out according to the methodology described by Taylor *et al.* (2005a).

3.2.3.1 Field Collection Procedures

Cobbles (<2cm-20cm) and boulders (<20cm) that were free from filamentous algae were used to collect diatoms for high and low flow samples as this biotope is considered to be preferential substrate for biomonitoring purposes (Kelly, Cazaubon, Coring, Dell'Uomo, Ector, Goldsmith, Guasch, Hürlimann, Jarlman, Kawecka, Kwandrans, Laugaste, Linstrøm, Leitao, Marvan, Padisák, Pipp, Prygiel, Rott, Sabater, Van dam & Vizinet, 1998). Five cobbles/boulders in flowing water with mucilaginous diatom growth were chosen from a 10 meter reach at each site for the purpose of representivity. The upper surface of each cobble was scrubbed with a toothbrush and rinsed into a tray using distilled water. The contents of the tray were then transferred to a small plastic storage bottle to which 10% ethanol was added.

3.2.3.2 Diatom Sample Cleaning Technique

Diatom samples were prepared for microscopy by using the hot hydrochloric acid (HCl) and potassium permanganate ($KMnO_4$) method (Hasle, 1978).

Diatom samples were re-suspended and 10 ml of suspension was decanted into separate 50 ml heat resistant glass beakers. An equal amount of saturated $KMnO_4$

solution was added to each beaker, mixed and left to stand for 24 hours. In a fume cabinet, 10 ml of concentrated HCl (32%) was added to each sample and covered with a watch glass. The samples were then gently boiled (approximately 90ºC) on a hot plate in a fume cabinet for between 1 to 3 hours, or until each sample had cleared sufficiently.

After the samples had cleared they were allowed to stand over night. The supernatant was decanted, after which the samples were re-suspended and transferred to 10 ml centrifuge tubes. The diatom samples were rinsed five times by centrifugation with distilled water at 2 500 rpm for 10 minutes cycles. After each centrifugation, supernatant was decanted and diatom material at the bottom of the centrifuge tube was loosened by means of a jet of distilled water from a wash bottle. After the last rinse, the diatoms were once again loosened by means of a jet of distilled water, vortexed and then poured into small glass storage vials.

3.2.3.3 Diatom Slide Preparation

Cleaned samples were re-suspended in distilled H_2O and a small portion (determined by the density of the diatoms in the suspension) of each sample was added to a clean test tube. A single drop of ammonium chloride (NH_4Cl; 10% solution) was added to each test tube containing diatom samples to neutralise electrostatic charges and reduce aggregation of the diatoms (McBride, 1988), after which each sample was diluted with distilled water until it appeared slightly cloudy.

Samples were well mixed with a vortex mixer to suspend the diatoms in the solution, and approximately 1 ml of the cleaned diatom suspension was placed on clean, dry cover-slips and dried at room temperature. After the cover-slips had dried they were placed on a hot plate at 350°C for 1 to 2 minutes to sublimate the residual NH_4Cl.

The diatom coated cover-slips were allowed to cool after which one drop of Pleurax mountant (high refractive mountant) was placed onto each cover-slip. A glass slide was then lowered onto the cover slip, inverted, and heated at approximately 120°C on a hot plate until the mounting medium simmered and the solvent evaporated. The slides were left to cool and the surplus mountant was removed with isopropyl alcohol.

3.2.3.4 Diatom Enumeration

For the purposes of this study, 300 to 600 diatom frustules were counted for ecological analysis (Prygiel, Carpentier, Almeida, Coste, Druart, Ector, Guillard, Honeré, Iserentant, Ledeganck, Lalanne-Cassou, Lesniak, Mercier, Moncaut, Nazart, Nouchet, Peres, Peeters, Rimet, Rumeau, Sabater, Straub, Torrisi, Tudesque, van der Vijver, Vidal, Vizinet & Zydek, 2002). Suggested rules for counting diatoms according to CEN (2004) were followed. A Zeiss photomicroscope I with differential interference contrast optics (DIC) was used for identification and enumeration at a magnification of 100 x 1.3 N.A (oil immersion objective). The microscope was attached to a JVC video camera with frame grabber and images were captured using Automontage software.

3.2.3.5 Diatom Identification

The taxonomic guide by Taylor, Harding and Archibald (2007b) was consulted for identification purposes in this study. Where necessary, Krammer and Lange-Bertalot (1986; 1988; 1991a & 1991b) were used for identification and for confirmation of species identification.

3.2.4 Data Analysis and Diatom Index Calculations

Multiple endpoints were used (species assemblages, auto-ecological metrics and diatom indices) to relate diatom assemblages to land-use impacts. The diatom indices used in this study, as well as reasons for their selection are listed in Table 3.1.

Table 3.1: Diatom based indices, abbreviations for each index and the reason for selection and inclusion in the study.

Diatom Index	Abbr.	Reason for Selection
Generic Diatom Index (Coste & Ayphassorho, 1991)	GDI	Functions at a genus level of identification The most straightforward index.
Specific Pollution sensitivity Index (CEMAGREF, 1982)	SPI	Contains the broadest species base of all of the indices.
Biological Diatom Index (Lenoir & Coste, 1996)	BDI	Incorporates 14 parameters of water quality, and therefore would best reflect water quality problems.
Eutrophication/Pollution Index (Dell'Uomo, 1996)	EPI	Reflects eutrophication.
Percentage Pollution Tolerant Valves (Kelly & Whitton, 1995)	%PTV	Indicates organic pollution and eutrophication.

Diatom community data were entered into OMNIDIA (Lecointe, Coste & Prygiel, 1993) software which incorporated the above indices, with the calculation of the index scores. In all cases (excluding the %PTV) the diatom indices were calculated using the weighted average formula of Zelinka and Marvan (1961) (Cited in Taylor *et al.,* 2007a):

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

Where *aj* = abundance of species *j* in sample; *vj* = indicator value and *sj* = pollution sensitivity of species *j.*

For all of the above indices (except %PTV which has a maximum value of 100) the maximum value is 20, where a score tending to zero indicates an increasing level of pollution or eutrophication. Class values for diatom index scores for SPI, GDI, BDI and EPI indicating varying levels of pollution are found in Table 3.2 (Eloranta & Soininen, 2002). These values were used in this study for the interpretation of the scores yielded by the various indices.

Table 3.2: Class values for diatom indices in the evaluation of water quality classes.

Index score (SPI, BDI, GDI, EPI)	Class	Trophy
>17	High Quality	Oligotrophy
15 to 17	Good Quality	Oligo-mesotrophy
12 to 15	Moderate Quality	Mesotrophy
9 to 12	Poor Quality	Meso-eutrophy
<9	Bad Quality	Eutrophy

3.2.5 Statistical Data Analyses

3.2.5.1 Environmental Statistics

Primer version 6 statistical suite was used on water quality data for sample sites at high and low flow. Temperature data was excluded after an initial statistical analysis because of the contribution of the variation in temperature between seasons to skewness in the data. Selected water quality data variables (DO (O_2 and %), conductivity, pH, NO_2, NO_3, COD, NH_3 and NH_4) were Log (V) transformed due to the skewness in data indicated by the scatter plot for variable pairs (Draftsman Plot). Principal Component Analysis (PCA) was carried out on normalised data. A lower triangular resemblance matrix was created based on Euclidean distance between samples. The resemblance matrix was subjected to two-dimensional non-metric Multi-Dimensional Scaling (NMDS). Finally, the BIOENV procedure using Biota and/or Environment

(BEST) matching based on Spearman's correlation was used to identify variables that best explained species ordination.

3.2.5.2 Diatom Community Statistics

Primer version 6 was used to construct Bray-Curtis similarity matrices from square root transformed diatom species abundance data recorded for each site at high and low flow occasions. Similarity matrices were subjected to group averaged hierarchical clustering (CLUSTER) and ordination by NMDS to summarise patterns in species composition. Two-dimensional NMDS uses an algorithm which refines the positions of the points until they satisfy the dissimilarity between samples (Clarke & Warwick, 2001). Stress values of 0.1 correspond to a good ordination with no real prospect of a misleading interpretation (Clarke & Warwick, 2001). Factors were assigned to Bray-Curtis resemblance matrices based on groupings from the CLUSTER analysis and NMDS ordination. Permutation-based hypothesis testing using One-way Analysis of Similarities (ANOSIM) was undertaken to determine the extent of the differences between diatom community structures for the different samples at different flow periods. Pairwise comparisons from ANOSIM were used to identify significant differences (p<0.05) between groupings of diatom community compositions. One way Analysis of Similarity Percentages (SIMPER) based on species contribution was used to identify the species of diatoms that primarily provided discrimination between sample clusters. K-dominance plots were included to indicate sites that have an increased dominance of species relative to the other samples and flow periods.

3.3 Results

3.3.1 Water Quality

Water chemistry was characterised by spatial and temporal variability in water variables among the 7 sites in both high and low flow seasons. Water quality data including system variables and nutrients for high flow are shown in Table 3.3 and data for low flow in Table 3.4. These tables indicate that variables were generally within target range, with the exception of a few noticeable values in the data:

- Increased NO_3 levels were noted for relative reference sites CRH, agricultural site C2L and urban influenced sites C4H, C4L.
- *Phosphate* levels were high for urban impacted sites C4H, C4L and C3H.
- *Conductivity, SO_4* and *Cl* levels were increased at the agriculturally influenced sites C1 and C2 for both seasons.

- A sharp increase in COD levels from high to low flow were noted for site C3 (2-17 mg O_2/l), and at C4 (0.5-5 mg O_2/l)

Referring to Table 3.5, it is evident from data collected that TSS increased for all sites except for CR from high flow to low flow. The Inorganic (TSIM) fraction of the TSS increased from high to low flow at agricultural sites C1, C2 and M2, as well as C3 which has an urban influence. The remainder of the sites, which include the relative reference sites and C4 showed an increase in the organic (TSOM) fraction between seasons.

Relationships between different sites and water quality variables are displayed using PCA bi-plots showing samples and water quality parameters (Figures 3.1, 3.3 and 3.4). The direction and length of the arrows in relation to the sites are indicative of increased values for the corresponding variables. The angles between the arrows are indicative of a positive correlation if the arrows are acute, and are negatively correlated when the angles are larger that 90%. Measure of fit is indicated by the length of the arrow in relation to the placement of the variable, and the distance between sampling sites approximates the dissimilarity of water chemistry as measured by Euclidean distance (Clarke & Warwick, 2001).

Table 3.3: Water quality data including system variables and nutrients for once off sampling at the high flow survey (April, 2006).

	Temperature (◦C)	pH	O_2 (mg/l)	O_2 (%)	Turbidity (NTU)	Conductivity (µS/cm)	TDS (ppm)	NO_2 (mg/l)	NO_3 (mg/l)	P (mg/l)	PO_4 (mg/l)	Cl (mg/l)	SO_4 (mg/l)	Ca (mg/l)	COD (as O_2) (mg/l)	NH_4 (mg/l)	NH_3 (mg/l)
CR H	18.3	6.69	8.50	91.50	8.0	128	65	0.0	19.6	0.01	0.030	113.0	6.5	10	0.5	0.020	0.00
C1 H	23.0	7.97	8.13	94.40	18	612	299	0.09	0.3	0.07	0.230	286.0	63.0	30	2	0.070	0.00266
C2 H	20.2	8.07	8.75	96.30	32	710	335	0.57	9.2	0.12	0.370	402.0	94.0	20	1	1.200	0.0636
C3 H	23.5	8.50	10.91	127.00	14	452	452	0.59	0.8	0.32	0.910	234.0	33.0	7	2	0.490	0.01862
C4 H	18.5	8.05	8.76	92.20	27	534	266	0.51	25	0.65	1.810	164.0	44.0	41	0.5	0.010	0.00053
MR H	18.3	7.88	8.28	87.80	33	431	312	0.08	0.6	0.04	0.140	144.0	13.0	24	0.01	0.090	0.00342
M2 H	18.3	7.88	8.28	87.80	33	431	312	0.08	0.6	0.04	0.140	144.0	13.0	24	0.5	0.090	0.00342

Table 3.4: Water quality data including system variables and nutrients for once off sampling at the low flow survey (August 2006).

	Temperature (◦C)	pH	O_2 (mg/l)	O_2 (%)	Turbidity (NTU)	Conductivity (µS/cm)	TDS (ppm)	NO_2 (mg/l)	NO_3 (mg/l)	P (mg/l)	PO_4 (mg/l)	Cl (mg/l)	SO_4 (mg/l)	Ca (mg/l)	COD (as O_2) (mg/l)	NH_4 (mg/l)	NH_3 (mg/l)
CR L	9.4	6.61	5.56	85.60	7	137	69	0.01	5.60	0.02	0.070	36.0	0.1	9	0.50	0.100	0.000060
C1 L	12.0	8.00	9.70	84.00	18	739	372	0.01	3.50	0.02	0.050	412.0	124.0	19	1.00	0.015	0.000390
C2 L	14.2	8.19	9.20	96.00	19	976	495	0.59	14.60	0.19	0.570	424.0	121.0	34	4.00	0.630	0.049770
C3 L	15.0	8.96	3.79	40.80	23	509	240	0.10	9.30	0.11	0.330	182.0	38.0	17	17.00	0.270	0.010800
C4 L	13.6	8.02	7.76	91.60	25	608	308	0.56	26.10	0.67	2.010	324.0	46.0	23	5.00	0.040	0.000880
MR L	18.0	8.22	7.29	85.00	10	256	129	0.01	1.10	0.01	0.030	38.0	0.1	19	0.50	0.015	0.000128
M2 L	14.4	7.69	7.18	86.70	18	496	227	0.01	5.50	0.02	0.050	172.0	28.0	25	0.25	0.030	0.001140

Table 3.5: Total Suspended Solids (TSS), Total Suspended Inorganic Matter (TSIM) and Total Suspended Organic Matter (TSOM) for all sampling sites for high (H) and low (L) flow periods.

	TSS (g/l)	TSIM (g/l)	TSOM (g/l)
CR H	0.018	0.0176	0.0004
C1 H	0.0108	0.0088	0.0020
C2 H	0.0456	0.0196	0.0260
C3 H	0.0104	0.0076	0.0028
C4 H	0.0396	0.026	0.0136
MR H	0.026	0.01	0.016
M2 H	0.0388	0.0192	0.0196

	TSS (g/l)	TSIM (g/l)	TSOM (g/l)
CR L	0.0016	0.0008	0.0008
C1 L	0.04	0.0256	0.0144
C2 L	0.0584	0.0356	0.0228
C3 L	0.0468	0.0196	0.0272
C4 L	0.0416	0.0256	0.016
MR L	0.0284	0.0048	0.0236
M2 L	0.0416	0.0284	0.0132

3.3.1.1 High and Low Flow

Principal Component Analysis ordination for water quality variables at both high and low flow showing (dis)similarity amongst study sites on the Crocodile and Magalies rivers are represented in Figure 3.1. The two dimensional PCA bi-plot describes 56.6% of the variation in data, where 41.1% is displayed on the first axis and 15.5% is displayed on the second axis.

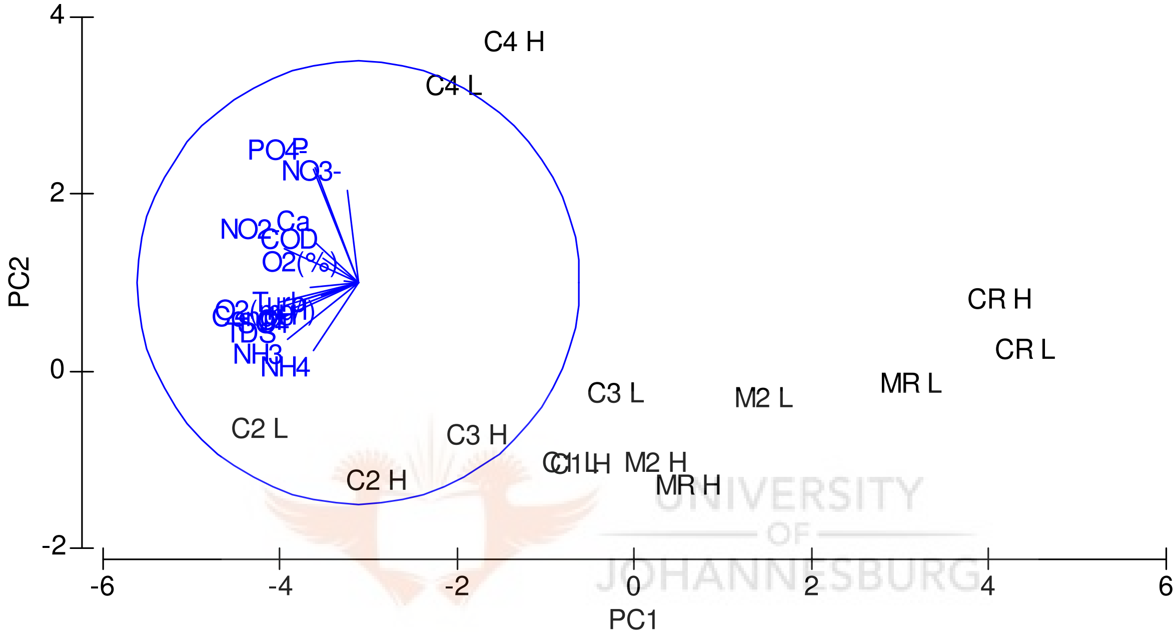


Figure 3.1: A PCA bi-plot of water quality variables showing (dis)similarity amongst study sites on the Crocodile (C) and Magalies (M) rivers for all sample sites at high (H) and low (L) flow periods.

The relative reference sites CRH, CRL, MRL, MRH and Magalies River agricultural test sites M2H and M2L were separated from the remainder of the sites along the PC1 axis, indicating their dissimilarity to test sites. Urban site C4 was more influenced by TP levels at both high and low flow than the other sites, and thus is separated from the other sites on the PC2 axis. No apparent trends in water quality based on the specific land use practices are indicated in the PCA for the remainder of the urban and agricultural test sites on the Crocodile River. There was a degree of spatial variation for the remainder of the test sites. The subset of water quality variables that best described the existing classification and placement of sites at high flow according to BIO-ENV matching are DO (% saturation), TP, PO_4^-, Cl and NH_3.

3.3.1.2 High Flow

A PCA ordination of water quality variables for study sites at high flow is indicated in Figure 3.2. The two dimensional bi-plot describes 66.9% of the variation in data, where 46.9% is displayed on the first axis and 20.1% displayed on the second axis.

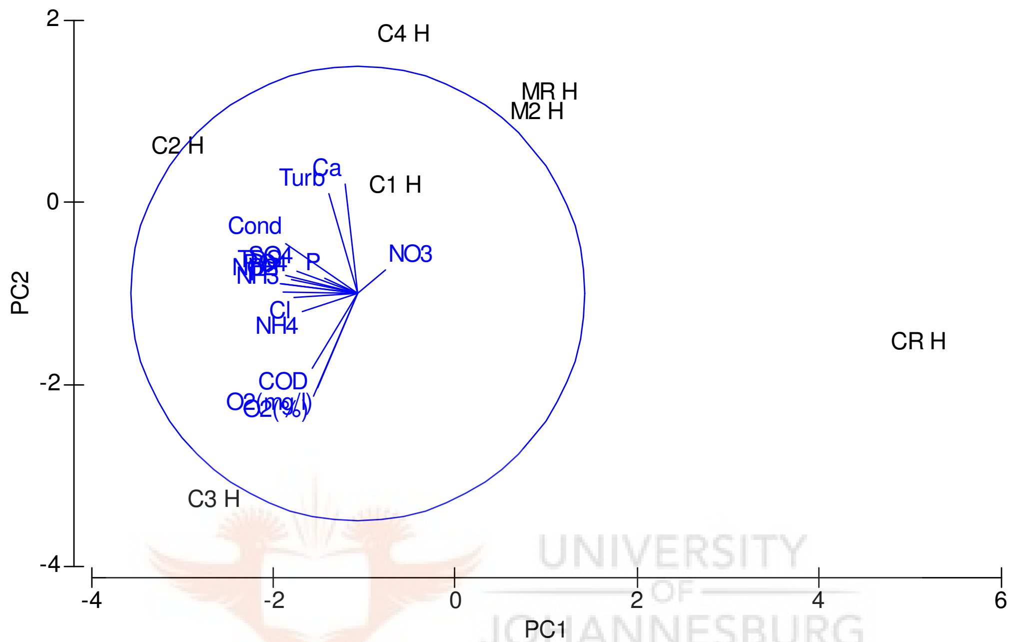


Figure 3.2: A PCA bi-plot of water quality variables showing (dis)similarity amongst study sites on the Crocodile (C) and Magalies (M) rivers for all sample sites at high (H) flow.

Study sites on the Magalies River are similar to each other in terms of water quality, indicating spatial variation. When referring to specific variables using a 2D configuration plot it shows that agricultural sites C1 and C2 varied in water quality due to the higher nutrient levels at C2 in comparison to C1, but were similar in terms of higher COD, conductivity and turbidity levels overall. Urban test sites C3 and C4 at high flow show dissimilarity on the PC2 axis that was contributed to by the difference in values for Ca, NH_3 and NH_4. Urban sites were, however, very similar in terms their TP and PO_4^- levels, which were higher than those of the agricultural sites. The subset of water quality variables that best describe the existing classification and placement of sites at high flow according to BIO-ENV matching remained as DO (% saturation), TP, PO_4^-, Cl and NH_3.

3.3.1.3 Low Flow

A PCA bi-plot of water quality variables for all sample sites at low flow is shown in Figure 3.3 The two dimensional bi-plot describes 74.0% of the variation in data, where 53.8% displayed on the first axis and 20.2 % is displayed on the second axis. Variables from a 2D configuration plot, overlaid on the PCA plot showed that relative reference sites CR and MR, as well as M2 were similar in terms of water quality at low flow. Water quality variables contributing to this similarity were relatively low levels of turbidity, TDS, conductivity, nutrients and COD.

Urban sites were separated from agricultural sites on the PC2 axis for low flow indicating trends in water quality that may be related to land use. Urban sites were placed due to the higher COD, PO_4^-, TP and NO_3 levels relative to the agricultural sites. Agricultural sites differed from the urban sites in terms of higher conductivity, TDS and salts (Cl and SO_4) levels. Relative reference sites are shown to be less influenced by water quality drivers according to the NMDS ordination. A trend was noted between water quality and land use in Figure 3.3, where sites that have agriculture as their main land use have strong positive correlations to DO, and where urban impacted sites show negative correlations to DO. According to BIO-ENV matching, the subset of water quality variables that best described the placement of sites at low flow were conductivity, pH, TDS and NH_3.

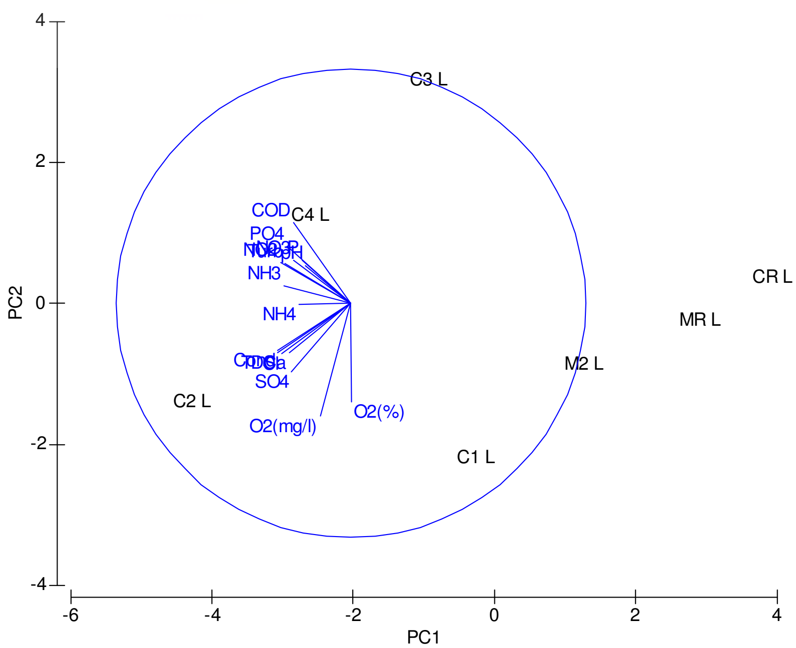


Figure 3.3: A PCA bi-plot of water quality variables showing (dis)similarity amongst study sites on the Crocodile (C) and Magalies (M) rivers for all sample sites at low (L) flow.

3.3.2 Diatom Community Composition

A total of 99 diatom species were identified from the 7 sites in this study (Appendix B). Species richness varied from 11 to 42 with an average of 23. The grouping of sample sites according to CLUSTER analysis and NMDS ordination based on diatom assemblages for high and low flow are shown in Figure 3.4a and 3.4 b. The NMDS ordination indicated that sites group mostly according to land use, with the exception of site C1L/F which was an outlier, and C2L/F which is an agricultural site that grouped with sites exhibiting urban impacts.

One-Way ANOSIM showed that there was a significant difference (*p<0.05*) between relative reference groups, agricultural groups and urban groups. There were no significant differences (*p>0.05)* between groups that were made up of sites exhibiting the same land use patterns.

(a) **(b)**

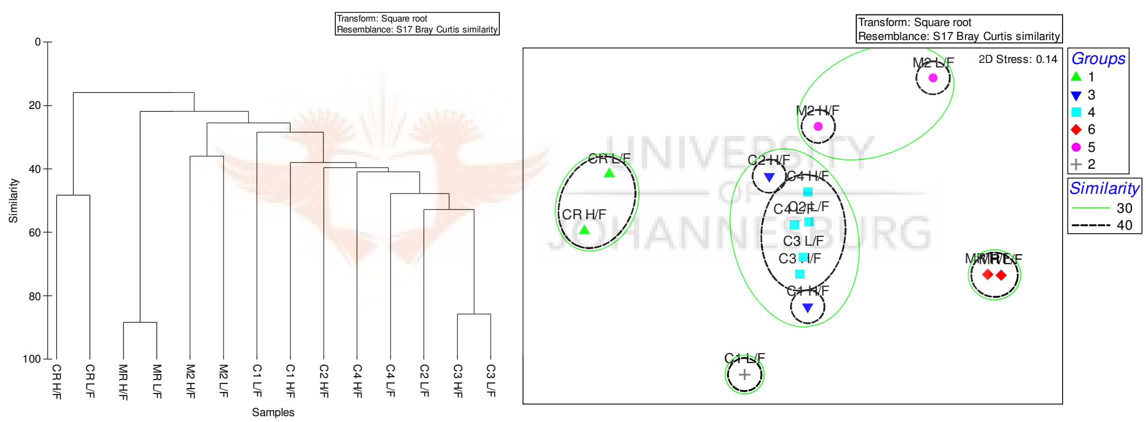


Figure 3.4: (a) Bray Curtis similarity matrix based on hierarchical cluster analysis indicating the similarity between samples in relation to the diatom community structure at each site for high and low flow; and (b) 2 Dimensional NMDS for diatom community structure indicating percentage similarity and groups.

The diatom species for high and low flow periods that contributed to the groupings of sample sites and similarity within the diatom groupings are shown in Table 3.6. The relative reference sites were contained in Group 1 (Crocodile River relative reference group) and Group 6 (Magalies River relative reference group), and these two groups showed entirely different species contributions in relation to each other. SIMPER analysis for the Magalies River reference site showed that *Achnanthes minutissima* (Syn. *Achnanthidium minutissimum*), *Gomphonema venusta* were dominant species, whereas *Cocconeis placentula var. euglypta* and *Navicula gregaria* were dominant for the Crocodile reference group.

The agriculturally influenced sites (with exception of C2L) were contained within three different groups, separated seasonally and spatially, with varying dominant taxa; Group 2 (dominated by *Diatoma vulgaris*), Group 3 (*Nitzschia frustulum* and *N. palea*) and Group 5 (*Navicula tripunctata* and *N. cryptotonella*). Urban sites were placed together in Group 4, along with agricultural site C2L. These sites were dominated by three species of relatively equal contributions, namely *D. vulgaris*, *N. tripunctata* and *Amphora pediculus*.

Table 3.6: Results obtained from SIMPER analysis with a 50% cut off for low contributions indicating the contribution of various diatom species to similarity within the diatom groupings.

	Sites	Land use	Species	Contribution %	Cumulative %
Group 1	CR L CR H	R R	*Cocconeis placentula var. euglypta*	19.36	19.36
			Navicula gregaria	16.09	35.45
			Gomphonema pumilum	14.64	50.09
Group 2	C1 L	A	*Diatoma vulgaris*	77.00	77.00
Group 3	C1 H C2 H	A A	*Nitzschia frustulum*	20.74	20.74
			Nitzschia palea	16.06	36.80
			Amphora pediculus	10.87	47.67
			Aulacoseira granulata	9.84	57.51
Group 4	C2 L C3 L C3 H C4 L C4 H	A U U U U	*Diatoma vulgaris*	10.62	10.62
			Navicula tripunctata	10.14	20.76
			Amphora pediculus	9.85	30.61
			Eolimna subminuscula	7.84	38.45
			Cocconeis pediculus	6.78	45.24
			Navicula cryptotonella	5.00	50.24
Group 5	M2 H M2 L	A A	*Navicula tripunctata*	15.15	15.15
			Navicula cryptotonella	12.22	27.37
			Achnanthes minutissima	10.16	37.53
			Achnanthidium pyrenaicum	10.16	47.69
			Gomphonema parvulum	6.78	54.47
Group 6	MR H MR L	R R	*Achnanthes minutissima*	21.80	21.80
			Gomphonema venusta	11.49	33.30
			Cocconeis placentula	9.84	43.14
			Cocconeis pediculus	9.73	52.87

Ranked species K-dominance plots for diatom communities indicated by Figures 3.6a and 3.6b, show relative species abundance as a percentage of the total abundance, plotted for each site. At high flow the site where there was an overwhelming dominance of a single species is CRH, whereas at low flow the sites that are dominated by a particular species are C1L and M2L.

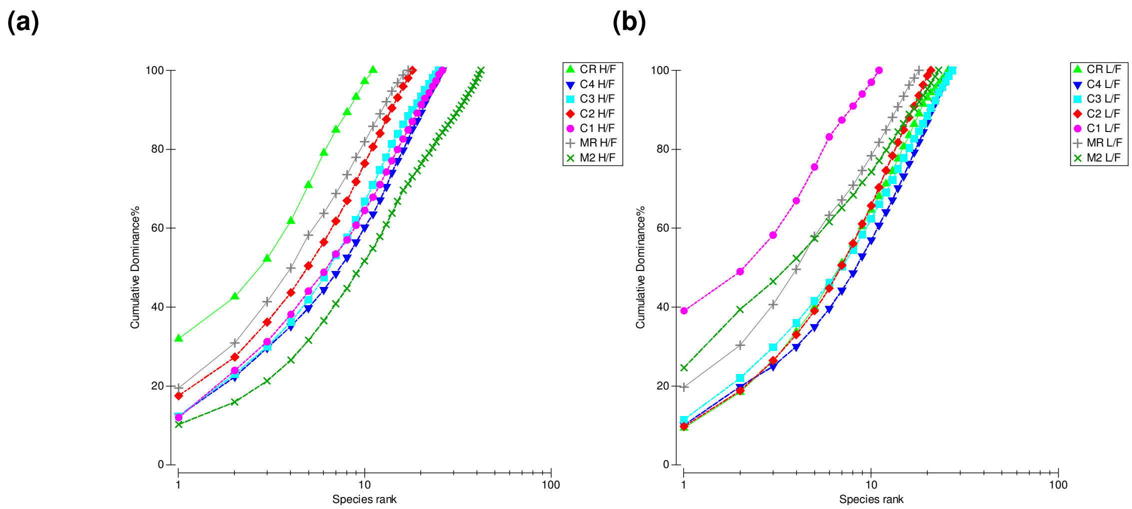


Figure 3.5: Ranked species K-dominance plot for diatom communities collected at (a) high flow (H/F), and (b) low flow (L/F) at sites on the Crocodile (C) and Magalies (M) rivers utilising abundances to indicate cumulative dominance.

3.3.3 Diatom Index Scores

The index scores for the selected diatom indices as well as their classes for each site on the Crocodile and Magalies rivers at high and low flow periods are shown in Table 3.6 and Figure 3.6a to 3.6(e). Table 3.2 should be used for interpretation of the scores. Index scores were calculated using the OMNIDIA software programme (Lecointe *et al.*, 1993). Diatom index scores are presented as values from 0 to 20, where a decreasing score indicates an increasing level of pollution or eutrophication.

Overall, the diatom index scores for the Magalies River relative reference (MR) and agricultural site (M2) indicated that the Magalies River was in an overall better class of ecological health than the Crocodile River (Figure 3.6). The Crocodile River agricultural sites (C1 and C2) were in a slightly more modified ecological state than urban impacted sites (C3 and C4) referring to Figure 3.5. The exception is urban site C4, which has the lowest scores for the SPI and EPI diatom indices (Figure 3.6a and 3.6d). Agricultural site C2 showed the lowest overall scores for GDI and an increased tolerance to organic pollution (Figure 3.6c and 3.6e). C4 was the more impacted site of the two urban related land use sites according to the diatom indices.

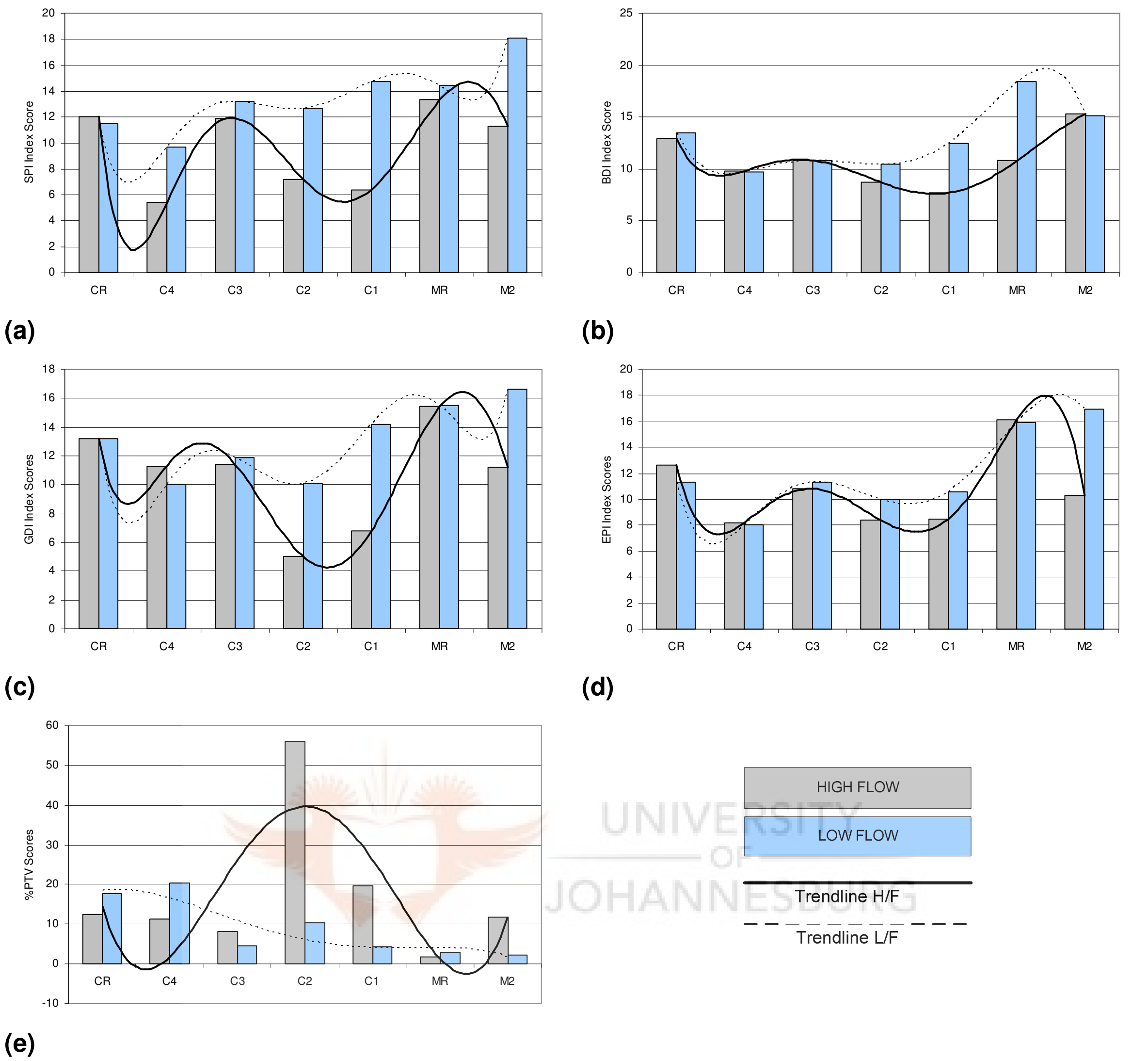


Figure 3.6: Graphs showing values for high and low flow index scores for (a) SPI (b) BDI (c) GSI (d) EPI and (e) %PTV diatom indices in the Crocodile and Magalies rivers. Polynomial trend lines are included to indicate trends in index scores between sites and seasons.

The overall diatom index scores for the Magalies River ranged from moderate to high integrity, and the percentage of diatom species that are tolerant to organic pollution (%PTV) were low (Table 3.7). It is important to note that M2 (agricultural test site) was classified as having a higher integrity than CR (reference site for Crocodile River).

During high flow, the overall integrity classes of the agricultural sites were “poor” according to diatom indices, whereas integrity classes increased in the low flow season to. Urban sites were generally classed from “moderate/poor” to “moderate” overall. There was an overall increase in the integrity from high flow to low flow noted for the diatom index classes.

Table 3.7: Diatom index scores and classes for sites on the Crocodile and Magalies rivers at high and low flow periods. Index scores were calculated using the OMNIDIA software programme (Lecointe *et al.*, 1993).

Site	Flow Period	Diatom Index Scores and Classes					Overall Class
		SPI	BDI	GDI	EPI	%PTV	
C1	High Flow	6.4	7.7	6.8	8.5	19.7	Poor
C2	High Flow	7.2	8.7	5	8.4	55.9	Poor
C3	High Flow	11.9	10.8	11.4	10.8	8.1	Moderate
C4	High Flow	5.4	9.8	11.3	8.2	11.2	Moderate/Poor
CR	High Flow	12	12.9	13.2	12.6	12.4	Moderate
M2	High Flow	11.3	10.8	11.2	10.3	11.8	Moderate
MR	High Flow	13.3	15.3	15.4	16.1	1.6	Good
		Diatom Index Scores and Classes					Overall Class
		SPI	BDI	GDI	EPI	%PTV	
C1	Low Flow	14.7	12.5	14.2	10.6	4.3	Good
C2	Low Flow	12.7	10.4	10.1	10	10.2	Moderate
C3	Low Flow	13.2	10.8	11.9	11.3	4.5	Moderate
C4	Low Flow	9.7	9.7	10	8	20.4	Moderate
CR	Low Flow	11.5	13.5	13.2	11.3	17.6	Moderate/Good
M2	Low Flow	18.1	18.4	16.6	16.9	2.2	Good/High
MR	Low Flow	14.4	15.1	15.5	15.9	2.8	Good

Sites on the Magalies River were in a state of oligotrophy (with the exception of M2 which at high flow was classed as eutrophic) and diatom communities present were made up of species that had a continuously high DO requirement (Table 3.8). There was a low DO requirement at high flow for M2, indicating impairment of the diatom community due to changes in water quality.

The agricultural sites were in a state of eutrophy, were β-mesosaprobous and had a low to moderate DO requirement (Table 3.8). Diatom communities at C1 at high flow showed that the salinity was tending to wards brackish-fresh, which would infer that there was an increase in halophilous species at this site. Site C2 was classed as nitrogen heterotrophic tolerant, indicating an increased number of diatom species that are dependant on periodically elevated concentrations of nitrogen. It is important to note that CR at low flow shows α-mesosaprobity, which indicates a site that is strongly polluted with restricted fauna due to an increase in organic pollution. See Appendix B for an interpretation of the descriptors in Table 3.8.

Table 3.8: Ecological descriptors obtained from OMNIDIA software based on the calculation by Van Dam et al. (1994) for high and low flow periods. (Ecological descriptor limits are addressed in Appendix B).

Site	Flow	pH	Salinity	Nitrogen uptake mechanism	Oxygen requirements	Sabrobity	Trophic state
C1	High Flow	Alkaliphilious	Brackish-fresh	Nitrogen autotrophic tolerant	Moderate	β-mesosaprobous	Eutrophic
C2		Alkaliphilious	Fresh-brackish	Nitrogen heterotrophic obligatory	Low	β-mesosaprobous	Eutrophic
C3		Alkaliphilious	Fresh-brackish	Nitrogen autotrophic tolerant	Moderate	β-mesosaprobous	Eutrophic
C4		Alkaliphilious	Fresh-brackish	Nitrogen autotrophic tolerant	Low	β-mesosaprobous	Eutrophic
CR		Alkaliphilious	Fresh-brackish	Nitrogen autotrophic tolerant	Low	β-mesosaprobous	Eutrophic
M2		Alkalibiontic	Fresh-brackish	Nitrogen autotrophic tolerant	Low	β-mesosaprobous	Eutrophic
MR		Circumneutral	Fresh-brackish	Nitrogen autotrophic tolerant	Continuously high	β-mesosaprobous	Oligo-eutrophic
C1	Low Flow	Alkalibiontic	Fresh-brackish	Nitrogen autotrophic tolerant	Moderate	β-mesosaprobous	Meso-eutrophic
C2		Alkaliphilious	Fresh-brackish	Nitrogen autotrophic tolerant	Moderate	β-mesosaprobous	Eutrophic
C3		Alkaliphilious	Fresh-brackish	Nitrogen autotrophic tolerant	Moderate	β-mesosaprobous	Eutrophic
C4		Alkaliphilious	Fresh-brackish	Nitrogen autotrophic tolerant	Moderate	β-mesosaprobous	Eutrophic
CR		Alkaliphilious	Fresh-brackish	Nitrogen autotrophic tolerant	Low	α-mesosaprobous	Eutrophic
M2		Circumneutral	Fresh-brackish	Nitrogen autotrophic tolerant	Continuously high	β-mesosaprobous	Oligo-mesotrophic
MR		Circumneutral	Fresh-brackish	Nitrogen autotrophic tolerant	Continuously high	β-mesosaprobous	Oligotrophic

Of particular interest was site C3 at low flow where individuals of *Cocconeis pediculus* (Figure 3.7a), *A. pediculus* (Figure 3.7b) and *N. tripunctata* (Figure 3.7c) that were noted as having reasonably severe deformities were observed.

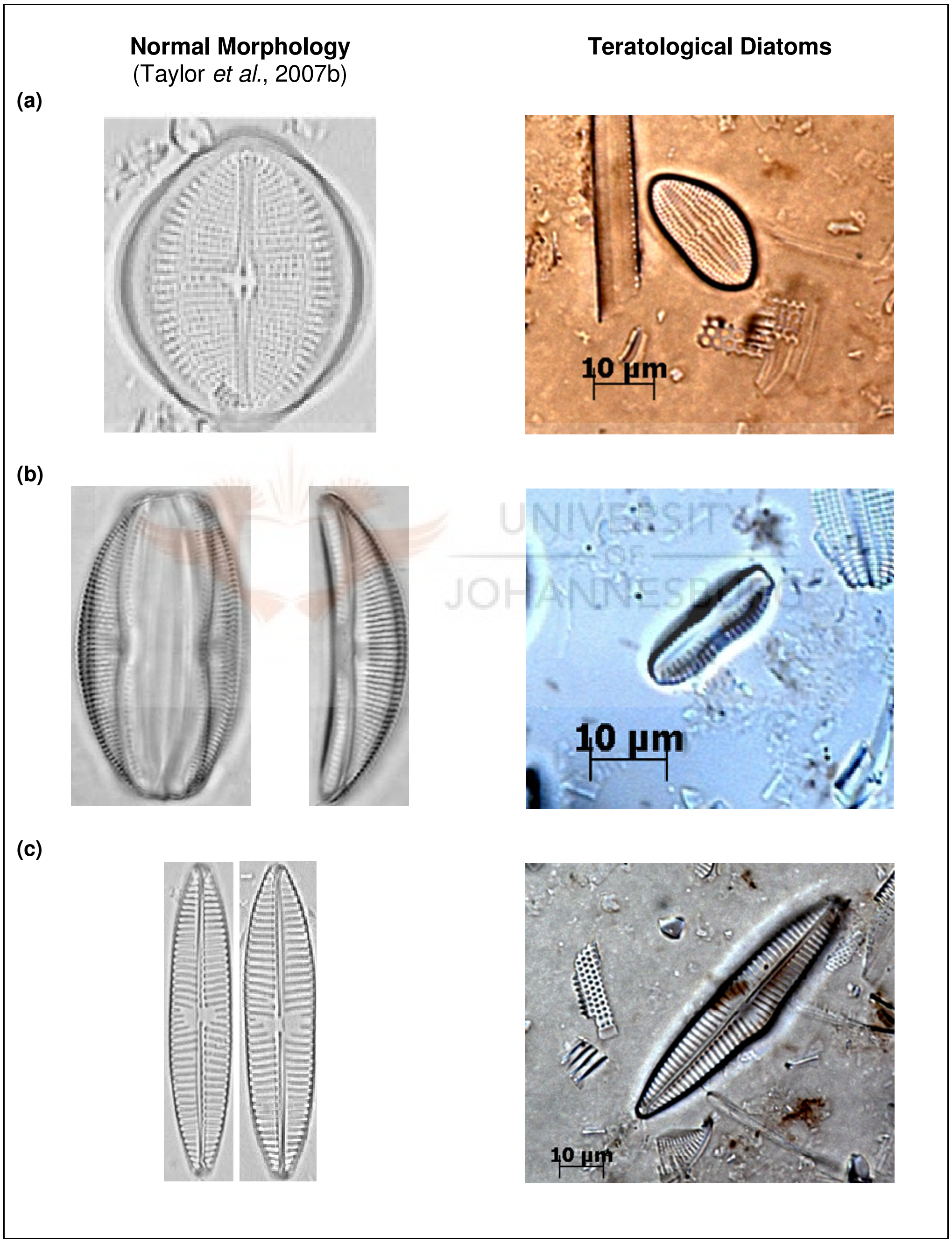


Figure 3.7: Species of diatoms found at site C3L showing normal and teratological forms of (a) Cocconeis pediculus, (b) Amphora pediculus, and (c) Navicula tripunctata. Morphologically normal specimens were taken from Taylor *et al.* (2007b).

3.4 Discussion

3.4.1 Water Quality

Referring to water quality data and results collected at high and low flow seasons for relative reference, agricultural and urban test sites in 2006, it is evident that overall agricultural sites suffered from increased conductivity, TSIM, SO_4 and Cl levels, whereas urban sites were impacted by elevated levels of nutrients (in particular TP) and COD. The respective site's water quality in relation to their land uses will be discussed below, and one should refer to Table 3.3, Table 3.4. Table 3.5, Appendix A (aerial and topographical imagery of the sites) and Appendix B (current and historical water quality graphs) were necessary.

3.4.1.1 Agricultural Sites

Site C1

As one would expect from a site dominated agricultural activities, conductivity values were relatively high (Schofield & Ruprecht, 1989; Williams, 1987; Williams, 2001). Water quality values increased from summer to winter (612 µS/cm to 739 µS/cm), TDS values increased from high to low flow (299pp – 372ppm) and salt concentrations increased sharply from high to low flow with values of 286 mg/l to 412 mg/l for Cl and 63 mg/l to 124 mg/l for SO_4 respectively. An increase in TSS from 0.0108 g/l at high flow to 0.04 g/l at low flow, most of which was in the form of the inorganic fraction was noted.

The seasonal trend in the pollution gradient in the water quality data (i.e. – an increase in variable concentrations from high to low flow) for this site may be explained by a combination of decreased flows in the winter months and abstraction of water for irrigation that leads to concentration of these variables. Data from this study agrees with studies done by Qader (1998) who found that reduced flows caused an increase in sedimentation and TDS concentrations downstream of abstraction points and reduced the dilution capacity of the river.

An increase in TSS and TSIM has been shown to be directly correlated with stripping of land and riparian zones for crop planting (Allan *et al*., 1997). Referring to aerial imagery and topographical maps in Appendix A, center-pivot farming occurs extensively along the length of the Crocodile River at, and preceding this site.

Nitrogen enrichment in the form of elevated levels of NO_3 also occurred from high to low flow (0.30 mg/l to 3.50mg/l). When considering that historic data for this section of river collected by DWAF from 1993 to 2006 showed that the 90th percentile for NO_3 was 1.011mg/l, and the median value 0.129 mg/l, this value has increased exponentially (DWAF, 2008). Inorganic nitrogen content may increase in rivers that are downstream from dams that are not efficiently regulated (Deksissa, Ashton & Vanrolleghem, 2003). Releases from Roodekopjes Dam, upstream of C1 as well as fertilizer utilisation in the area may explain this increase in NO_3 levels. Flows from Vaalkop Dam on the Elands River may also have impacts in terms of intermediate levels of nutrients which are speculated to come from surrounding platinum mines (RHP, 2005).

Site C2

Water quality impacts for site C2 were much the same as for C1, with a downward trend in water quality from high flow to low flow. Conductivity values were the highest at C2, increasing from 710 µS/cm at high flow to 976 µS/cm at low flow. TDS values increased from 335ppm to 495 ppm, and salt concentrations increased sharply from high to low flow with values of 402 mg/l to 424 mg/l for Cl and 94 mg/l to 121 mg/l for SO_4 respectively. TSS showed the highest value at this agricultural site at both high and low flow, increasing from 0.0456 g/l at high flow to 0.0584 g/l at low flow, with a high inorganic fraction noted. When referring to historical data collected from the DWAF weir at Brits, these water quality variables have increased (DWAF, 2008).

Overall, nutrient levels at this site were higher than at C1, with an increase in COD levels. Brits town and sewage works are situated upstream of this site, and in conjunction with the intensive cultivation and orchards leading up to this site this may contribute to the increase in nutrients and COD. According to DWAF (1996), oxidizable organic matter originating from waste discharges cause spikes in COD. Increases in inorganic nitrogen concentrations are usually met with increase in the COD and pH values, which agrees with data collected at this site.

Site C2 at low flow particularly, showed elevated nitrogen and TP levels, which would explain its grouping with urban sites which are more likely to be affected by increases in nutrient values due to point source discharges of domestic and sewage effluents (DWAF, 1996).

One would expect the cumulative effect of the intensive agricultural practices prior to C1 to show a decrease in the water quality from C2 to C1. However, this is not the case and water quality at the downstream agricultural site C1 appears to be of a better quality than that of C2. An explanation is that the Elands River joins the Crocodile River just before site C1, and water from the Elands is known to be in a “fair” state, while that of the Crocodile River is in a “poor” state (RHP, 2005). This confluence may have a dilution effect on pollutants in the middle Crocodile River. The combination of cumulative agricultural and urban water quality impacts at site C2 appear to have an additive and interactive effect on water quality at this site.

Site M2

Impacts in terms of water quality at M2 were nominal in comparison to agricultural test sites C1 and C2. Elevated levels of NO_3 were noted from high to low flow (0.60 mg/l to 5.5 mg/l), where historical data showed that the median NO_3 concentration from 1972 to 2005 was 0.074 mg/l (DWAF, 2008). There was a fractional increase in TSS (more specifically the inorganic fraction) from 0.0388 g/l to 0.0416 g/l. No other striking temporal trends noted in the data for this site.

The relatively low water quality impact at this agricultural test site is of interest, as there is intensive cultivation and livestock farming occurring along the length of the Magalies River upstream of this site. A study on land use and instream integrity in the Crocodile (West) Marico WMA, looking at the relative extent of different land uses at different spatial scales (ranging from 100m to catchments) showed that cultivated land was not a significant predictor of instream integrity at any scale (Amis, Rouget, Balmford, Thuiller, Kleynhans, Day & Nel, 2007).

This section of the Upper Crocodile sub-management area is less populous and exponentially less urbanized than quaternary catchments where the other agricultural test sites have been selected (DWAF, 2004). The site borders on the Magaliesberg Nature Area and this reserve may have remediation effects on water quality. Amis and colleagues (2007) did find that the total area under natural vegetation was the best predictor of instream health, as it inferred good riparian integrity and hence good water quality control. The River Health Programme (2005) found that the water quality on the Magalies River closer to the Hartbeespoort Dam was “good”, with localized impacts from lodge developments and return flow from pig farms, chicken farms and flower farms in the area being the main contributors to water quality impacts.

3.4.1.2 Urban Sites

Site C4

Site C4 is on the Crocodile River above the Hartbeespoort Dam wall, and below the confluence of the Hennops River. Referring to Table 3.3, Table 3.4 and Table 3.5, nitrogen and phosphorous values where very high overall, staying relatively constant in terms of values between the two sampling trips.

A value of over 10 mg/l for inorganic nitrogen is considered to be indicative of hypertrophy, in which there is very low species diversity, but in which algae flourishes (DWAF, 1996). Levels of NO_3 alone were above 25 mg/l at this site which is at a level that is toxic to human beings (Dodds & Welch, 2000). The immediate surroundings are natural, but there is an urban influence in terms of water quality from upstream land use, and this part of the river receives effluent from the Northern Sewage works which services the extensive urban settlements at Alexandra and Diepsloot. This would explain the consistently high values noted with regard to nutrients, as nutrients are being received from a point source.

DWAF historical water quality data collected from 1972 to 2006 show that the 90th percentile and median values for NO_3 recorded from water received from the Hennops was 7.969 mg/l and 4.563 mg/l respectively, with the highest recorded value measuring 27.58 mg/l (DWAF, 2008). Water quality is known to be "poor" at this point of the Crocodile River, because of high levels of nutrients received from the Hennops and Jukskei rivers. The high nutrient levels are caused by sewage spillages and industry discharges into the sewer system. Increased development in Soweto and inadequate infrastructure is the likely cause for these increases in NO_3 levels (RHP, 2005).

Sewage effluent received from the sewage works often contain NO_3 concentrations that are above the TWQR's. Industrial effluent received from the urban area increases the need for oxygen because of certain chemical species entering the ecosystem. This severe nutrient and chemical loading is accompanied by increases in COD values (DWAF, 2008). As was noted, there was a rise in the COD from 1 mg/l in summer to 5 mg/l in winter.

Site C3

C3 is downstream of Hartbeespoort Dam wall and the main impairments in water quality noted were changes from summer to winter of NO_3 levels (0.80mg/l to 9.30 mg/l), a sharp decrease in DO (10.91 mg/l to 3.79 mg/l) in a manner consistent with COD increase (2 mg/l to 17 mg/l).

The shift in COD levels indicates that the oxygen deficit has increased from <15% to <75% between seasons. These values also indicate a shift from a "slightly" polluted oligosaprobic to a "strongly" polluted ά-mesosaprobic river reach (Taylor *et al.*, 2007b). The water quality impacts due to land use at this site are associated with the development and maintenance of the residential areas surrounding the dam and river, and impacts due to urban run off and sewage effluent. It is well known that these activities are responsible for nutrient and COD increases (Morrison, Fatoki, Persson & Ekberg, 2001).

A ten fold increase in TSOM was noted for this site from summer to winter. This increase could be related to sewage inputs and algae blooms in the Hartbeespoort Dam. Algal blooms in this dam are common and well documented and may increase the organic fraction of the suspended sediments. The ongoing intensive construction prior to this site would contribute to the TSS values.

3.4.1.3 Relative Reference Sites

Site CR

With the exception of high NO_3 levels in the summer months (19.60 mg/l), water quality parameter values were within the TWQRs for aquatic ecosystems, and were generally quite low (DWAF, 1996). The adjacent land use is recreational; however the upstream land use is urban. A source of the high NO_3 levels is urban run-off as this is known to cause increases in nutrients (Carpenter, Caraco, Correll, Howarth, Sharpley & Smith, 1998).

Site MR

As was expected for this site, there were no major water quality impacts with exception of somewhat higher TDS (312 ppm and 129ppm) and conductivity (431 µS/cm and 256 µS/cm) values, and an increase in the organic fraction of the TSS from 0.016 g/l to 0.0236 g/l for this reach of the river.

Upstream of this site is a small aquaculture operation. Organic matter fractions in suspended solids have been shown to increase downstream from fish farms (Brown, 1996 cited in Dallas & Day, 2004). An increase in the organic fraction of the TSS in winter could also be due to leaf litter from the dense riparian canopy that covers the river. Illegal water abstraction of ground water from the aquifer supplying Maloney's eye is more than likely a contributing reason for the increase in TDS and conductivity values from reference conditions (Magalies River Forum, *Pers Comm.*) [1].

[1] Magalies River Forum Meeting, Personal Communication. Magaliesberg, March 2007.

All of the other water quality parameters fell within the TWQRs as set out by DWAF (1996). The dolomitic source of the Magalies River (Maloney's Eye), is a few kilometres up-stream of this site, hence water variable concentrations were expected to be low as the upper reaches of rivers are generally oligotrophic (Davies & Day, 1998). Contributing to this good water quality is the land adjacent to the Magalies River which is in a natural condition. As discussed earlier, natural land use is the best predictor of good stream integrity (Amis *et al.* 2007).

3.4.1.4 Relationships between Water Quality and Land use

High and Low Flow

When comparing each site's water quality variables spatially and temporally, there is an important separation between the two Magalies River sites (MR and M2) and the relative reference site on the Crocodile (CR), and other test sites on the PC1 axis (Figure 3.1). This is an indication that the water quality at the relative reference sites and M2 clearly differs in constitution from the test sites. The reason for this separation would be that the water quality variables at these sites are lower, indicative of oligotrophic to mesotrophic conditions that tend to move away from pollution gradients that are shown in Figure 3.1.

A distinct separation between site C4 and the remainder of the Crocodile River test sites (C1, C2, and C3) was noted along the PC2 axis (Figure 3.1). The placement of urban site C4 was due to consistently high nutrient levels that distinguished it from the other sites, in particular phosphorous levels. This is most likely from the influence of the sewage works that are associated with this site.

Interestingly enough, agricultural site C2 and urban site C3 showed more similarity in terms of placement than C2 and C1 did. Referring to Table 3.2 and Table 3.3, C2 and C3 were more similar in terms of nutrient levels (nitrogen levels in particular) than the agricultural sites were. Site C2 is impacted by sewage inputs from Brits town upstream, and this could be a possible factor influencing its similarity with the urban site C3.

Agricultural site C1 is more similar to the sites with better water quality than to the other more impacted sites. This shows that a recovery takes place downstream from agricultural site C2 to site C1. As discussed earlier, this is thought to be from the influence of the water of the Elands River that joins the Crocodile before site C1 (RHP, 2005). Another plausible reason could be attributed to the Roodekopjes Dam that is upstream from C1. Dams may have the ability to settle out sediments and nutrients before water re-enters the river.

Approximately 43.4% of the variation in the water quality for high and low flow combined is not explained in Figure 3.1. This unknown factor is thought to be contributed to by pesticides and heavy metals that may be present in the water, but that were not included in measured parameters for the purpose of this study. It is speculated that that pesticide and heavy metal concentrations would offer more explanation in terms of the interpretation of Figure 3.1, as they are impacts that would be directly associated with the land use adjacent to the study sites.

High Flow

At high flow a separation of sites CR, MR and M2 along the PC1 axis is again noted, indicating dissimilarity in variable concentrations in comparison to higher values noted for test sites (Figure 3.2). The close placement of both of the Magalies River sites on the PCA bi-plot signifies a spatial dissimilarity to other sites on the Crocodile River. The Magalies River is said to have better water quality than that of the Crocodile River (RHP, 2005).

The reason that there are no distinct patterns that link water quality to land use in the high flow PCA is because each of the remaining test sites (C1, C2, C3 and C4) have water variable concentrations (combinations of concentrations) that are unique to each site. This is thought to be contributed to by a combination of water quality impacts from run-off from land after summer rains, and releases from respective dams that are upstream of site C1, C2 and C3. Land run off would contain pollutants that would be associated with the practices of each specific adjacent land use, while dam water would contain a mixture of pollutants from a larger scale.

Urban site C3 had the highest DO concentration noted for any of the sites, and these notably higher values contribute to its dissimilarity in relation to other test sites. The higher DO is due to the high cobble contribution to the instream habitat at this site which contributes to mechanical mixing.

Low Flow

Figure 3.3 shows that a pollution gradient exists between CR, MR and M2, and the remainder of the test sites along the PC1 axis. As mentioned previously, the water quality values for these sites were low.

Low flow data shows a separation of the agricultural sites (C1 and C2) and urban sites (C3 and C4). The placement of the agricultural sites is driven by variables such as salts and conductivity, whereas urban sites placement were more driven by nutrient and COD. This indicates a more specific pollution gradient were distinct impacts are seen for each land use type. The variables contributing to placement here are textbook examples of well know specific pollution problems associated to each land use. Irrigation in the dry season is known to cause salinity gradients in agricultural regions due to abstraction and concentration of salts around the roots of crops (Moore *et al.,* 1990 cited by Lemly*,* 1994; Williams, 2001).

3.4.2 Diatom Community Composition in Relation to Land Use

Thus far this chapter has attempted to provide an outline of how water chemistry varies between sites with different land uses. The discussion pertaining to diatom community structure that follows is based on the diatom community response to water chemistry as outlined by three objectives in the introductory chapter, namely: (2) to compare community structure data for diatoms taken from agriculturally impacted sites to sites impacted by urban inputs and natural sites; (4) from the above information elucidate in what way the community structures of diatoms are impacted or modified (if any) due to agricultural inputs into the aquatic system; and (6) to identify suitable indicators of change due to agricultural pollution in these diatom communities.

3.4.2.1 Site Groupings

Reference Groups

Group 1 (Figure 3.4a, Figure 3.4b, and Table 3.6) contains the relative reference site for the Crocodile River (CR) at high and low flow. *Cocconeis placentula* var. *euglypta*, *N. gregaria* and *Gomphonema pumilum* contributed to 50.9% of the make up of this group. The species in **Group 1** are all tolerant of eutrophic to hyper-eutrophic environments and *N. gregaria* and *G. pumilum* can tolerate critically to strongly polluted waters (Taylor *et al*., 2007b). Figure 3.5a indicating K-dominance curves for sample at high flow also showed disturbance at this site to be the greatest in terms of the dominance of C. *placentula* var. *euglypta*. Considering the adjacent river land use, this result was not expected.

Immediate surroundings of this site are recreational/natural with a relatively intact riparian zone; however, on a larger scale the upstream activities are of a high intensity urban nature which would explain the presence of these diatom species. This indicates that scale and continuum play an extremely important role and that instream health was not predicted by natural land cover at this scale. The extent to which land use influences the instream integrity is scale dependant and generally is more predictable on a catchment scale rather than a localised scale as in this study (Allan *et al.*, 1997; Amis *et al*, 2007).

The Magalies relative reference site (MR) for both seasons are contained in **Group 6** (Figure 3.4a and 3.4b and Table 3.6). *Achnanthes minutissima* (21.80%) and *G. venusta* (11.49%) accounted cumulatively for 33.3% of the total diatom species contribution by abundance. The diatom *A. minutissima* is numerically dominant in upland streams and is typically found in rivers with low salinities and phosphorus concentrations, and dominates when turbidity values are low (Eloranta & Soininen, 2002; Blinn & Bailey, 2001). Taylor *et al.* (2007b) also noted that *A. minutissima* is generally found in clean, fresh waters that are well oxygenated. The findings of this study are in agreement with the findings of Lavoie *et al.* (2004), who found *A. minutissima* to be prevalent at reference sites. In a study on organic and agricultural pollution, this species was found only in the uppermost river sites where conditions were oligotrophic (Rott *et al.*, 1998).

Because *G. venusta* is thought to be an endemic species to South Africa, environmental preferences have not been established in European indices used for calculating integrity (Taylor *et al.*, 2007b; Taylor, Prygiel, Vosloo, de la Rey & van Rensberg, 2007c). In a study on the relevance of diatom-based pollution indices to South Africa (Taylor *et al.*, 2007c), *G. venusta* was shown to be associated with good water quality and with low levels of water quality variables at sites in the Crocodile (West) Marico WMA.

The presence of *Cocconeis placentula* and *C. pediculus* in the remainder of the similarity percentage make up suggests that possible underlying nutrient and salinity problems exist. *Cocconeis placentula* can tolerate mesotrophic to eutrophic conditions, whereas *C. pediculus* may tolerate moderate to high salinities (Van Dam, Mertens & Sinkelda, 1994; Hill, Stevenson, Pan, Herlihy, Kaufmann & Johnson, 2001; Taylor *et al.*, 2007b). Higher than expected conductivity and TDS values, and an increase in NO_3 from the high to low flow season confirms the indicator values of these species for this headwater site.

Agricultural Groups

Agricultural sites were separated into Group 2, Group 3 and Group 5 (Figure 3.4a, Figure 3.4b and Table 3.6). An unexpected positioning of C1L was noted when referring to NMDS and CLUSTER ordination based on diatom community structure for samples at high and low flow periods. It was expected that agricultural site C1L would group with other agricultural sites on the

Crocodile River at low flow (i.e. C2L). However, C1L was dissimilar to any of the other sites in terms of diatom community composition. This outlier group (**Group 2**) was because of the strong domination of *D. vulgaris* (77%) at this site (Table 3.6). This dominance of *D. vulgaris* is also noted in the K-dominance plots for low flow, indicating disturbance at this site (Figure 3.5b).

Environmental preferences for *D. vulgaris* include electrolyte contents of 100µS/cm to 500 µS/cm and mesotrophic to eutrophic conditions (Taylor *et al*., 2005b; Hill *et al*., 2001). According Table 3.3, Table 3.4 and Table 3.5, C1L exhibited a marked increased in Cl and SO_4 levels for the low flow period, almost doubling from high flow figures, and showed an increase of close to 400% in TSS concentrations.

These findings oppose evidence of *D. vulgaris* being one of the most sensitive species in relation to increases in sediment loads and embededness in the USA (Blinn & Herbst, 2003). According to indicator values from the Netherlands, *Diatoma* sp. have relatively high indicator values for pH, organic nitrogen, oxygen, saprobity and trophic state (van Dam *et al*., 1994). However, South African studies have linked *D. vulgaris* specifically *to* freshwaters with elevated levels of phosphate-phosphorus (Taylor *et al*., 2007b). According to Dr. Taylor (*Pers Comm*)[2] this diatom species is routinely found in high abundances below Bloemhof Dam, which occurs in an irrigated agricultural region. This somewhat contrasting information suggests that cosmopolitan indicators, such as *D. vulgaris*, that are used in European and American diatom indices of pollution may not have the same value in a South African context. Apprehension has been expressed as to the viability of using data concerning the ecological preferences of diatoms between the Northern and Southern Hemispheres (Kelly *et al*., 1998).

When considering the remainder of the diatom community composition at C1L, impacts point in the direction of nutrient problems, more than salinity and conductivity problems as indicated by the actual water variable figures (Table 3.3, Table 3.4, Table 3.5 & Appendix B). *Amphora pediculus*, *Cocconeis pediculus*, *C. placentula* var*. euglypta* and *N. gregaria* make up the majority of the remainder of the community. The combination of these sub-dominant species with dominant *D. vulgaris* suggests that there were major problems related to eutrophication directly preceding sampling. At the low flow site visit, it was noted that there was a bloom of filamentous algae on cobbles at this site which is indicative of over fertilization. Increases in algal

[2] Dr. J.C Taylor, Personal Electronic Communication. School of Environmental Sciences, Division of Botany, University of the North West, November 2007.

biomass were recorded when artificial channels of stream reaches that were artificially fertilized (Hart & Robinson, 1990).

As discussed above, NO_3 levels rose from high to low flow far exceeding historic levels for this section of river. Preparation of land with fertilizer, and subsequent irrigation and run-off may be the cause of the increase in NO_3 levels (Dallas & Day, 2004). Problems with interpretation of such results were shown by Biggs (1996). Over time many large-scale indirect factors such as climate and geology, limit or obscure the expression of direct small-scale, factors such as nutrients and flow. This ultimately makes data interpretation difficult and ambiguous.

The high flow Crocodile River agricultural sites C1H and C2H were placed in **Group 3**. The groups comprised of *N. frustulum*, *N. palea*, *A. pediculus* and *Aulacoseira granulata* which are associated with increased salts, caused most probably from run-off from these highly cultivated areas. The composition of pollution tolerant taxa at this agricultural site points directly to salinity problems, which were noted in the water quality data.

Nitzschia frustulum and *N. palea* were linked to high conductivity gradients (5 - 7.5 mS/cm) in lowland streams with moderate to heavy agricultural practices. The above species and *A. granulata* were also found to be important indicators for high phosphorous environments in streams in Australia (Blinn & Bailey, 2001). Slightly elevated TP levels were noted at C2H.

When observing the individual composition of the diatom assemblages at C1H and C2H, one notes that *Navicula recens* and *Nitzschia liebertruthii* are dominant at the respective sites (Appendix B). The presence of these species reiterates the presence of a salinity gradient at these agricultural sites, as Taylor *et al.* (2007b) describes these two diatoms a occurring in "very" electrolyte rich to brackish waters. The significant contribution of the genus *Nitzschia* to this grouping also suggests siltation problems, as this genus is known to have many species that are motile that may escape the effects of siltation (Hill *et al.*, 2001).

Group 5 was composed of agricultural site M2 at high and low flow. As noted, water quality impacts for this agricultural site were less severe than sites on the Crocodile River. There was an increase in NO_3 between the summer and winter months, however, the remainder of the water quality values were relatively constant. The combination of the present dominant species points strongly to a eutraphentic trophic state, bordering on hyper-eutraphentic. The most dominant diatom of this group's assemblage is *N. tripunctata*, which is considered to be "a good indicator of eutrophic waters that can tolerate critical levels of pollution" (Taylor *et al.*, 2007b).

Two species belonging to the genus *Navicula* (*N. tripunctata* and *N. cryptotonella*) cumulatively make up the initial 27.37 % of the assemblage of Group 5 (Table 3.6). *Navicula* spp. have been used in siltation indices as they are motile and can escape habitats that have silt issues (Kutka & Richards, 1996; Hill *et al.*, 2001). Bahls (1993) reported that catchments that generate higher levels of silt that end up in receiving streams have a higher percentage of motile diatoms present, even if there are no other pollution gradients of disturbance present. *Gomphonema parvulum*, which made up 6.78% of the composition of Group 5, is also a sediment increaser taxa that is tolerant of "extremely polluted waters" (Telpy & Bahls, 2006; Taylor *et al.*, 2007b). Rott *et al.* (1998) showed *N. tripunctata* and *N. cryptotonella* were excellent indicator species in relation to agricultural disturbances. The frequency of occurrence for above taxa at sites associated with agriculture was 80%, and these species occurred at their highest abundances at these sites.

The presence of the dominant pollution tolerant species are counter acted by two diatoms that are indicative of oligotrophic, fresh waters; namely *A. minutissima* and *Achnanthidium pyrenaicum* (Syn. *Achnanthes biasolettianum*). The presence of these pollution sensitive species infers that water quality is still of a high enough quality to support sensitive species, however, this would also indicate that there is a decreasing trend in water quality in this area due to nutrient inputs.

Site M2L shows a large degree of dominance by a single species (*A. minutissima*) in K-dominance plots for low flow (Figure 3.5b). This species is a common pioneer species in head waters, and often dominates surfaces that have previously been impacted by physical abrasion or by pollution. The dominance of this species for low flow would indicate the recovery of this site from an unknown impact.

Urban Group

Species making up the composition of **Group 4**, which contained mostly urban impacted sites, were not entirely unique to this group. One diatom species that does stand out however, is *E. subminuscula*. This species is a very good indicator of industrial organic pollution and of strongly polluted waters (Gevrey, Rimet, Park, Giraudel, Ector & Lek, 2006; Taylor *et al.*, 2007b). Urban sites did exhibit high nitrogen and phosphorous levels overall, and it is well known that compounds of nitrogen and phosphorus are often present in high concentrations in organic discharges.

Examining more closely the species composition of C3 and C4 at high and low flow, one notices the contribution of *Fistulifera saprophila*, *Mayamaea atomus* and *Eolimna minima* to the urban sites (Appendix B). These species are considered to be some of the most pollution tolerant diatoms and are generally indicative of organic pollution (sewage), or are associated with organic detritus (Taylor *et al*., 2007b). The increases in COD from summer to winter at these sites would select for the above species.

The grouping of site C2L with urban sites in Group 4 was interesting. This site suffers from impacts of agriculture and urban practices with sewage discharges contributing to an increase in the organic pollution at this site. Rott *et al*. (1998) found that sites that were impacted by both agricultural and urban practices were highly affected in terms of water quality. It appears that in the low flow season that water quality, and hence diatom assemblage is more affected by organic inputs rather than inorganic nutrients from farming practices as in the low flow season.

3.4.2.2 Diatom Community Integrity

A range of diatom indices that were tested by Taylor (2004) and Taylor *et al*. (2007a) were selected for representation of the extent of aquatic pollution at each of the study sites for the Magalies and Crocodile rivers. The indices selected were the Specific Pollution sensitivity Index (SPI) (CEMAGREF, 1982), the Biological Diatom Index (BDI) (Lenoir & Coste, 1996), the Generic Diatom Index (GDI) (Coste & Ayphassorho, 1991), the Eutrophication and Pollution Index (EPI) (Dell'Uomo, 1996) and the Percentage Pollution Tolerant Valves (%PTV) (Kelly & Whitton, 1995). Reasons for selecting these particular indices are outlined in Table 3.1 and follow the same process of selection as Taylor (2004) and Taylor *et al*. (2007a).

Figure 3.6a to 3.6e and Table 3.7 indicate graphs and display trends in water quality as represented by the selected diatom pollution based indices. These data indicate that the Magalies River was more ecologically sound than the Crocodile River according to the diatom indices. The relative reference site MR remained in a “Good” class overall, whereas the agricultural site showed recovery from high to low flow moving from a “Moderate” integrity to a “Good/High”. The water quality integrity for M2L as indicated overall by diatom indices superseded that of MR. This data is in agreement with the water quality data collected for the Magalies River in this study.

Huizenga (2004) used historical data to show that the Magalies and Skeerpoort rivers did not show any sign of water quality pollution or change from the 1970's to present and had good to excellent water quality index scores. According to the causal principle (Schonfelder, 2000) this would provide an ambient environment that would favor the colonisation of diatoms that have ecological preferences for oligotrophic to mesotrophic water. The confluence of the Skeerpoort and the Magalies rivers before site M2 may be a contributing factor the good water quality noted at this site for low flow, as the Skeerpoort is known to have a good water quality and a high ecological integrity (RHP, 2005).

Referring to the Crocodile River, the indices that represent general water quality are the SPI, BDI and GDI (Figure 3.6a to 3.6c). These indices show very similar trends in water quality between the seasons. What is interesting to note is the recovery in water quality integrity (mostly noted in the low flow samples) after the influence of large dams. For example, urban site C4 (upstream) is separated from urban site C3 (downstream) by Hartbeespoort Dam; and agricultural site C2 (upstream) is separated from site C1 (downstream) by Roodekopjes Dam. This trend may be attributed to the settling out of suspendoids and nutrients in these large dams, adsorption of some nutrients to sediments and phosphate removal by algae and macrophytes (Andersen, Dunbar & Friberg, 2004).

The EPI (Figure 3.6d) is designed for the measurement of the effect of nutrients on ionic strength and is influenced most significantly by phosphorous levels (Taylor, 2004; Dell'Uomo, 1996; Dell'Uomo, Pensieri & Corradetti, 1999). The EPI showed a similar trend in water quality in relation to the positioning of dams. From the comparison of high and low flow index scores for the Crocodile River, one notes that there is an increase in the EPI after Hartbeespoort Dam wall, where after there is a downward trend to the first agricultural site with a very slight recovery after the Roodekopjes Dam wall.

The %PTV is defined as the sum of diatom taxa regarded as being tolerant to organic pollution. Once again, the trend noted in the %PTV Scores (Figure 3.6e) indicates that the percentage of pollution tolerant valves decreases immediately after the influence of large dams. The only indication of heavy organic pollution (likely due to sewage inputs) was a site C2 at high flow. This site had a value of 55.9%, indicating serious organic pollution. At C2 there are urban and agricultural impacts at different spatial scales associated with this site. Nutrient impacts from agriculture arise from a combination of smaller sewage inputs, livestock run-off, emissions of

NH_3 to air and leaching and runoff of nitrogen (Jarvie, Whitton & Neal, 1998). Nutrients from urban activities include large inputs of phosphorus to surface waters due to point sources of sewage and industrial effluents entering river systems (Dallas & Day, 2004). These results once again reiterate that the cumulative organic inputs of agricultural and urban impacts have synergistic effects on water quality that have severe impacts on primary producer communities such as diatoms.

There was a general increase in overall classes/scores from high to low flow (Table 3.7). A study in a catchment with mixed land use by Boyacioglu (2006) showed that in high flow conditions, water pollutants mainly originated from urban land use, while water quality was contributed to by agricultural pollutants during the low flow period. The reason noted for the results obtained for high flow was due to runoff from an increase in impermeable surfaces which weakened the buffering capacity of storm-water in urban areas. This data goes against common belief in that a change in river flow is often inversely related to the concentration of constituents in the water (Stednick, 1991). Taking into consideration data from the current study and the study by Boyacioglu (2006), this would imply that agricultural pollution has a lesser impact on overall water quality than urban practices do.

3.4.2.3 General Ecological Description and Trophic Classification

Using diatoms to monitor eutrophication and saprobity in rivers may be undertaken by the collection of a single diatom sample per season as diatoms have varying tolerance for nutrient increases and organic enrichment (Taylor, 2004; Van Dam *et al.,* 1994). The changes in diatom communities that represent changes in nutrient and organic inputs are more holistic than chemical water quality monitoring as they indicate any impacts that may have occurred in the previous 6 weeks. This section refers to Table 3.8 and Appendix B (for explanations of the ecological descriptors).

Along a longitudinal gradient from the head waters to the middle reaches of rivers, there should be an increase in nutrients and organic material (Vannote, Minshall, Cummins, Sedell & Cushing, 1980). Looking at the Magalies River as a tributary of the Crocodile, the trophic state was oligotrophic at MR (mountain stream) and M2 (upper/middle reaches) improved from a state of eutrophy to oligo-mesotrophy between seasons (Table 3.8). The saprobity was β-mesoprobous for all sites on the Magalies for all seasons, indicating a slight to moderately polluted system in terms of organic matter.

The Crocodile River was eutrophic from the headwaters (CR) to the site situated most downstream in the middle reaches (C1) for both seasons with the exception of agricultural site C1. This site showed a slight improvement in its trophic status from eutrophic at high flow to mesotrophic at low flow. According to Kelly (1998), in low flow conditions, rivers should resemble lentic systems and nutrients are usually retained in rivers in low flow. This contradicts the findings for site C1. The reason for this is that run off from rain fall in the summer months is a contributor of nutrients to this point. At low flow, there is less run off from surrounding land, and thus less inorganic nutrients entering the system from over-fertilization. These nutrients would then be flushed off of the agricultural lands after the first heavy spate and return to the river causing the river to return to a eutrophic state in the summer months.

The relative reference site for the Crocodile (CR) in the upper reaches of the Crocodile River should be in a state of oligotrophy according to its order (Vannote *et al.*, 1980), however this site is in a eutrophic state. This eutrophy is indicated in the presence of species that are tolerant to elevated levels of nutrients and that have low DO requirements in Table 3.7. The saprobity at CR at low flow was α-mesoprobous. This was the only site on the Crocodile River that showed definite saprobity problems as the rest were β-mesoprobous for both seasons. An α-mesoprobous state suggest that the river has been exposed to organic pollution and is in the initial stages of recovery where fauna is restricted and bacterial counts are high (Kolkwitz & Marsson, 1908 cited in Dallas & Day, 2004). As discussed in section 3.4.2.2.1, this site does suffer from impacts that are related to high density urban activities which would explain the change in saprobity from high to low flow.

3.4.2.4 Diatom Deformities

The presence of cell wall deformities in *C. pediculus*, *A. pediculus* and *N. tripunctata* noted at site C3 at low flow are shown in Figure 3.7a to 3.7c and deserve some comment. Deformities in frustules of diatoms are generally ascribed to silicon limitation, heavy metals and extreme pH (Ruggiu Lugli´e, Cattaneo & Panzani, 1998; Dickman, 1998). Referring to water quality data in Table 3.3, pH may be ruled out as a contributing factor to the deformities as it was within TWQRs as set out by DWAF (1996). Silicon was not measured for the purpose of this study. Considering that there are numerous mines (one of which is an open cast chrome mine) close to the Hartbeespoort Dam area, heavy metal contamination may very well be a plausible explanation for the deformities noted in diatoms at this site. Section 3.3.1 showed that COD increased to a level of 17mg/l at C3L from 2mg/l at C3H which coincides with the observation of deformities in the diatom species.

Researchers have concluded that abnormal cell morphology of diatoms might be a valid indicator of ecosystem health. In case studies in Hong Kong and Hungary more deformed species with teratological frustules were found close to heavy metal polluted sources (Dickman, 1998; Szabó, Kiss, Taba & Ács, 2005).

For deformities to occur in cell walls of diatoms, metal concentrations must be high enough to cause the development of irregular frustules, however, the concentrations need to be low enough to permit at least one division and this range of concentration is often very narrow (Pickett-Heaps, Schmid, & Edgar,1990). Converse to the point made in the above paragraph, many authors consider this along with the fact that diatoms may have natural variations in morphology to be a limiting factor in the use of teratological diatom frustules as indicators of pollution due to heavy metals (Dickman, 1998).

3.5 Summary and Conclusion

Site CR was chosen as a relative reference site, but displayed signs of water quality impacts related to urban run off, and is not included in the synopsis as this makes the results somewhat ambiguous.

Particular water quality impacts were noted for each specific land use. In line with the objectives stated in Chapter 1, comparison of community structure for diatoms taken from sites with varying land uses using NDMS and SIMPER analysis showed differences from one another. Considering the make up of the relative reference site MR, which was comprised of diatom species that had preferences for clean, fresh water, community structures of diatoms were modified and showed specific change in relation to this reference site due to agricultural and urban water quality impacts.

Agriculture could be split into high and low intensity practices, where high intensity agriculture was indicated by the presence of motile species of the genus *Nitzschia*, and low intensity agriculture was indicated by motile species of the genus *Navicula*. Urban sites contained a combination of species that were tolerant of nutrient and organic spikes in water quality.

Sites that were impacted by high intensity agriculture were in a “poor/moderate” class overall, indicating that water quality impacts at these sites were more severe than at urban sites which showed an overall “moderate” class for diatom community structure. The relative reference site was classed as “good” in terms of water quality and diatom assemblage.

There is an increasing acceptance of the view that chemical measurements only are poor determinants of the biological impacts of pollutants. As a result, there is an ever-increasing change away from the reliance on chemical water quality data to assess the health of ecosystems, to a more holistic approach that uses biological communities which provide a direct way of observing the impact of contaminants. Because benthic diatoms remain in one place for number of months, they show cumulative effects and an ecological “recall” of the water quality over this period. This was evident in the current study as different pollution types were associated with specific diatom species

Chapter 4 : Macroinvertebrate Community Structure

4.1 Introduction

4.1.1 Macroinvertebrates as Biological Indicators

Because macroinvertebrates integrate natural and anthropogenic effects temporally, they have been used widely to characterise catchment and water quality conditions related to land use (Rosenberg & Resh, 1993). Results from a study by Black, Munn and Plotnikoff (2004) suggested that macroinvertebrates could be used as indicators of land cover optima and environmental conditions. The study specifically showed that at both local and catchment scales, macroinvertebrate community composition in large streams were correlated to the percentage of agricultural land use cover. The findings also indicated that taxa were present that could be identified to indicate specific levels of environmental variables in relation to catchment land use at varying scales.

There are numerous ways of assessing freshwater ecosystem disturbance via the use of macroinvertebrate community structure. Diversity indices such as the Shannon-Weiner index (Wilhm & Dorris, 1968), Margalef's species richness (Margalef, 1951 cited in Dallas & Day, 2004) and Pielou's evenness (Pielou, 1966); and biotic indices such as the South African Scoring System Version 5 (SASS5) (Dickens & Graham, 2002) and the Macroinvertebrate Response Assessment Index (MIRAI) (Thirion, 2007) have been widely used in South Africa. A less utilised approach to gauge ecosystem disturbance is the use of macroinvertebrate functional feeding groups (FFGs) which can be used to address the functional role that invertebrates play in the food base of a stream ecosystem (Cummins & Wilzbach, 1985).

Functional feeding groups of macroinvertebrate communities are predictable according to the River Continuum Concept because of the natural change in productivity of a river longitudinally (Vannote *et al*., 1980). Typical traits representing adaptations of macroinvertebrate taxa may be useful in forming a measure of impact across varying communities with different taxonomic distribution (Statzner, Dole´dec & Hugueny, 2001). Increased inputs of nutrients and organic matter via anthropogenic sources (i.e. urban and agricultural practices) may cause a shift in the percentage composition of FFGs downstream of the impact due to increased organic and nutrient inputs. Considering macroinvertebrate assemblages through their functional traits (such as FFGs) rather than in terms of family or species data provides a more applicable approach that may be compared to other data from different geographical regions (Santoul., Cayrou, Mastrorillo & Ce're'ghino, 2005).

4.1.2 Macroinvertebrate Responses to Agricultural Land use Patterns

A study undertaken in an agricultural zone of southwestern Australia showed that the most significant environmental factors that dictated the distribution macroinvertebrates were salinity and land use (Kay, Halsem, Scanlon & Smith, 2001). Macroinvertebrate communities were uniform and consisted of families that tolerated a broad range of ambient conditions, of which most were particularly tolerant to high salinities, some in orders of magnitude that were greater than has been reported for running freshwaters.

Sedimentation is believed to the one of the most important factors that dictates the distribution and abundance of macroinvertebrates at a local and reach scale (Minshall, 1984 cited in Zweig & Rabeni, 2001). The reason for this is that an increase in sedimentation causes a reduction in habitat integrity, type and quantity. Small increases in sediment deposition may reduce the population density and diversity of macroinvertebrates (Lenat & Crawford, 1994; Quinn, Cooper, Davies-Colley, Rutherford & Williamson, 1997) and cause changes in dominance from the Ephemeroptera, Plecoptera and Trichoptera taxa, to Oligochaetes. Changes due to sedimentation are however, difficult to quantify as sedimentation is usually accompanied by other impacts upon freshwater ecosystems (Zweig & Rabeni, 2001)

According to Wang, Robertson and Garrison (2007), the relationships between nutrient concentrations and assemblages of macroinvertebrates and fish in wadeable streams have not been well documented. Studies that have been carried out on inputs of inorganic nutrients may interact with light availability and temperature to augment in-stream primary production resulting in changes in the trophic structure of benthic communities (Johnson, Richards, Host & Arthur, 1997; Sponseller, Benfield & Valett, 2001).

Controlled experiments on nutrient enrichment that have been undertaken have indicated that macroinvertebrate and periphyton abundance rose with increased nutrients (Dudley, Cooper & Hemphill, 1986). Changes in the make up and abundance of periphyton algae have also been found to reduce macroinvertebrate drift and food quality (Kerans, 1996). Increased periphyton can also affect sensitive macroinvertebrates by depleting oxygen through nocturnal respiration (Wang, Lyons & Kanehl, 1997).

Recently, numerous studies have been undertaken in South Africa on pesticide contamination in rivers (Schulz, 2001; Schulz *et al.,* 2001; Schulz *et al.,* 2001a; Dabrowski, Peall, Reinecke, Liess & Schulz, 2002; Schulz, Thiere, & Dabrowski, 2002). Reduced invertebrate density was noted in the Lourens River (Western Cape) that is known to suffer from pesticide inputs (Schulz *et al.*, 2002). The reduced diversity was mainly attributed to by various insect taxa, such as Simuliidae and Chironomidae. In contrast, *Aeshna* sp., *Dugesia* sp., Ceratopogonidae and *Cheumatopsyche* sp. were unaffected. Schulz *et al.* (2002) also noted that species composition and abundances of macroinvertebrates differed significantly between control and test sites.

It has been shown that organophosphates associated with agricultural practices were responsible for sediment and water toxicity in samples collected from a river in highly cultivated areas, and that toxicity was ultimately linked to number of macroinvertebrate community metrics (Anderson, Hunt, Phillips, Nicely, de Vlaming, Connor, Richard, Tjeerdema, 2003; Phillips, Anderson, Hunt, Nicely, Kosaka, de Vlaming, Connor, Richard & Tjeerdema, 2004; Anderson, Phillips, Hunt, Connor, Richard & Tjeerdema, 2006). Significant negative correlations were made between the number of Ephemeroptera taxa, taxonomic richness and the percentage Chironomidae, and concentrations of chlorpyrifos in rivers receiving agricultural drainwater (Anderson *et al.,* 2003). Ultimately, pesticide pollution can affect the structure of aquatic biocoenoses as has previously been established by (Leonard, Hyne, Lim, Pablo & Van den Brink, 2000).

The objectives of this chapter are to compare and relate the community make up of macroinvertebrate data to specific land use practices (agricultural vs. urban vs. natural) to elucidate whether communities of macroinvertebrates differ between the dissimilar land use patterns. Functional Feeding Group make up of these assemblages between different land uses will also be compared to see whether FFG make up is indicative of land use at different sites. This will be undertaken by using raw abundance data, and refining macroinvertebrate data by using diversity indices and biotic indices.

4.2 Materials and Methods

4.2.1 Study Sites

Study sites and the selection there of are described in Chapter 2. Please refer to Table 2.1, Table 2.2 and Figure 2.1.

4.2.2 Habitat

A habitat assessment was conducted on site using the Integrated Habitat Assessment System version 2 (IHAS v.2) to enrich macroinvertebrate data obtained (McMillan, 1998). The IHAS measures the availability and integrity of each biotope (stones, vegetation and gravel, sand and mud) as well as physical stream integrity and was specifically designed to be used with SASS5 benthic macroinvertebrate studies. The quality and suitability of each biotope is expressed in terms of a percentage, where a score of 100% represents ideal habitat availability. Table 4.1 indicates the classes, descriptors and percentage integrity used in this study for indicating habitat integrity.

Table 4.1: Categories, key colours and category descriptions presented within this study for habitat.

Category/ Key Colour	Category description and Integrity Score (%)	
A	Natural	90-100
B	Largely Natural	80-89
C	Moderately Modified	60 - 79
D	Largely Modified	40-59
E	Seriously Modified	20 - 39
F	Critically Modified	<20

4.2.3 Macroinvertebrates

4.2.3.1 Field Collection

The aquatic invertebrate index (SASS5) (Dickens & Graham, 2002) was implemented to collect macroinvertebrate samples at two sampling seasons; high (April 2006) and low flow (August 2006). The SASS5 protocol is a biotic index of the condition of a river, based on the resident macroinvertebrate community, whereby each taxon is allocated a score according to its level of tolerance to river health degradation.

A standardised invertebrate collection net (1,000µm mesh with a 300 x 300 mm square opening) was used for the collection of the aquatic macroinvertebrates. All of the available biotopes were sampled as described by Dickens & Graham (2002). The biotopes were divided into stones (S), vegetation (Veg) and gravel, sand and mud (GSM). Before and after disturbing the site, approximately 1 minute of “hand-picking” for specimens that may have been missed by the sampling procedures was carried out.

Samples collected where placed into an identification tray where debris was removed and macroinvertebrates were identified using macroinvertebrate field guides (Gerber & Gabriel, 2002). For each biotope, identification took place for a maximum of 15 minutes, and if no new taxa were identified for a period of 5 minutes identification was stopped. This data was then used to calculate the biotic index values, namely; biotope SASS5 scores, total SASS5 scores, the number of taxa and the Average Score Per Taxon (ASPT).

Each site's level 1 Ecoregion was identified according to Kleynhans, Thirion and Moolman, (2005) (Table 2.1). Each site's SASS5 scores were interpreted using biological banding scores per Level 1 Ecoregion and expressed as a percentage of a "reference" SASS5 score and APST value (Dallas, 2007). The SASS5 scores per Ecoregion that translates to an "A" category according to Dallas (2007) are shown in Table 4.2. Classes assigned to the SASS5 score per site are shown in Table 4.3.

Table 4.2: Ecoregions (Kleynhans et al., 2005) and respective ASPT and SASS5 scores indicating reference conditions per Ecoregion (Dallas, 2007).

Ecoregion (Levels 1) (Kleynhans *et al*., 2005)	**ASPT**	**SASS 5 Score**
Highveld	6	125
Bushveld Basin	6.9	240
Western Bankenveld	6.4	245

Table 4.3: Categories, key colours and category descriptions presented for biological data (macroinvertebrates and riparian vegetation) (Louw, Kleynhans & Thirion, 2007).

Category / key colour	**Category description and Integrity Score (%)**	
A	Very good	Unmodified state - Unimpacted state, conditions natural.
B	Good	Largely natural - Small change in community characteristics, most aspects natural.
C	Moderate	Moderately modified - Clear community modifications, some impairment of health evident,
D	Poor	Largely modified - Impairment of health clearly evident. Unacceptably impacted state.
E	Very poor	Seriously modified - Most community characteristics seriously modified, unacceptable state.
F	Critical	Critically modified - Extremely low species diversity - unacceptable state.

Samples collected from each biotope were placed into bottles, preserved with 10% neutral buffered formalin and stained with Rose Bengal. These were transported back to the laboratory where the macroinvertebrates were sorted, enumerated per family to obtain figures for statistical analyses.

4.2.3.2 Macroinvertebrate Response Assessment Index

Ecological reference conditions allude to minimally disturbed habitats or conditions based on selected physical, chemical and biological attributes (Reynoldson, Norris, Resh, Day & Rosenberg, 1997). Ecological reference conditions were set for varying Level 1 Ecoregions (Bushveld Basin, Highveld and Western Bankenveld) from data obtained from the Freshwater Conservation Plan for the Crocodile (West) Marico WMA (Smith-Adao, Nel, Roux, Schonegevel, Hardwick, Maree, Hill, Roux, Kleynhans, Moolman, Thirion & Todd, 2006; Mrs. H. Roux, *Pers Comm*[3]*;* Mrs. C. Thirion, *Pers Comm*[4]).

Abundance data collected from the implementation of SASS5 protocol at each site for high and low flow was used to populate the Macroinvertebrate Response Assessment Index (MIRAI) (Thirion, 2007). The MIRAI is a rule-based spread sheet index that makes use of a rating approach comprising of four different metric groups that measure the change in invertebrate assemblage from the reference assemblage. The MIRAI assesses assemblage in terms of four metric groups, namely: (1) flow modification, (2) habitat modification and (3) water quality modification, as well as (4) system connectivity and seasonality.

To compare reference and present conditions, the abundance and frequency of occurrence were compared. An increase or decrease in abundance or frequency of occurrence is considered as a change compared to natural conditions. Each metric in each respective metric group was then rated from 0 to 5, where 0 indicates no change from reference conditions and 5 an extreme change from reference. Each metric and metric group was then ranked in order of importance and weighted according to its importance in determining the Ecological Category (EC) of the macroinvertebrate assemblage. The final outcome of the model is the EC expressed as a percentage of similarity to reference conditions, and the invertebrate EC which is expressed as a category from A to F (Table 4.3).

4.2.3.3 Functional Feeding Groups

Functional Feeding Groups were assigned to the macroinvertebrate families collected using the SASS5 method from the two monitoring trips. In order to assign the FFGs relevant literature (Merritt, Cummins & Berg, 1996; Todd, 2000) was used (Table 4.4).

[3] Mrs. Hermien Roux, Personal Electronic Communication, North West Nature Conservation, North West Province, January 2008.

[4] Mrs. Christa Thirion, Personal Electronic Communication, Resource Quality Services, Department of Water Affairs and Forestry, Pretoria, February 2008.

Table 4.4: Macroinvertebrate families and their respective FFGs per family.

TAXA	FFGs
Aeshnidae	PRE
Amphipoda	SHO
Ancylidae	SC
Athericidae	PRP
Baetidae	COG, SC
Belostomatidae	PRP
Brachyura (Crabs)	SHO
Caenidae	COG
Ceratopogonidae	PRE, COG
Chironomidae	COF, PRE, PRP, COG
Chlorocyphidae	PRE
Chlorolestidae	PRE
Coenagrionidae	PRE
Corduliidae	PRE
Corixidae	PIH, PRE, PRP, SC
Culicidae	COF, COG
Dixidae	COG
Dytiscidae	PRE
Ecnomidae	COF
Elmidae/Dryopidae	SHH, COG, SC
Ephydridae	COG, SHO, PRE, HEM, SC
Gerridae	PRP, PRS
Gomphidae	PRE
Gyrinidae Adults	PRE, SCS
Helodidae Larvae	SC, COG, SHH, PIH
Heptageniidae	SC, COG
Hirudinea	PRE
Hydrachnellae	PA
Hydraenidae Adults	SC, COG
Hydrophilidae Adults	COG
Hydropsychidae	COF, PRE

Table 4.4 (cont): Macroinvertebrate families and their respective FFGs per family.

TAXA	FFGs
Hydroptilidae	PIH, SC, COG
Leptophlebiidae	COG, SC
Libellulidae	PRE
Lymnaeidae	SC
Muscidae	PRP
Naucoridae	PRP
Nepidae	PRP
Notonectidae	PRP
Oligochaeta	DT, COG, COF
Perlidae	PRE
Philopotamidae	COF
Physidae	SC
Planariidae	PR
Planorbidae	SC
Pleidae	PRP
Psephenidae Larvae	SC
Psychodidae	COG
Psychomyiidae	COG
Simuliidae	COF
Sphaeriidae	COF
Syrphidae	COG
Tabanidae	PRP
Tipulidae	SHD, COG
Trichoptera	COG, COF, SHH, SC, PRE, SHO
Tricorythidae	COG
Veliidae	PRP
COF = Collector filterers; COG = Collector gatherers; DT = Detritivores; FG = Filter gatherers; HEM = Herbivore miners; PIH = Piercer herbivores; PRE = Predator engulfers; PRP = Predator piercers; PRS = Predator scavengers; SC = Scrapers; SCS = Surface film scavengers; SHD = Shredder detritivores; SHH = Shredder herbivores; SCS = Surface film scavengers	

4.2.4 Statistical Analyses of Functional Feeding Groups

Multivariate statistical procedures were used to assess changes in macroinvertebrate FFGs between sites and seasons using Primer version 6. Bray-Curtis similarity matrices were constructed from square root transformed macroinvertebrate FFG data recorded for each site at high and low flow occasions. Similarity matrices were subjected to group averaged hierarchical CLUSTER analysis and ordination by NMDS to summarise patterns in FFG composition. Factors were assigned to Bray-Curtis resemblance matrices based on groupings from the CLUSTER analysis and NMDS ordination.

Permutation-based hypothesis testing using One-way Analysis of Similarities (ANOSIM) was undertaken to determine the extent of the differences between macroinvertebrate FFGs for the different samples between flow periods. Pairwise comparisons from ANOSIM were used to identify significant differences (p<0.05) between groupings of macroinvertebrate FFGs. One way Analysis of Similarity Percentages (SIMPER) with a 50% cut off based on FFG contribution was used to identify the macroinvertebrate FFGs that primarily provided discrimination between sample clusters.

4.2.5 Statistical Analyses of Macroinvertebrate Community Data

The macroinvertebrate community structure was assessed by making use of univariate and multivariate analyses. Univariate diversity and evenness indices were used to describe macroinvertebrate species-abundance relations using Primer version 6. Univariate analyses undertaken was Margalef's index (d) (Margalef, 1951 cited in Dallas & Day, 2004), which is a measure of the number of species present for a given number of individuals, the Shannon-Wiener diversity index (H') (Wilhm & Dorris, 1968), and Pielou's evenness index (J') (Pielou, 1966).

Multivariate statistical procedures were used to assess changes in macroinvertebrate communities between sites using PRIMER version 6. Bray-Curtis similarity matrices were constructed from log transformed macroinvertebrate family abundance data recorded for each site at high and low flow occasions. Similarity matrices were subjected to group averaged hierarchical clustering (CLUSTER) and ordination by NMDS to summarise patterns in species composition. Factors were assigned to Bray-Curtis resemblance matrices based on groupings from the CLUSTER analysis and NMDS ordination.

Permutation-based hypothesis testing using One-way Analysis of Similarities (ANOSIM) was undertaken to determine the extent of the differences between macroinvertebrate community structures for the different samples at different flow periods. Pairwise comparisons from ANOSIM were used to identify significant differences (p<0.05) between groupings of macroinvertebrate community compositions to remain consistent with the p value for the RDA analysis in Chapter 5. A One way Analysis of Similarity Percentages (SIMPER) based on species contribution was used to identify the families of macroinvertebrates that primarily provided discrimination between sample clusters. K-dominance plots were included to indicate sites that have an increased dominance of taxa relative to the other samples and flow periods.

For the purpose of visually illustrating the relationship between macroinvertebrate community data and habitat integrity, respective data were subjected to Redundancy Analysis (RDA) using Canoco version 4.5. Redundancy Analysis was carried out on log transformed data and the significance of RDA axes was tested using unrestricted Monte Carlo permutation testing (499 permutations, ρ= 0.05). Redundancy Analysis is an ordination technique that uses best fit values from multiple linear regression between variables, and includes a second axis (in this case habitat) (Ter Braak & Smilauer, 2002).

4.3 Results

The results for habitat integrity, macroinvertebrate integrity and macroinvertebrate FFGs are presented in the following section. It is important to note that CR, which was initially included as relative reference site in Chapter 3, will be classed as a relative reference site with urban water quality influences (R/U) for the purpose of this chapter. This is due to the outcome of the diatom community analysis in section 3.5.

4.3.1 Habitat

The results for IHAS indicating habitat integrity and integrity categories are shown in Table 4.5 and Figure 4.1. Overall, habitat integrity at the sites was good, with the exception of M2H (which fell into a moderately to largely modified C/D category), the sites fell into largely natural (B category) to natural (A category) categories (Table 4.5). There were noteworthy increases in habitat integrity between high and low flow seasons at C4 and M2. It is important to note that at high flow, M2 on the Magalies River has highly impaired flow due to low rainfall, a series of illegal weirs and farm dams preceding this site, and over abstraction of water for farming in the area.

Referring to Figure 4.1, reference sites showed the highest IHAS scores, and thus the highest habitat integrity and availability to macroinvertebrates overall. The IHAS scores between test sites were comparatively very similar. Temporal trends indicated that very little change was noted between seasons, with the exception of M2H. There was a very slight improvement at most of the sites from high flow to low flow indicating improvement of availability was linked to the decrease in flow from summer to winter.

Table 4.5: Results obtained from the application of the IHAS index indicating integrity scores and classes for habitat components and overall habitat at high flow (H) and low flow (L).

IHAS		CRH	C4H	C3H	C2H	C1H	MRH	M2H
	Stone in-current score (20)	20	16	16	17	16	20	16
	Vegetation (15)	8	8	6	6	12	9	9
	Other habitats (20)	17	13	11	12	14	16	8
	IHAS Score (55)	45	37	33	35	42	45	33
	Stream Condition (45)	28	27	32	25	26	39	23
	Total (100)	**73**	**64**	**65**	**60**	**68**	**84**	**56**
	Category	**B**	**B**	**B**	**B/C**	**B**	**A/B**	**C/D**
		CRL	**C4L**	**C3L**	**C2L**	**C1L**	**MRL**	**M2L**
	Stone in-current score (20)	20	18	17	15	16	20	16
	Vegetation (15)	7	9	8	10	11	12	11
	Other habitats (20)	18	16	11	10	11	16	11
	IHAS Score (55)	45	43	36	35	38	48	38
	Stream Condition (45)	34	30	28	30	32	40	33
	Total (100)	**79**	**73**	**64**	**65**	**70**	**88**	**71**
	Category	**B**	**B**	**B**	**B**	**B**	**A/B**	**B**

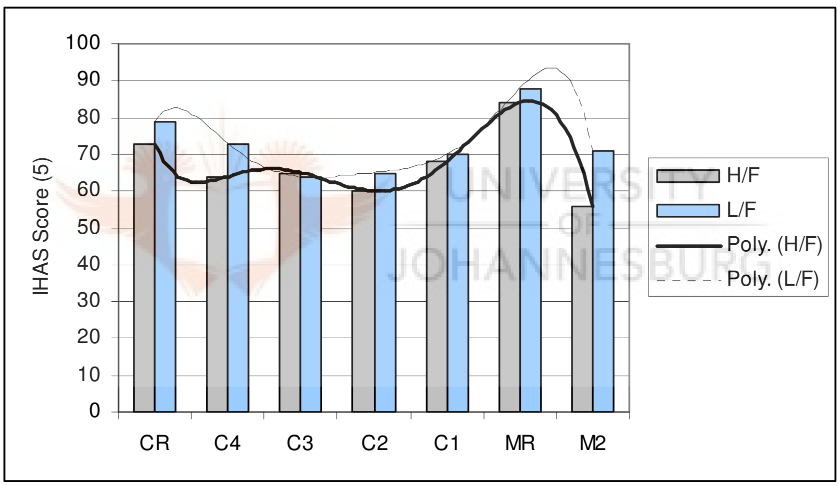


Figure 4.1: Representation of the IHAS habitat scores for high flow (H/F), and low flow (L/F) at sites on the Crocodile (C) and Magalies (M) rivers representing habitat integrity and availability for macroinvertebrates.

Figure 4.2a and 4.2b show the (dis)similarity between sites on the Crocodile and Magalies Rivers in relation to habitat during high and low flow periods. The RDA bi-plot describes 62.4% of the variation in data, where 34% is displayed on the first axis and 28.4% is displayed on the second axis. There was an overall good diversity of habitat that was noted at each site visit. Reference sites were separated from test sites (with the exception of C4L) on the first RDA axis, and test sites had a degree of temporal separation associated with their placement, indicating the importance of flow for these sites (Figure 4.2a). Figure 4.2b indicates that the macroinvertebrate families of Philopotamidae, Athericidae and Tipulidae showed strong, positive correlations to overall habitat integrity and availability (IHAS Scores), where stream condition was correlated with Ecnomidae and Leptophlebiidae. The families Hirudinea and Baetidae showed strong negative correlations to habitat integrity.

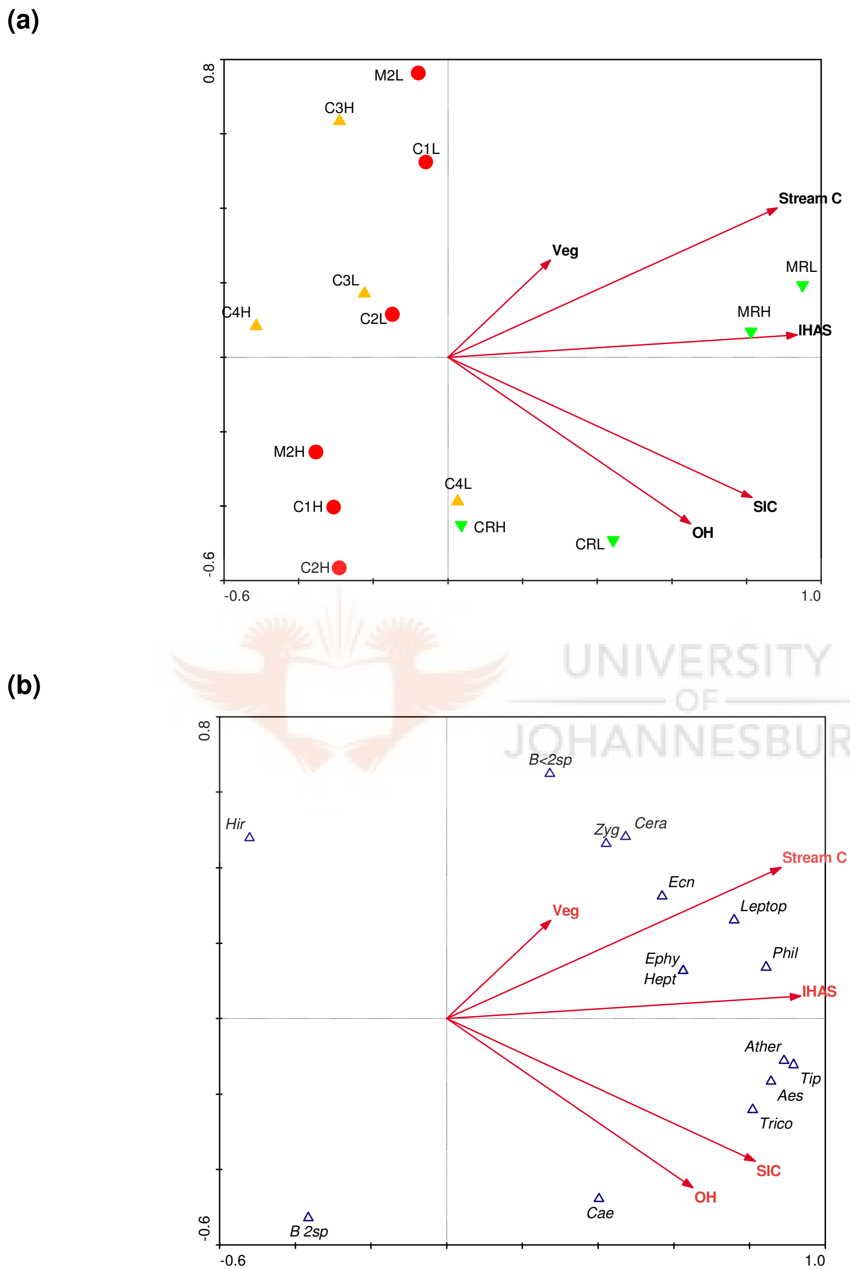


Figure 4.2: Redundancy analysis ordination of habitat components at each site indicating (a) habitat components in relation to land use based on macroinvertebrate community structure; and (b) macroinvertebrate family affinities to particular habitat parameters based on macroinvertebrate community structure data for high and low flow scenarios. [H – High flow; L – Low flow; Veg – Vegetation; Stream C – Stream Condition; IHAS – Integrated Habitat Assessment Index; SIC – Stones in current; OH – Other Habitat].

4.3.2 Macroinvertebrate Diversity

The results of univariate diversity indices are shown in Figure 4.3a to 4.3e. A total of 53 macroinvertebrate families were identified from the 7 sites in this study (Appendix C). Taxonomic richness varied from 14 to 29 with an average of 19.6. When considering the number of invertebrate families (Figure 4.3a), at high flow there is a longitudinal trend towards increased taxonomic richness at test sites on the Crocodile River (i.e. an increase from C4H to C1H). This indicates that agricultural sites C1H and C2H have higher species richness than urban sites C3H and C4H. At low flow, total species once again shows a decreasing trend after large dams. Figure 4.3b indicates that the total number of individuals were greatly increased seasonally, with site C4L having a total of almost 15 000 individuals; most of which were contributed to by the Oligochaeta family.

Margalef's species richness (Figure 4.3c) integrates species diversity and abundances. At high flow species richness increased longitudinally on the Crocodile River from C4H to C1H, indicating that there was a higher richness at agricultural sites in comparison to urban sites. At low flow no trends related to land use were noted for species richness, however, there was a temporal decrease in richness from high to low flow.

Pielou's evenness (Figure 4.3d) indicates how individuals are distributed over the species in the samples. Evenness for high flow showed that there was a more or less even distribution between samples on the Crocodile River, with very slight increases in evenness on the Crocodile River after the presence of Hartbeespoort Dam and Roodekopjes Dam. At low flow, evenness at the agricultural sites was greatly reduced, especially at C1L which dropped from a J' value of 0.68 to below 0.1. Sites on the Magalies River were essentially even at both flows. A temporal decrease was noted for evenness data from high to low flow on both the Crocodile and Magalies rivers.

The Shannon-Wiener diversity index (Figure 4.3e) follows the same trends as Pielou's evenness and reveals that agricultural site C1 had the greatest reduced diversity at low flow. H' loge diversity values noted for test sites at high flow showed a slight increase in diversity after the placement of big dams on the Crocodile River. Low flow values for the Crocodile River show that there is an increase in species diversity before the Hartbeespoort Dam from site CRL to C4L, with a longitudinal decline after Hartbeespoort Dam from C3L to C1L. This indicates that urban sites had a higher diversity than agricultural sites at low flow. The Magalies agricultural site M2L had a higher species diversity at low flow than the relative reference site MRL, although at high flow MRH showed the highest species diversity overall. A temporal trend in species richness was noted with a reduction in species diversity from high to low flow.

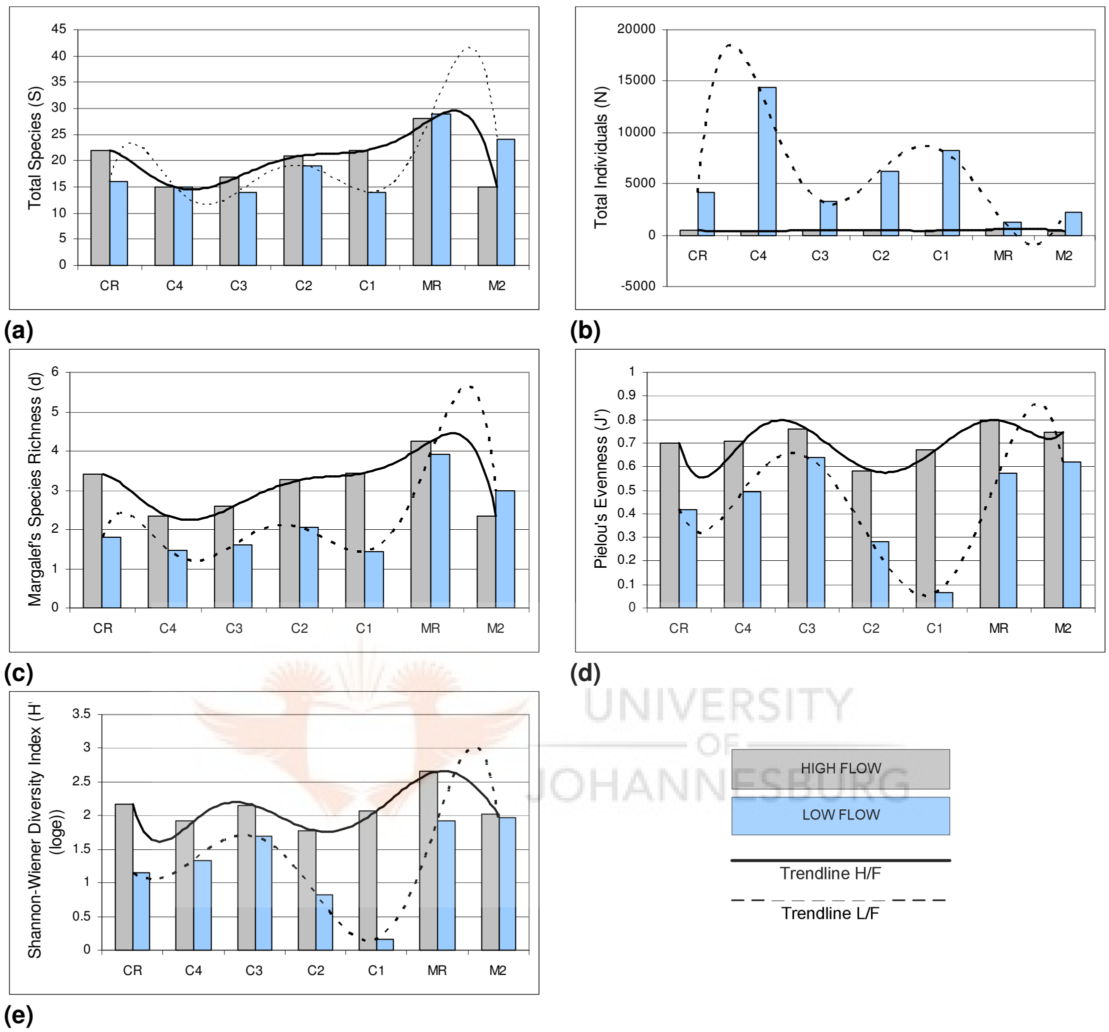


Figure 4.3: Univariate diversity index values for macroinvertebrate (a) Total Species (S) (b) Total Individuals (N); (c) Margalef's species richness (d); (d) Pielou's evenness (J'); and (e) Shannon-Wiener diversity index (H' (loge)). The values indicated in the figure are for high and low flow for sites at the Crocodile and Magalies rivers. Polynomial trend lines are included to indicate trends in index scores between sites and seasons.

4.3.3 Macroinvertebrate Community Composition

The grouping of sample sites at 40% similarity according to CLUSTER analysis and NMDS ordination, based on macroinvertebrate community structure, at high flow is shown in Figure 4.4a and 4.4b. The NMDS ordination indicated that sample sites group according to land use based on macroinvertebrate community data, with the exception of site CRH which was a relative reference site that showed diatom community impacts that were related to urban inputs

(Section 3.4.2.1). Site CRH grouped with sites C1H, C2H and M2H that were agriculturally impacted. One-Way ANOSIM showed that there were no significant differences ($p<0.05$) between the different relative reference group (Group 2), agricultural group (Group 3) and urban group (Group 1) at high flow.

(a) **(b)**

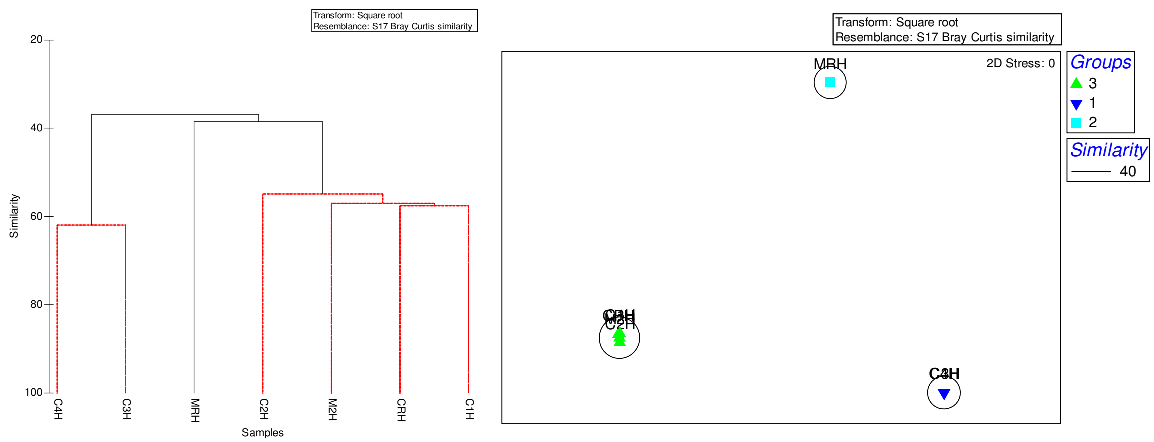


Figure 4.4: (a) Bray Curtis similarity matrix based on hierarchical cluster analysis indicating the similarity between samples in relation to the macroinvertebrate community structure at each site for high flow; and (b) 2 Dimensional NMDS for macroinvertebrate community structure data indicating percentage similarity and groups.

The macroinvertebrate families for the high flow period that contributed to the groupings of sample sites and similarity within the macroinvertebrate groupings are shown in Table 4.6. As mentioned, sites at high flow were separated into the varying groups according to land use. SIMPER analysis for the Magalies River reference site (Group 2) showed that the dominant and distinguishing family contributing to the out grouping of this site was Tricorythidae. The urban group (Group 1) and agricultural group (Group 3) both showed Baetidae, Chironomidae and Simuliidae as the main contributing families, however, further down the list of contributing families it was noted that the Hydropsychidae and Hirudinea were unique to urban sites, whilst Hydropsychids were absent from agricultural sites which instead showed higher contribution from air breathers (Potamonautidae, Corixidae, Veliidae and Gyrinidae).

Table 4.6: Results obtained from SIMPER analysis with a 90% cut off for low contributions indicating the contribution of various macroinvertebrate families at high flow (H) to similarity within the macroinvertebrate groupings. [U – Urban; A – Agricultural; R- Relative Reference].

	Sites	Land use	Family	Contribution %	Cumulative %
Group 1	C3 H C4 H	U U	Baetidae (>2p) Chironomidae Simuliidae Hydropsychidae 1 sp Hirudinea Oligochaeta Coenagrionidae	22.04 18.51 14.14 12.42 8.62 7.56 7.56	22.04 40.55 54.69 67.11 75.73 83.29 90.85
Group 2	MR H	R	Tricorythidae Baetidae (>2sp) Hydropsychidae (>2 sp) Simuliidae Chironomidae Gerridae Ceratopogonidae Ancylidae Gomphidae Aeshnidae Turbellaria Philopotamidae	28.94 11.27 9.53 6.07 4.33 3.47 3.47 3.47 2.60 2.60 2.60 2.60	28.94 40.21 49.74 55.81 60.14 63.64 67.11 70.58 73.18 75.78 78.78 80.98
Group 3	C1 H C2 H M2 H CR H	A A A R/U	Baetidae (2sp) Simuliidae Chironomidae Gyrinidae Oligochaeta Coenagrionidae Veliidae Caenidae Potamonautidae Ancylidae Corixidae	26.69 14.06 11.85 10.40 7.08 5.72 4.51 3.31 2.72 2.12 1.73	26.69 40.74 52.60 63.00 70.08 75.80 80.31 83.62 86.34 88.47 90.20

The grouping of sample sites according to CLUSTER analysis and NMDS ordination (based on macroinvertebrate community structure at a 40% similarity) at low flow are shown in Figure 4.5a and 4.5b. There is a higher degree of spatial variation at low flow than at high flow (Figure 4.4a and 4.4b). On the Crocodile River, NMDS ordination indicated that sample sites group according to land use based on macroinvertebrate community data and there positioning in relation to Hartbeespoort Dam wall. Urban sites C3L, C4L and CRL were placed in Group 3, and agricultural sites C1L and C2L in Group 2. Spatial separation occurred between the Crocodile and Magalies rivers at low flow as is noted on the NMDS ordination of MRL and M2L (Group 1). One-Way ANOSIM showed that there were no significant differences (p<0.05) between the different groupings, but indicated that groups on the Crocodile River (Group 2 and Group 3) were more similar to each other than to sites of the Magalies River (Group 1).

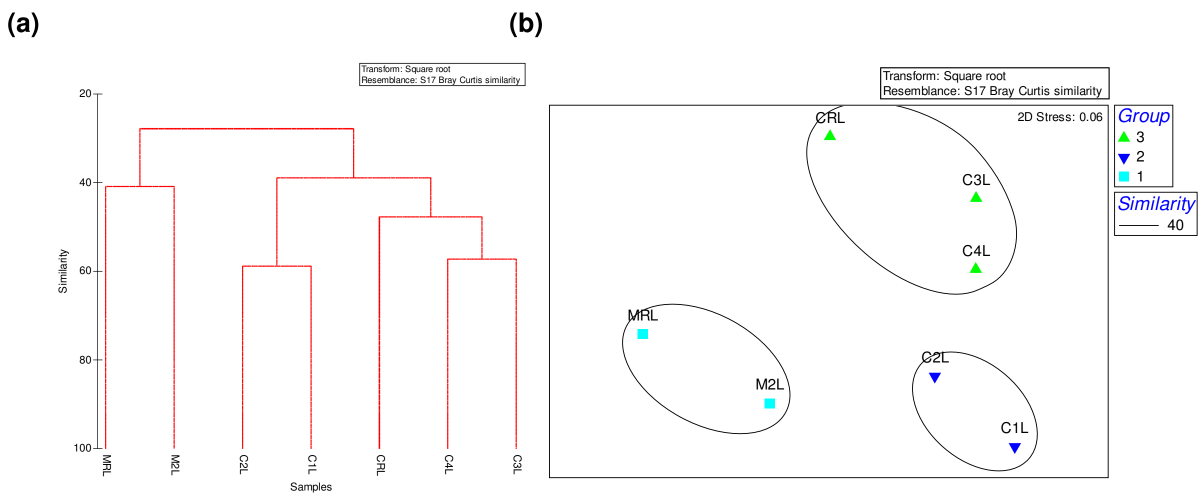


Figure 4.5: (a) Bray Curtis similarity matrix based on hierarchical cluster analysis indicating the similarity between samples in relation to the macroinvertebrate community structure at each site for low flow; and (b) 2 Dimensional NMDSfor macroinvertebrate community structure data indicating percentage similarity and groups.

The macroinvertebrate families for the low flow period that contributed to the groupings of sample sites and similarity within the macroinvertebrate groupings are shown in Table 4.7. Sites at low flow were initially separated spatially, and then into the varying groups according to land use on the Crocodile River. The Magaliesberg River (Group 1) was characterised by numerous Baetidae species, where Agricultural sites on the Crocodile River (Group 2) showed an overwhelming dominance of Oligochaeta which contributed 78.03% to Groups 2's overall make up. Referring back to previous the section, site C1L exhibited lowered species richness (Figure 4.3c) lowered species diversity (Figure 4.3e) and lowered evenness scores (Figure 4.3d) due to the high contribution of the Earth Worm family to the make up of this site. Urban sites in Group 3 varied from agricultural sites by the domination of one species of Baetidae, and Chironomidae. Although urban sites also showed contribution of Oligochaeta to the grouping of the sites, it was in a much lower dominance in comparison to the agricultural sites.

The grouping of sample sites according to CLUSTER analysis and NMDS ordination based on macroinvertebrate community structure at both high and low flow combined are shown in Figure 4.6a and 4.6b. The NMDS ordination for combined seasonal data indicated that there was an initial distinct temporal separation of samples at high and low flow in relation to community composition. Subsequent separation of sites into land use types was evident at the respective flow intervals.

The grouping of sample sites according to CLUSTER analysis and NMDS ordination based on macroinvertebrate community structure at both high and low flow combined are shown in Figure 4.6a and 4.6b. The NMDS ordination for combined seasonal data indicated that there was an initial distinct temporal separation of samples at high and low flow in relation to community composition. Subsequent separation of sites into land use types was evident at the respective flow intervals.

Table 4.7: Results obtained from SIMPER analysis with a 90% cut off for low contributions indicating the contribution of various macroinvertebrate families at low flow (L) to similarity within the macroinvertebrate groupings. [U – Urban; A – Agricultural; R-Relative Reference].

	Sites	Land use	Family	Contribution %	Cumulative %
Group 1	MR L M2 L	R A	Baetidae (>2sp) Chironomidae Hydropsychidae(>2sp) Simuliidae Turbellaria Oligochaeta Caenidae Ceratopogonidae Leptophlebiidae Tabanidae Porifera	23.98 12.64 12.02 10.95 8.94 7.23 5.26 3.51 2.48 2.48 1.75	23.98 36.62 48.64 59.59 68.53 75.76 81.02 84.52 87.00 89.48 91.23
Group 2	C1 L C2 L	A A	Oligochaeta Chironomidae Turbellaria Hydropsychidae(1 sp)	78.03 5.71 5.15 4.53	78.03 83.73 88.88 93.41
Group 3	C3 L C4 L CR L	U U R/U	Baetidae (1 sp) Chironomidae Oligochaeta Hydropsychidae(1 sp) Caenidae Turbellaria	30.70 26.10 11.70 9.04 7.77 5.80	30.70 56.80 68.51 77.55 85.32 91.12

There were two exceptions to the above stated trends that were noted. Site CRH, which was expected to group with urban sites at high flow, instead grouped with agricultural sites. Secondly, agricultural site M2L was out grouped completely but showed more similarity to urban sites at high flow. One-Way ANOSIM showed that there were no significant differences (p<0.05) between any of the sites at either of the flow periods. This signifies a high level of homogeneity, especially for the Crocodile River.

The macroinvertebrate families for the combined flows that contributed to the groupings of sample sites and similarity within the macroinvertebrate groupings are shown in Table 4.8. This table indicates that spatial variation plays a major role in macroinvertebrate grouping based on community structure at family level. Family contributions either changed very little, or did not change when high and low flow period data was combined; hence there is an initial temporal variation after which spatial variation in this data occurs.

(a) **(b)**

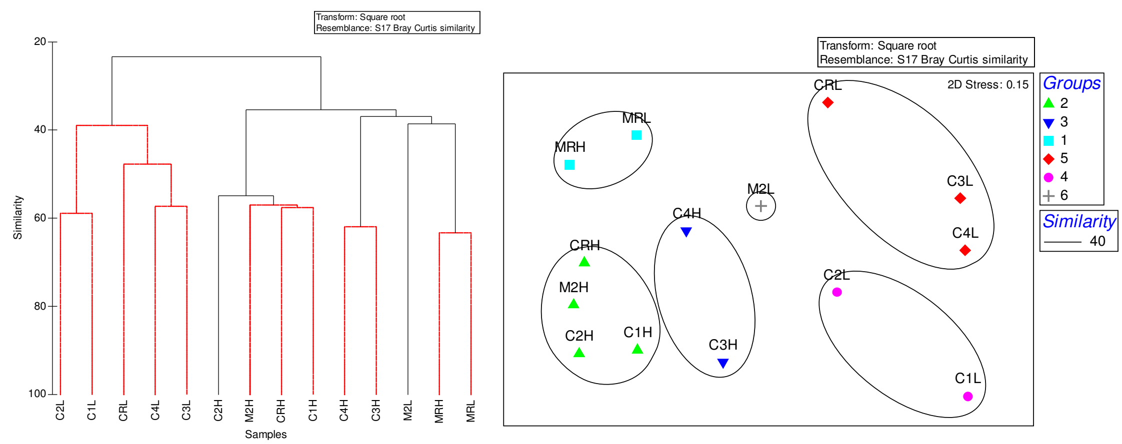


Figure 4.6: (a) Bray Curtis similarity matrix based on hierarchical cluster analysis indicating the similarity between samples in relation to the macroinvertebrate community structure at each site for high and low flow combined; and (b) 2 Dimensional NMDS for macroinvertebrate community structure data indicating percentage similarity and groups.

Table 4.8: Results obtained from SIMPER analysis with a 60% cut off for low contributions indicating the contribution of various macroinvertebrate families at high flow and (H) low flow (L) to similarity within the macroinvertebrate groupings.

	Sites	Land use	Family	Contribution %	Cumulative%
Group 1	MR L MR H	R R	Tricorythidae Baetidae (>2p) Hydropsychidae (>2sp) Simuliidae Chironomidae Ceratopogonidae Turbellaria	17.46 10.89 9.26 7.99 6.76 6.04 5.23	17.46 28.36 37.62 45.61 52.37 58.41 63.65
Group 2	C1 H C2 H M2 H CR H	A A A R/U	Baetidae (2sp) Simuliidae Chironomidae Gyrinidae	26.69 14.06 11.85 10.40	26.69 40.74 52.60 63.00
Group 3	C3 H C4 H	U U	Baetidae > 2p Chironomidae Simuliidae Hydropsychidae 1 sp	22.04 18.51 14.14 12.42	22.04 40.55 54.69 67.11
Group 4	C1 L C2 L	A A	Oligochaeta	78.03	78.03
Group 5	C3 L C4 L CR L	U U R/U	Baetidae 1sp Chironomidae Oligochaeta	30.70 26.10 11.70	30.70 56.80 68.51
Group 6	M2 L	A	Baetidae Simuliidae Chironomidae	30.5 16.3 15.9	30.5 46.8 62.7

Ranked species K-dominance plots for macroinvertebrate communities indicated by Figures 4.7a and 4.7b show relative species abundance as a percentage of the total abundance, plotted for each site. There was no dominance of a particular family at high flow, with an even distribution of families at each site. Low flow data indicates that there is less evenness in terms of abundance at most sites on the Crocodile River (CRL, C1L, C2L and C4L) that indicates an overall longitudinal disturbance exists. Agricultural site C1L showed a noted increase in abundance of one family that contributed to almost 70% of the overall community make up at this site.

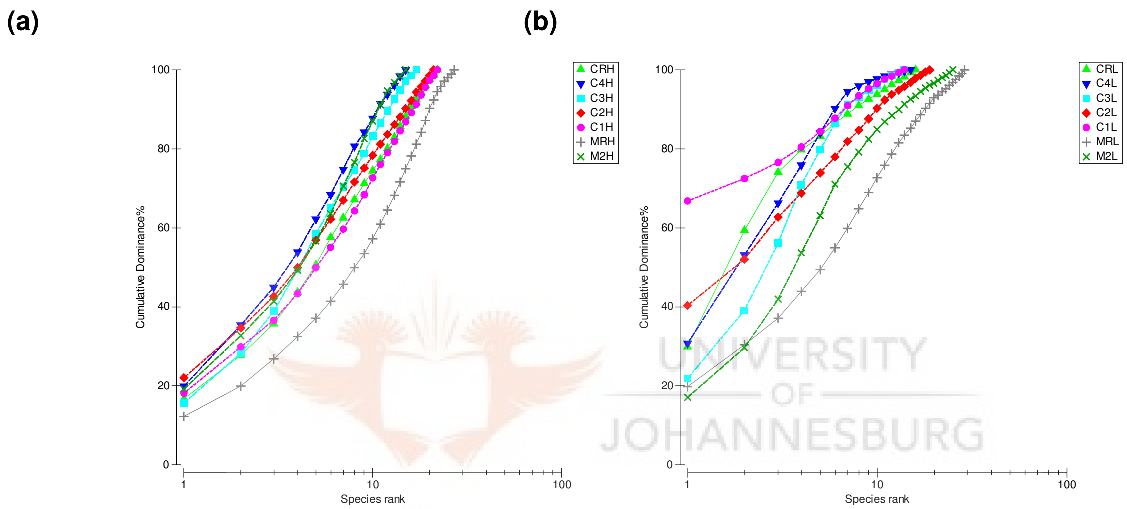


Figure 4.7: Ranked species K-dominance plot for macroinvertebrate communities collected at (a) high flow (H/F), and (b) low flow (L/F) at sites on the Crocodile (C) and Magalies (M) rivers utilising abundances to indicate cumulative dominance.

4.3.4 Functional Feeding Groups

Referring to Figure 4.8a and 4.8b, analysis of FFGs between sites and seasons showed temporal differences in macroinvertebrate feeding groups. Overall, there was a high degree of similarity between the sites with regard to FFG structure as indicated by CLUSTER analysis, with an 80 to 90 percent similarity between the FFG groups. No trends were noted in terms of land use in relation to the FFG make up of macroinvertebrates at sites, and no significant differences ($p<0.05$) were noted from One-Way ANOSIM.

(a) (b)

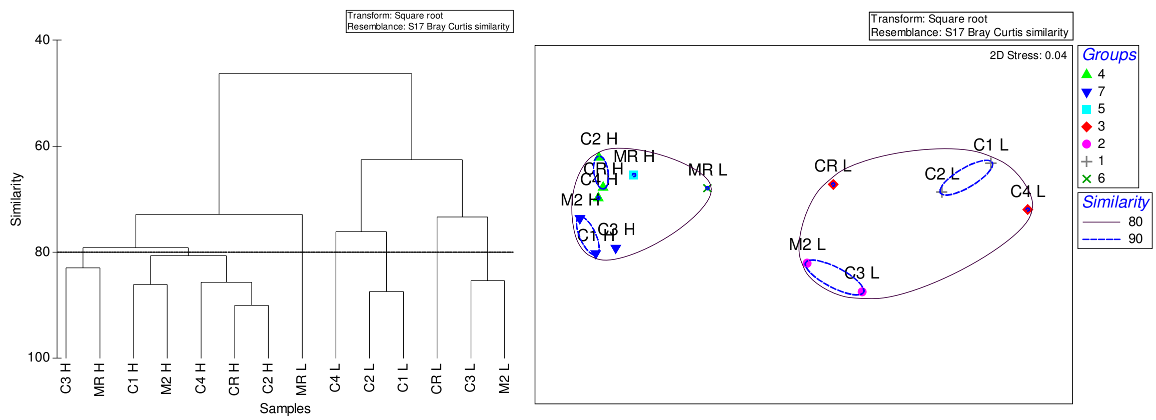


Figure 4.8: (a) Bray Curtis similarity matrix based on hierarchical cluster analysis indicating the similarity between samples in relation to the macroinvertebrate FFGs at each site for high flow; and (b) 2 Dimensional NMDS for macroinvertebrate FFG data indicating percentage similarity and groups.

The macroinvertebrate FFGs for high and low flow periods that contributed to the groupings of sample sites and similarity within the macroinvertebrate FFG groupings are shown in Table 4.9. Low flow results showed that the Crocodile River agricultural sites C1L and C2L grouped together in Group 1 with a dominance of collector gathers (COG) and collector filterers (COF) making up the FFG composition. Sites on the Crocodile River above Hartbeespoort Dam that had an urban influence grouped (Group 3) together. Interestingly enough, agricultural site M2L on the Magalies showed a high similarity to urban site C3L on the Crocodile River for low flow. Reference site MRL was the only site at low flow to show more similarity to high flow sites, however, this site was out grouped due to the predominant contribution of COG (65.65%). There was an overwhelming overall contribution of COG and COF to all of these sites for the low flow period.

Referring to Figure 4.8a, Figure 4.8b and Table 4.9, at high flow there was no obvious land use trend that was noted relating to FFGs. Agricultural site C2H showed more similarity to urban sites CRH, and C4H (group 4), while conversely the urban site C3H showed more similarity to agriculturally influenced sites once again (Group 7). The Magalies River relative reference site was out grouped as in low flow (Group 5) due to the dominance of COG macroinvertebrate feeders at this site. What is different from low flow to high flow data is the contribution of predator engulfers (PRE) to the SIMPER make ups.

Table 4.9: Results obtained from SIMPER analysis with a 50% cut off for low contributions indicating the contribution of various macroinvertebrate FFGs at high flow (H) to similarity within the macroinvertebrate FFG groupings. [H – high flow; U – Urban; A – Agricultural; R- Relative Reference; COG – Collector Gatherers; COF – Collector Filterers; SC - Scrapers; PRE – Predator Engulfers].

	Sites	Land use	FFG	Contribution %	Cumulative %
Group 1	C1L C2L	A A	COG COF	29.98 28.58	29.98 58.55
Group 2	M2L C3L	A U	COF SC COG	23.17 23.06 21.06	23.17 46.23 67.61
Group 3	CRL C4L	R/U U	COG SC	43.62 23.55	43.62 67.17
Group 4	CRH C4H C2H	R/U U A	COG SC PRE	27.07 20.29 18.39	27.07 47.36 65.75
Group 5	MRH	R	COG PRE	41.99 18.24	41.99 60.23
Group 6	MRL	R	COG	65.65	65.65
Group 7	C3H C1H M2H	U A A	COF COG SC	23.63 18.81 16.57	23.63 42.44 59.01

4.3.5 Macroinvertebrate Indices

The SASS5 scores, number of taxa, ASPT and ECs for each site at high and low flow periods are indicated in Table 4.10. The SASS5 categories were assigned by using Table 4.2 and Table 4.3. Figures 4.9a and 4.9 b graphically represent SASS5 scores and ASPTs for high and low flow, respectively.

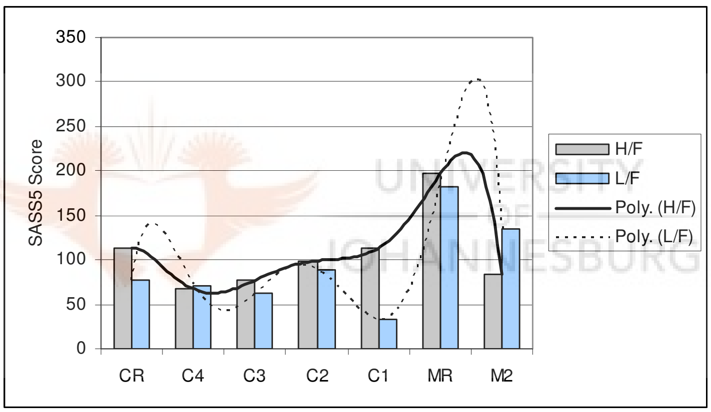
It is evident from the results that SASS5 scores follow the same trend as species richness results (Figure 4.3a). At high flow, relative reference sites on the Crocodile (CRH) and Magalies (MRH) rivers showed the highest SASS5 scores overall for the respective rivers (Figure 4.9a). Higher SASS5 scores were noted for agricultural sites C1H, C2H and M2H than were noted for urban sites C3H and C4H, indicating an increasing longitudinal trend from C4H to C1H. At low flow however, no trends were noted between SASS5 score and land use, but slight decreases in SASS5 scores were noted after Hartbeespoort Dam and Roodekopjes Dam on the Crocodile River.

Figure 4.9b indicates that at high flow, agricultural sites showed higher ASPTs than urban sites on the Crocodile River. At low flow there was a slight decrease in ASPT values that were noted after the presence of large dams (i.e. from C4L to C3L; and from C2L to C1L).

Table 4.10: Results obtained from the application of the SASS5 index indicating SASS5 Scores, Number of Taxa, Average Score Per Taxon (ASPT), Ecological Category and Ecoregion level 1 types for each site at high flow (H) and low flow (L).

		Site	SASS Score	No. Taxa	ASPT	Category	Ecoregion Level 1
SASS5	HIGH FLOW	CRH	114	20	5.7	D	Highveld
		C4H	67	17	3.94	E	Western Bankenveld
		C3H	77	19	4.05	D/E	Western Bankenveld
		C2H	99	19	5.21	B/C	Bushveld Basin
		C1H	113	23	4.29	C/D	Western Bankenveld
		MRH	197	27	7.29	A	Western Bankenveld
		M2H	84	16	5.25	C/D	Western Bankenveld
	LOW FLOW	Site	SASS Score	No. Taxa	ASPT	Category	Ecoregion Level 1
		CRL	77	16	5.13	D/E	Highveld
		C4L	71	15	4.73	D	Western Bankenveld
		C3L	62	14	4.43	D/E	Western Bankenveld
		C2L	89	19	4.68	C	Bushveld Basin
		C1L	33	10	3.3	E/F	Western Bankenveld
		MRL	183	29	6.31	A/B	Western Bankenveld
		M2L	135	26	5.25	C	Western Bankenveld

(a)



(b)

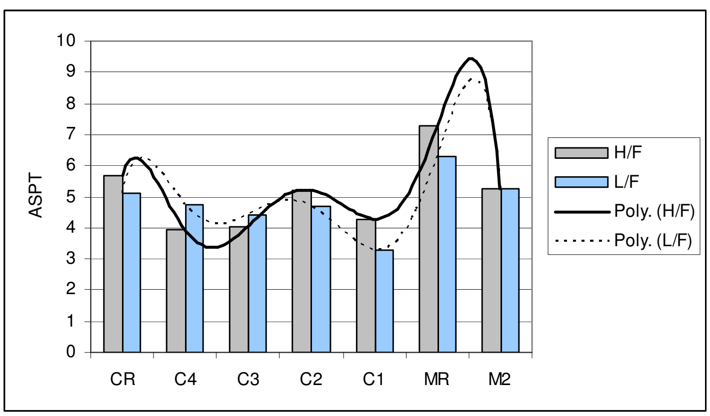


Figure 4.9: (a) Representation of the SASS5 macroinvertebrate scores for high flow (H/F), and low flow (L/F) at sites on the Crocodile (C) and Magalies (M) rivers; and (b) Representation of the ASPTs for high flow (H/F), and low flow (L/F) at sites on the Crocodile (C) and Magalies (M) rivers. Polynominial trend lines are included to indicate trends in data from site to site and season to season.

The MIRAI scores, metric group scores and ECs for each site, based on combined high and low flow data, are indicated in Table 4.11. The ECs were derived using Table 4.2 and Table 4.3. Figures 4.10a and 4.10b graphically represent MIRAI and metric group scores. Referring to Table 4.11, the Magalies River relative reference site MR showed the highest overall EC and was placed in a C category, indicating that it was in a moderately modified state. This site showed a 73.7% similarity to natural conditions in terms of macroinvertebrate assemblage. Even though MR showed a degree of difference from natural conditions, it still retained basic ecosystem functions and showed a difference of 25.29% from the next highest test site EC (i.e. C1 at 48.41%)

MIRAI values indicated that urban sites (C3, C4 and CR) were in a slightly more impaired ecological state than agricultural sites (C1, C2 and M2). Urban sites ranged from a D to an E category (largely to seriously modified macroinvertebrate communities with loss of ecosystem function). The agricultural sites all fell into a D category indicating that were largely modified and in an unacceptably impacted state.

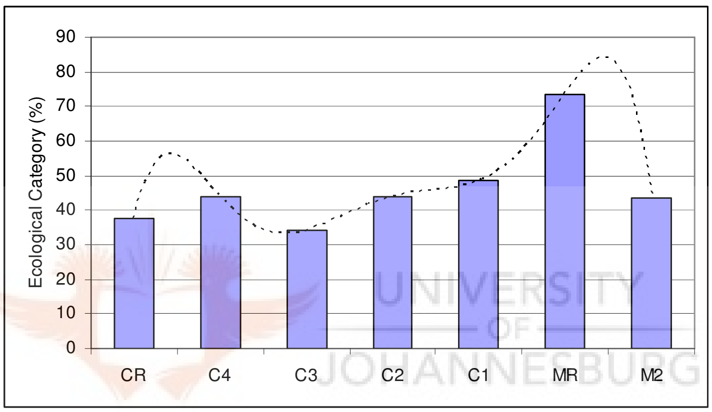
Polynomial trend lines in Figure 4.10a showed an increase in ecological health on the Crocodile River before Hartbeespoort Dam, changing in integrity from 37.5% (CR) to 43.86%. After Hartbeespoort Dam an increasing longitudinal trend exists from C3 to C1 (changing from 34.2% to 48.4%). On the Magalies River a decreasing trend was noted from MR (73.7%) to M2 (43.5%).

According to MIRAI, underlying problems that are shown by changes from reference conditions in the metric groups are as follows: the most notable modification in flow was noted at urban site CR and C4 before the Hartbeespoort Dam; habitat modification was most severe at CR according to the macroinvertebrate assemblages present; water quality at urban sites was in a more degraded state than agricultural sites; and, changes in connectivity and seasonality were most severe at urban site C3 and agricultural site M2. This being said, all test sites were similarly impacted in terms of the different metric groups in comparison to reference data. Relative reference site MR did show some modification that was mostly attributed to by seasonality of the upper reaches of the Magalies River. Overall, MIRAI scores followed similar patterns as SASS5 data for high flow.

Table 4.11: Results obtained from the application of the MIRAI index indicating metric group scores, overall MIRAI scores and ECs for each site.

		METRIC GROUPS					
		Flow Modification (%)	Habitat Modification (%)	Water Quality (%)	Connectivity & Seasonality (%)	EC (%)	MIRAI Category
MIRAI	CR	36	34.2	38.5	55	37.5	E
	C4	40	52.8	32.2	50	43.86	D
	C3	47.3	42.2	34.2	15	34.2	E
	C2	45.6	42.8	42.8	80	43.9	D
	C1	49.3	43.2	42.4	60	48.41	D
	MR	71.1	67.3	82.4	60	73.7	C
	M2	44.1	61.3	48.8	20	43.5	D

(a)



(b)

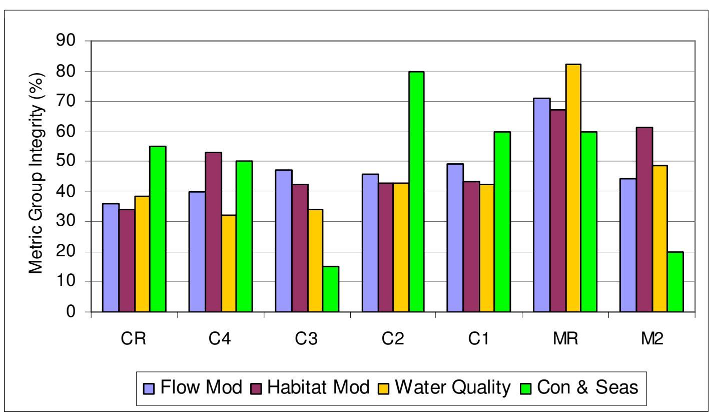


Figure 4.10: (a) Representation of the MIRAI scores for at sites on the Crocodile (C) and Magalies (M) rivers; and (b) Representation of the metric group scores.

4.4 Discussion

4.4.1 Habitat

The IHAS index was developed to provide physical habitat information that is comparable with the SASS index (McMillan, 1998). There was a general increase in habitat quality from high to low flow that was noted in this study. This same trend was also noted on the Elands River by O'Brien (2003). Reduced flows would increase the available riffle and rapid habitat for invertebrates to occupy, which would otherwise be inundated at high flow scenarios (McMillan, 1998). Thus, the flow regime is an important component of the habitat template. Habitat integrity is discussed in terms of varying land use types in the following section.

4.4.1.1 Relative Reference Sites

Relative reference site MR showed the highest IHAS score for both flow periods and was assigned score of 84% and 88% respectively, indicating that the habitat was in a largely natural condition (Table 4.5 and Figure 4.1). The relative reference site consisted of lengthy cobble beds with fast flowing water, broken by slower flowing runs and pools dominated by sand and mud. Marginal vegetation was in a good state and showed good diversity at this site with little disturbance, however aquatic vegetation was not present. Stream condition was also in a good condition due to the low immediate surrounding impacts and high cover of vegetation in the riparian zone. This is shown in Figure 4.2a, where stream condition, vegetation and overall IHAS scores are strongly positively correlated to site MR at both flow periods. The extent of cover by natural vegetation (and thus natural land use) was the most powerful predictor of instream habitat integrity according to Amis *et al.* (2007), however, in the same study it was noted that the extent of natural land use was a better indicator of riparian integrity than instream integrity.

Taxa that were strongly associated with habitat integrity at this site are shown in Figure 4.2b. The Heptageniidae, Leptophlebiidae, Ecnomidae, Philopotamidae and Ephydridae taxa show preferences for stream condition and overall IHAS scores, inferring that they are indicator taxa for overall habitat integrity. The Heptageniidae, Leptophlebiidae, Ecnomidae and Philopotamidae all have preferences for the stones (cobble) habitat and moderate to high water quality, whilst Ephydridae shows preference for GSM and low water quality (Thirion, 2007). This indicates that the in the present study Heptageniidae, Leptophlebiidae, Ecnomidae and Philopotamidae are more indicators of good stones habitat, and more specifically riffle habitat because this was the most predominant stones habitat at MR. Roy, Rosemond, Leigh, Paul and Wallace (2003) suggested that invertebrates that showed preference for riffle habitat were most sensitive to changes resulting from land cover change and thus were important for detection of these changes. The results of the current study agree with these findings.

4.4.1.2 Agricultural Sites

There was degradation noted in habitat from the reference site MR and relative reference/urban site CR to the test sites in terms of percentage integrity (Figure 4.1 and Table 4.5). Agricultural sites showed that the habitat integrity scores were mostly affected by modified stream conditions and general habitat metrics (GSM, Stones out of Current (SOOC) and algal presence). It was visually observed that the agricultural sites generally had higher amounts of finer sediments contributing to their sediment makeup and suffered from stream disturbances and clearing of riparian zones (Appendix C).

As was noted in the water quality analysis, higher suspended sediment (TSS and TSIM) loads were recorded at agricultural sites (Section 3.3.1). In a study by Roy *et al.*, (2003) it was found that there was a greater richness of invertebrate taxa at minimally impacted sites compared to sediment impacted sites. The reason for this is that poorly managed agricultural practices cause sediment to enter the systems and be deposited, altering substrate composition and filling interstices on cobble and rocks surfaces, which ultimately changes the fitness of substrates for invertebrate taxa and increases macroinvertebrate drift due to substrate instability (Richards & Bacon 1994 cited in Wood & Armitage, 1997). Figure 4.2a and 4.2b indicated that agricultural sites showed negative correlations to habitat integrity and that the ubiquitous Baetidae family (where 2 species occurred) was an indicator of habitat integrity at high flow. The Baetidae family show low water quality preferences and are generalists in terms of habitat (Thirion, 2007).

Site M2, in addition to sediment increases showed lowered flows due to the placement of an illegal farm weir upstream of this site. The lowered flows, and increased sediments severely altered flow and riffle availability, turning the site into a homogenous mixture of pools and slow flowing areas dominated by silty substrate. This kind of agricultural site habitat would be limited to biota that have no migratory requirements and are silt tolerant (Poff, 1997).

4.4.1.3 Urban Sites

Urban sites C3 and C4, and relative reference/urban site CR, suffered habitat impacts mostly due to flow modification. Increased flow was contributed to by increased canalization, urban run-off and storm water inputs from upstream areas, showing signs of erosion on the banks of the Crocodile River. Increased connected impervious surfaces raise the flows causing riffle bed instability (mobility), and construction in catchments that are under development pressure have increased inputs of sediments into urban streams which makes the general habitat unsuitable for aquatic macroinvertebrates (Woods & Armitage, 1997; Wang *et al.*, 2001). As noted by Poff (1997), increased “flashiness” leads to disturbance of cobble and gravel beds which ultimately leads to a presence of macroinvertebrates with traits for “flood resistance”. Geomorphologic and chemical variables associated with the percentage of urban land cover have been correlated with riffle insect richness and density, and pool insect density (Roy *et al.* 2003). Taxa richness in riffle habitat studied by Roy *et al.* (2003) was negatively related to fine bed sediment size (riffle and pool), bed mobility and TSS.

The placement of Hartbeespoort Dam in relation to C3 is an important factor regulating habitat at this site. Dam operating regimes (water release regulation) would cause the flow of the Crocodile River beneath the dam to be more or less constant throughout the year for the purpose of irrigation in the downstream agricultural areas (Dallas & Day, 2004). This would indicate that the natural flow variation according to rain fall would be impeded. Thus, habitat at this site is affected mainly due to extreme flow modification which puts a constant pressure on habitat due to instability of the boulders and cobbles at this site. There was also a large increase of marginal vegetation and algal presence on rocks at C3L (low flow) which was linked to the increases in NO_3 noted in Section 3.3.1.3.

4.4.2 Macroinvertebrates

4.4.2.1 Macroinvertebrate Diversity

Reference Site

Univariate diversity index results indicated that MR at high flow showed the highest figures for all univariate indices (with exception of abundance) as indicated in Figure 4.3a to 4.3e. At low flow however, agricultural site M2 slightly superseded MR in terms of Shannon-Weiner diversity and Pielou’s evenness (Figure 4.3d and 4.3e), which indicated that there was some form of ecological stress from high to low flow. Natural temporal variation does occur from season to season in lotic systems (Chutter, 1998). There was an increase in TSOM measured from high to low flow (Section 3.3.1) which could have contributed to the lowered diversity. Increases in the organic fraction of suspended sediments may cause a shift in processes and functional feeding groups, selecting for organisms that feed on organic particulate matter. A shift would also be seen in obligate or facultative taxa in which generalists would be selected over obligate specialists (Merritt *et al*., 1996). This could lead to lowered diversity and richness overall.

Margalef's species richness is based on a calculation that takes into consideration the total number of species (in this case family) in relation to the total number of individuals (Dallas & Day, 2004), where Shannon-Weiner includes the number of individuals per species in the calculation of diversity (Wilhm & Dorris, 1968). These indices are based on the assumption that as species diversity decreases, so does the ecological stress, thus inferring that at high flow the ecological condition and water quality at MR was the best out of all the sites under consideration in this study.

Agricultural Sites

On the Crocodile River at high flow, taxonomic richness (Figure 4.3a) and Margalef's index (Figure 4.3c) showed that agricultural sites had higher values than urban sites. Lenat and Crawford (1994) studied invertebrate communities in relation to land use, and found that taxa richness was "moderately" reduced at agricultural sites, and "severely" reduced urban sites. However, at low flow agricultural sites were highly disturbed showing low diversity and uneven distribution of macroinvertebrate families, with a sharp increase in abundance of invertebrates. Shannon diversity for low flow indicated that agricultural sites were in a more disturbed state than urban sites. The drop in diversity coincides with pesticide spraying in North West Province, where winter crops are sprayed from August each year (Ansara-Ross *et al.,* 2008). Shreiver *et al*. (2007) showed that sites with high run-off potential and long-term intense agrochemical spraying activities showed decreased taxonomic richness and Shannon-Weiner diversity. A study by Schulz and Leiss (2001) linked decreased invertebrate diversity with pesticide contamination in mesocosm studies. Assuming microcosm experiments may be extrapolated to field experiments, this may be a viable explanation for decreased richness and diversity at agricultural sites at low flow. The dilution factor due to seasonality may also play a role in concentration of pollutants and decrease in diversity.

Referring to Section 3.4.1.1, specific water quality impacts for agricultural sites at low flow included increased NO_3, salts, TSS and TDS concentrations, as well as increased conductivity values. Lenat (1984) identified sedimentation and subsequent changes in water quality as causes for decreased macroinvertebrate richness in streams in agricultural areas. Macroinvertebrate fauna found in agricultural regions where salinity was high were mostly salt tolerant (Kay *et al.*, 2001), thus selection for halo-tolerant species would occur and decrease diversity in macroinvertebrate assemblages.

Agricultural site M2 on the Magalies River showed increases in richness, diversity and evenness in comparison to Crocodile River agricultural sites for low flow. At high flow M2 showed lower values for number of taxa (Figure 4.3a) and Margalef's richness (Figure 4.3c), but showed an increase in diversity in terms of evenness (Figure 4.3d) and Shannon-Weiner diversity (Figure 4.3e). This is in agreement with the findings from the diatom community structure in Section 3.5 (Figure 3.8), where it was concluded that agricultural intensity could be split into high (Crocodile River sites) and low intensity (Magalies River site) based on the community data.

The increase in Shannon-Weiner diversity and evenness noted after the presence of Hartbeespoort Dam and Roodekopjes Dam at high flow is of interest (Figure 4.3e). A similar trend was noted in the diatom community in Section 3.4.2.2, which was ascribed to phosphate removal by algae and macrophytes, settling out of suspendoids and nutrients due to the lentic nature of the dams, and possible adsorption of toxicants and nutrients to sediments (Andersen *et al.*, 2004).

Urban Sites

Sites C4, C3 and CR have low overall species richness and diversity, which declined further at low flow. As mentioned above, Shannon Diversity at low flow indicated that urban sites were less slightly stressed than agricultural sites (Figure 4.3e). The factors controlling macroinvertebrate diversity at urban sites are different to agricultural sites and are mainly attributed to enrichment through the addition of nutrients and particulate organics, and toxicants from impervious surfaces (Dallas & Day, 2004). Nutrient enrichment and COD were reflected as the main water quality impacts affecting these sites (Section 3.4.1.1).

CR had the highest univariate diversity values at high flow for urban sites, but showed lower values than other urban test sites at low flow (Figure 4.3). Because of the placement of CR, this site is affected by urban storm water run off from impervious surfaces. In a review of impacts on urban streams, Walsh (2000) suggested that macroinvertebrate communities of streams that drain urban land are not only affected by the pollutants in urban run-off, but also by the "efficiency" of pollutant delivery due to the impervious nature of urban catchments. A spate in urban areas could cause high spikes in contaminants entering a river, thus lowering diversity by selecting for pollution tolerant species.

The position of site C4 is associated with urban sewage impacts. C4 had the highest abundance values, indicating enrichment, which was reflected in the water quality impacts (Section 3.4.1.1). An assessment of macroinvertebrate communities in streams impacted by urban land use in Brazil showed low values of richness and diversity, and high densities of tolerant organisms due to artificial eutrophication associated with sewage discharge (Moreno & Callisto, 2006). The comparison of impacts and diversity values from CR to C4 indicates that sewage inputs affect macroinvertebrate diversity more severely than storm water run-off. Storm water run-off affects communities more intensely at low flow than high flow, possibly due to the dilution factor.

4.4.2.2 Macroinvertebrate Community Composition in Relation to Land use

The preceding text has provided discussion of how habitat and macroinvertebrate diversity are linked to land use patterns. The discussion pertaining to macroinvertebrate community structure that follows in Section 4.4.2.2 is based on the macroinvertebrate community response to water chemistry and habitat as outlined by objectives in Chapter 1, namely: to compare community structure data for macroinvertebrates taken from agriculturally impacted sites to natural and urban sites; subsequently, to elucidate in what way the community structures of macroinvertebrates are modified (if any) due to agricultural inputs into the aquatic system; and identify suitable indicators of change due to agricultural pollution in macroinvertebrate communities.

High flow

Figure 4.4a and 4.4b show sample groupings of macroinvertebrate families at high flow. As mentioned in Section 4.3.3, study sites group according to land use for this season, with the exception of site CR. Dominance curves were very similar at all sites (Figure 4.7a). Land use accounted for 47% of variability amongst urban sites in macroinvertebrate communities in urban streams in America (Kratzer, Jackson, Arscott, Aufdenkampe, Dow, Kaplan, Newbold & Sweeney, 2006).

Group 1 contains the urban sites for the Crocodile River C3H and C4 at high flow grouped at a 40% similarity. Referring to Table 4.6, macroinvertebrate families that contributed to the make up of each group at high flow were Baetidae (22.04%), Chironomidae (18.51%), Simuliidae (14.14%), Hydropsychidae (12.42%), Hirudinea (8.62%), Oligochaeta (7.56%) and Coenagrionidae (7.56%). Kratzer *et al.* (2006) and Moreno and Callisto (2006) found that the most abundant and diverse families in rivers and reservoirs in urban watersheds were the

Oligochaeta, Chironomidae and Gastropoda. The reason for this was because these families are tolerant of hypoxic conditions and are detritivores which feed on fine particulate organic matter (FPOM). The increase of FPOM in urban streams is due to the organic enrichment from to sewage inputs (Walsh, 2000) which often contribute to oxygen depletion (Dallas & Day, 2004).

These above mentioned families (with the exception of the Gastropoda) do contribute to the community composition at high flow, but are not the main contributors to urban make up. The family make up for all test sites overlapped, with the exception of the Hydropsychidae and Hirudinea which were unique to the make up of urban sites at high flow. The Hydropsychids are known to be collectors that are dependant on FPOM in deposited sediments (Merritt *et al*., 1996; de Moor & Scott, 2003) which is an impact of sewage inputs (Walsh, 2000). Leeches are potential indicators of heavy metal pollution and low dissolved oxygen content (Oosthuizen & Sidall, 2002) which are water quality impacts from run-off and sewage inputs.

Agricultural sites and site CR were contained in **Group 3**. Land use accounted for 40% of variability in macroinvertebrate communities in the agricultural regions and correlated with underlying geology in a study by Kratzer *et al*. (2006). The inclusion of CR in Group 3 indicates that at high flow, macroinvertebrate families at the relative reference/urban site are impacted by similar changes in water quality due to agricultural disturbance. Because land associated with CR is part of a botanical garden, herbicide and pesticide application may occur and would be washed into the Crocodile River in the summer months.

The initial make up of the agricultural group was contributed to by Baetidae (26.69%), Simuliidae (14.06%) and Chironomidae (11.85%) which was similar to Group 1's make up (Table 4.6). The contribution and diversity of air breathers and families that are tolerant to hypoxia at this site was higher than at urban sites and included the families of Chironomidae, Gyrinidae (10.4%), Oligochaeta (7.08%), Veliidae (4.51%), Potamonautidae (2.72%), Ancylidae (2.12%) and Corixidae (1.73%). Relationships between pesticide contamination and community structure based on differences in the physiology and mobility of the species was noted by Berenzen, Kumke, Schulz and Schulz (2005). It was also shown that breathing type may influence the uptake of contaminants (hence mortality) into the bodies of macroinvertebrates (Buchwalter, Jenkins & Curtis, 2002).

The Magalies relative reference site (MR) is separated from other land use samples and contained in **Group 2**. The contribution of the Ephemeroptera and Trichoptera by the

Tricorythidae (28.94%), Baetidae (11.27%) and Hydropsychidae (9.53%) families agrees with results of studies by Kratzer *et al.* (2006) and Berenzen *et al*. (2005). These studies found that Ephemeroptera, Plecoptera and Trichoptera (EPT) taxon richness was increased at reference sites in comparison to impacted sites (Kratzer *et al.,* 2006). Berenzen *et al.* (2005) also noted that diversity in the order Diptera was indicative of natural conditions. In the present study, exclusion of the Plecoptera family from the community make up of the relative reference site indicates that there were water quality impacts that excluded this highly sensitive family from the site.

Low flow

Figure 4.5a and 4.5b show sample groupings of macroinvertebrate families at low flow. As mentioned in Section 4.3.3, study sites group according to land use with the exception of M2 which grouped with the MR, and indicated spatial separation of the Magalies River from the Crocodile River.

Group 1 contains relative reference MR and agricultural site M2 grouped at a 40% similarity (Figure 4.5a and 4.5b). The grouping of M2 with MR was due to the fact that there was increased habitat quality (Table 4.5) for M2 at low flow which was associated with an increase in macroinvertebrate richness (Figure 4.3). Referring to Table 4.7, macroinvertebrate family contributions for low flow for Group 1 overlapped between reference, urban and agricultural sites. The differences that were noted were higher species diversity amongst the Baetidae and Hydropsychidae family at the reference sites, and the relatively minor contribution of the more sensitive Leptophlebiidae family (2.48%) to the group's make up.

Crocodile River agricultural sites C1 and C2 were placed in **Group 2**. The main difference between high and low flow is the exclusion of the Baetidae family, and overwhelming dominance of the Oligochaeta family (Table 4.7) which was indicated by the K-dominance curves (Figure 4.7b). Dabrowski, Bollen. and Schulz (2005) showed that at high flow, the availability of pesticides to the mayfly species *Baetis harrisoni* was reduced due to the reduced activity of pesticides under high flow conditions, inferring that pesticide spray drift was more toxic to Baetids at low flow. This would explain the decrease in the numbers of Baetids at agricultural sites at low flow. The predominance of the Oligochaetes (78.03%) at low flow indicates that the agricultural sites are suffering more from organic disturbance than agrochemical disturbance. The Oligochaetes have been identified as a tolerant family that has been associated with hypoxic conditions, organic pollution, nutrient enrichment and urban land use in previous studies

(Lenat & Crawford, 1994; van Hoven & Day, 2002). It was noted by Lenat and Crawford (1994) that agricultural sites also showed increases in Oligochaetes in comparison to reference conditions.

Urban land use sites C3, C4 and CR made up the composition of **Group 3**. The make up of the urban sites at low flow (Table 4.7) was very similar to high flow (Table 4.6) indicating that the urban sites suffer from the same impacts between seasons. There was a difference in the contribution of the Chironomidae family to the urban land use sites at low flow. Chironomids show a high degree of tolerance to pollution contaminants, particularly organic pollution (Lenat, 1983). This organic input would be explained by the constant input from sewage works to the Crocodile River from the urban areas. From the presence of the dominant macroinvertebrate families at high and low flow, these impacts were speculated as being increased FPOM in deposited sediments which is due to sewage inputs (Walsh, 2000; de Moor & Scott, 2003) and low dissolved oxygen content (Oosthuizen & Sidall, 2002) which are impacts from run-off and sewage inputs.

High and Low flow

Figure 4.6a and 4.6b indicated the role of seasonality in the grouping of sites, showing the separation of sites based primarily on flow. The exception was **Group 1**, which contained the reference site MR at both flows due to the unique contribution of the Tricorythidae to the reference site (Table 4.8). The remainder of the Groups were separated in much the same fashion as they were in Figure 4.4 and 4.5 for the separate flow scenarios. The influence of temperature on the ecology of aquatic insects has been documented by Quinn, Steele, Hickey and Vickers (1994). Fluctuations in stream temperature create thermal conditions that are conducive to changes in macroinvertebrate diversity according to their optimal water temperature; and create potential for niche segregation amongst a range of macroinvertebrate taxa (Ward & Stanford, 1982 cited in Sponseller *et al.*, 2001).

Due to the fact that there were no significant differences (as indicated by ANOSIM), and the family make up between test sites overlapped to a large degree (as shown in SIMPER analysis) it can be said that homogeneity exists between test sites. As noted in studies of macroinvertebrates at sites with different land uses, often, there are too many confounding impacts at catchment scale to significantly separate these communities (Black *et al.*, 2004). Inclusion of historical macroinvertebrate and land use data would also increase the significance of results (Berenzen *et al.*, 2005). Lenat and Resh (2001) state that comparison of information can be complicated by a lack of knowledge at the species level, thus increased taxonomic resolution increases the confidence and significance of results.

4.4.2.3 Macroinvertebrate FFGs

As outlined in Chapter 1, the aim of this section will be to compare the FFG traits of macroinvertebrates taken from agriculturally impacted sites, relative reference and urban sites. The river continuum concept suggests that macroinvertebrate communities should be predictable along a longitudinal gradient of a stream (Vannote *et al*., 1980), thus the abundances of FFGs should change in response to a shift in primary production downstream; and to seasonal changes in particulate organic matter input in autumn (Cummins & Klug, 1979). Referring to Figure 4.8a and 4.8b, one can see that analysis of FFGs does show temporal separation, caused by a change in abundances of FFGs from high to low flow.

Referring to FFGs at high flow, similarity between sites is extremely high and no land use patterns between sites were evident. Table 4.9 indicates that the reference site on the Magalies River (MR) at high flow was placed in **Group 5**, and at low flow was placed in **Group 6**. Site MR at high flow showed a very high contribution of COGs (41.99%) and PREs (18.24%) to the make up at this site, while at low flow the contribution of COGs was 65.65%. Immediately this information indicates an impact related to primary production at MR. One would expect to see the shredders making up the highest proportion of FFGs in headwater streams due to falling leaves from the dense riparian canopy and the Coarse Particulate Organic Matter (CPOM) being the main sources of food in this reach of river (Vannote *et al.,* 1980; Davies & Day, 1998; Compin & Ce´re´ghino, 2007). Collectors, such as COGs feed on sedimented FPOM, and patterns observed by Delong and Brusven (1998), showed that grazers and gatherers were common and shredders were rare in impacted streams. The suggestion by FFG make up of increased FPOM at this site may be related to the aquaculture farm upstream that contributes to an increase in FPOM due to inputs of fish waste.

At high flow, the PREs make up a significant proportion of Group 5. In headwater streams, the common school of thought dictates that one should not see such a high percentage of predators indicating that the system is allochthonous and food sources for predators are scarce (Vannote *et al*., 1980; Davies & Day, 1998). The contrary was discovered by Compin and Ce´re´ghino (2007) who found that the presence of predators did not show clear patterns along the longitudinal span of river systems and that predators were clustered along rivers, presumably where food was available to them.

Group1, Group 2 and **Group 7** (which consisted mainly of agricultural sites at high and low flow and urban site C3 at both flows) were characterised by high contributions of COFs overall (Figure 4.8a, Figure 4.8b and Table 4.9). Filterers are dependant on the presence of suspended FPOM and CPOM in the flowing water current (Moog, 1995 in Schmidt-Kloiber, Graf, Lorenz & Moog, 2006). Delong and Brusven (1998) found that there was a low variability from site to site because of the abundance of filterers and gatherers throughout the length of rivers in agricultural areas. Riparian vegetation removal and increased inputs of nutrients can result in increased primary productivity in agriculturally impacted rivers, thus contributing to a change in the sources and types of organic matter (Delong & Brusven, 1998). Filterers are dependant on Ultra-Fine Particulate Organic Matter, or UPOM (Cummins & Klug, 1979) which increases longitudinally (Vannote *et al.*, 1980). The agricultural sites in this study are situated further down the course of the Crocodile River and thus the processing of particulate organic matter has resulted in organic material that is finer and can be more easily suspended in the water column for access by COFs. The increase of the collector gatherer FFG is has been shown to be proportional to a decrease in river quality (Hering, Moog, Sandin & Verdonschot*.,* 2004).

COGs and SCs were predominant FFGs for **Group 4** which was made up of urban sites CR, C4 and agricultural site C2 at high flow (Figure 4.8a and 4.8b and Table 4.9). The COG and SCs were also principal contributors to **Group 3** which consisted of urban sites at low flow. This may also be related to the longitudinal placement of these sites in comparison to agricultural sites, but also indicates a change in food availability and habitat. Collector gatherers are dependant upon sedimented FPOM (as opposed to UPOM by COFs) as a source of food (Moog, 1995 cited in Schmidt-Kloibe *et al.,* 2006). The change of contributing FFGs from COFs at agricultural sites to COGs at urban site indicates a change in the stability of the substrate at urban sites, which allows gatherers to burrow (Doviack & Perry, 2002). The SC feeding type contributing to these groups showed a response to ecological variables, where a decrease in SC abundance along a gradient of 45% to 5% indicated a decrease in river integrity (Hering *et al.,* 2004).

4.4.2.4 Macroinvertebrate Indices and Community Integrity

The results from the application of the SASS5 and MIRAI macroinvertebrate indices are shown in Table 4.10, Table 4.11, Figure 4.9 and Figure 4.10. Macroinvertebrate abundance data for high and low flow scenarios are shown in Appendix C.

Initially it was assumed that water quality impacts would fall into groups based on the type of adjacent land use (relative reference, urban or agricultural related) and pollution sources associated with each site (type of pollution; and point or non-point). Referring to high flow data, SASS5 data, MIRAI scores and water quality metric group scores (from the MIRAI index in Table 4.11) of the relative reference site had the highest scores overall. This site was located in a headwater zone of the Magalies River where there was little impact before the situation of the site. The water quality metric group of MIRAI indicated that water quality at urban sites was in a more degraded state than at agricultural sites. Agricultural sites tended to show a slightly higher integrity than urban sites overall, indicating that they were largely impacted but showed downstream recovery from the intense impacts of urban land use. Urban sites were in a seriously modified ecological state according to macroinvertebrate assemblages and were mostly situated downstream of a point source impact (i.e. sewage treatment works). This pattern in data agrees with that found by Dallas (1995) in which urban and agricultural sites were generally separated on the basis of their proximity to point sources.

The major contributing factors indicating change from historical conditions were the loss of expected sensitive macroinvertebrate taxa at the test sites. These taxa include the Heptageniidae, Perlidae and Psephenidae families. These families are indicators of excellent water quality (Gerber & Gabriel, 2002) and their absence from sites indicates a decline in water quality. In a study on land cover optima by Black *et al.* (2004), it was discovered that Heptageniidae were positively associated with the percentage natural land cover, and designated as indicator taxa. Another family that indicated change between relative reference and test sites was the Tricorythidae family, as their sensitivity is moderate (bordering on high) with a SASS5 sensitivity of 9 out of 15 (Gerber & Gabriel, 2002). Relative reference site MR did show some modification that was mostly attributed to by seasonality impacts of the upper reaches of the Magalies River due to over abstraction of ground water from Maloney's eye. This site would historically have had very constant flows all year due to the groundwater source. The loss of the Perlidae family from MR indicates a serious and definite change in flow and water quality from reference, as Perlids are known to be highly sensitive to changes in flow and pollution and show preference for fast flowing habitats (Gerber & Gabriel, 2002; Thirion, 2007).

The outcome of the SASS5 index does not show definite cause-effect relationships between macroinvertebrate communities and their ambient environment. In contrast, the MIRAI provides "habitat-based cause-and-effect" information to assist in interpreting and understanding the variation of aquatic macroinvertebrate communities from natural conditions (Thirion, 2007).

Referring specifically to MIRAI metric group data (Table 4.11 and Figure 4.10), the comparison of present and historical reference data for macroinvertebrate families showed a modification in flow at urban sites CR and C4 before the Hartbeespoort Dam. This contributed to an overall change in the flow dependant macroinvertebrate species. These sites are associated with high intensity urban land use, and thus suffer changes in flow due to higher urban run off (Walsh, 2000). A change in flow will lead to loss of certain flow sensitive species and increases in others due to change in the in wetted perimeter, hydraulics and habitat availability (Thirion, 2007). At CR, the macroinvertebrate communities (according to the change from historical data) indicated that habitat modification was a serious problem as well. This is thought to be related to increased flows and flash floods from the increase in flows indicated above. This would lead to substrate instability and a decrease in viable habitat for macroinvertebrates.

4.5 Summary and Conclusion

As in Chapter 3, Site CR is again not included in the summary as results obtained from macroinvertebrate analysis are ambiguous, and it is impossible to distinguish between the factors influencing the macroinvertebrate make up at this site.

Habitat quality impacts particular to each land use were noted in relation to macroinvertebrate habitat. The comparison of macroinvertebrate communities, FFGs and riparian vegetation showed that differences between sites with different land uses were not significant. Nonetheless, some differences in refined data were noted for the varying land uses. Considering the macroinvertebrate community make up of the relative reference site MR, which was comprised of macroinvertebrate families that were more sensitive and showed preferences for higher water quality, community structures of macroinvertebrates were modified and showed change in relation to MR, and thus land use. It was also found that the relative reference site was missing important indicator species such as the Perlidae family, and showed decreased abundances of Psephenidae and Heptageniidae families. This indicates that there were water quality impacts shown at this level of trophism, even at sites were adjacent land use is largely natural.

An increased diversity in air breathing macroinvertebrates was shown at sites with agricultural practices at high flow, where urban sites were differentiated from agricultural sites due to the presence of the Hydropsychidae and Hirudinea families. At low flow macroinvertebrate families making up communities overlapped between land uses. One difference noted at low flow was that the contribution of the Chironomidae was higher at urban sites in comparison to agricultural sites, indicating organic water pollution.

The MIRAI showed that agriculture sites were in a D category overall, indicating that water quality impacts at these sites were largely modified. Urban sites were placed in an overall E category indicating that sites were seriously modified and in a worse state than agricultural sites. The relative reference site was classed in a C category and showed a moderately modified ecological state.

Macroinvertebrates FFGs indicated that a change in the input of UPOM at agricultural sites, and a change in the presence of FPOM at urban sites were responsible for the shift in the FFG dominance.

The results of this study suggest that land use caused changes in the structure of the benthic community in the Crocodile and Magalies rivers, although these changes could not be significantly differentiated between the different land uses. This study suggests that a multi-faceted approach to community analysis may be a useful approach to assessment of the effects of specific land use on the ecological condition of a stream.

Chapter 5 : The Relationship between Riparian Vegetation and Macroinvertebrate Community Structure.

5.1 Introduction

5.1.1 Riparian Vegetation and Macroinvertebrate Integrity

Generally, riparian landscapes are degraded by urban and agricultural land use practices which cause changes in ecosystem function (Poff, 1997). These activities, particularly agricultural activities can result in bank erosion and increased sedimentation which may lead to loss of habitat and subsequent loss of species (Stevens & Cummins, 1999). In addition, destruction of riparian zones in agricultural areas involves the additions of fertilizers and pesticides which cause changes in the soil that may lead to it becoming even more erodable (Correll, Jordan & Weller, 1992).

The influence of riparian vegetation integrity on macroinvertebrate communities is important for understanding the interactions of aquatic ecosystems with their adjacent land uses, as the riparian ecotone has many functions in terms of the hydrology, maintenance of water quality, nutrient input and habitat for instream communities (Gregory, Swanson, McKee & Cummins, 1991; Rios & Bailey, 2006). The scale and character of the relationship between riparian vegetation and benthic macroinvertebrates was investigated by Rios and Bailey (2006). This study showed that taxon richness and Simpson's diversity of macroinvertebrate communities where positively correlated to increased tree cover in the riparian zone of study sites. Results of a study by Aguiar, Ferrreira and Pinto (2002) corroborated this evidence by showing that total macroinvertebrate variation was explained solely by riparian variables 18% of the time. In this study, riparian features had greater influence than other environmental characteristics on the composition of macroinvertebrate assemblages because riparian features are closely related to food types.

The objectives of this chapter will be to ascertain whether riparian integrity plays a role in the integrity of macroinvertebrate assemblages and FFGs.

5.2 Materials and Methods

5.2.1 Field Identification Procedures

The Riparian Vegetation Response Assessment Index (VEGRAI) as developed by Kleynhans *et al.*, (2007), is used as a tool to assess riparian zone health and functionality in relation to in-stream integrity. Site selection and reconstruction of the reference condition was undertaken by making use of topographical maps and aerial photos (Appendix C). Once reference conditions had been reconstructed, a site visit was undertaken (Kleynhans *et al.*, 2007) and ground truthing was done to ascertain the extent of the site upstream and downstream. The marginal and non-marginal zones were also delineated in the walk about by noting changes in flow, geomorphology, elevation, vegetation structure and species. Species were identified using relevant South African tree and aquatic plant field guides (Van Wyk & Van Wyk, 1997; Thomas & Grant, 1998; Bromilow, 2001; Coates-Palgrave, 2002; Gerber, Cilliers, van Ginkel & Glen, 2004)

5.2.2 Riparian Index Calculations and Data Analysis

The VEGRAI is a rule-based spreadsheet model of the EcoStatus suite that makes use of a series of metrics and metric groups to describe the status of the riparian vegetation in the current and reference state. These two states are ultimately compared to elucidate a change from reference condition due to an impact regime.

Once the reference conditions were set and sites selected, the marginal and non-marginal metric groups were assessed in terms of their “woody” and “non-woody” vegetation components. For VEGRAI level 3, the marginal and non-marginal zones were assessed by rating cover, abundance and species composition metrics in response to their change from the reference condition.

The woody and non-woody vegetation components for each zone were then ranked and assigned a relative weight in order of their importance within the respective zone. This ranking and weighting exercise generates a percentage change from reference condition for each riparian zone. The overall riparian EC was generated by again ranking and weighting each zone relative to its importance at the particular study site. This change in each vegetation component for each zone is then integrated to generate an overall EC and overall percentage change from the reference condition (Table 4.3).

Community-based statistical analyses were undertaken as described in Section 4.2.5 (Chapter 4).

5.2.3 Statistical Analyses

For the purpose of visually illustrating the relationship between macroinvertebrate community data, FFGs and riparian vegetation integrity, respective data were subjected to Redundancy Analysis (RDA) using Canoco version 4.5. Redundancy Analysis was carried out on log transformed data and the significance of RDA axes was tested using unrestricted Monte Carlo permutation testing (499 permutations, ρ= 0.05). Redundancy Analysis is an ordination technique that uses best fit values from multiple linear regression between variables, and includes a second axis (in this case riparian integrity) (Ter Braak & Smilauer, 2002).

5.3 Results

5.3.1 Riparian Vegetation Community Structure

The results of the VEGRAI (Table 5.1) showed that riparian vegetation at sites ranged from an A category (natural) to a D category (largely modified) (Table 4.3). Marginal zone intactness was good overall with sites ranging from 77.6% to 96.7% integrity, while non-marginal intactness showed overall lower integrities ranging from 46.2% to 87.7%. Selected relative reference sites on the Crocodile and Magalies Rivers showed the highest ECs, where CR showed a 78.6% similarity to extrapolated reference conditions and MR showed a 92.2% similarity. Dominant riparian species at each site are indicated in Appendix C

Table 5.1: Results obtained from the application of the VEGRAI index indicating integrity scores for marginal, non-marginal and total intactness, and ecological categories for riparian vegetation at sites on the Crocodile (C) and Magalies (M) rivers.

		CR	C4	C3	C2	C1	MR	M2
VEGRAI	Marginal Zone Intactness (%)	80	93.6	77.6	90.3	87.6	96.7	81.2
	Non-Marginal Zone Intactness (%)	77.2	71.3	51.4	76.4	50.8	87.7	46.2
	Total (%)	**78.6**	**77**	**61.3**	**83.4**	**69.2**	**92.2**	**54.3**
	Category	**B/C**	**B/C**	**C/D**	**B**	**C**	**A**	**D**

Grouping of sites based on riparian vegetation using CLUSTER analysis and NMDS ordination is shown in Figure 5.1a and 5.1b. The NMDS ordination separated relative reference sites MR (Group 5) and CR (Group 4) from test sites. Urban sites were very similar in terms of riparian vegetation community structure (Group 3), were agricultural sites C2 and M2 grouped at 45% similarity but excluded C1 (Group 1).

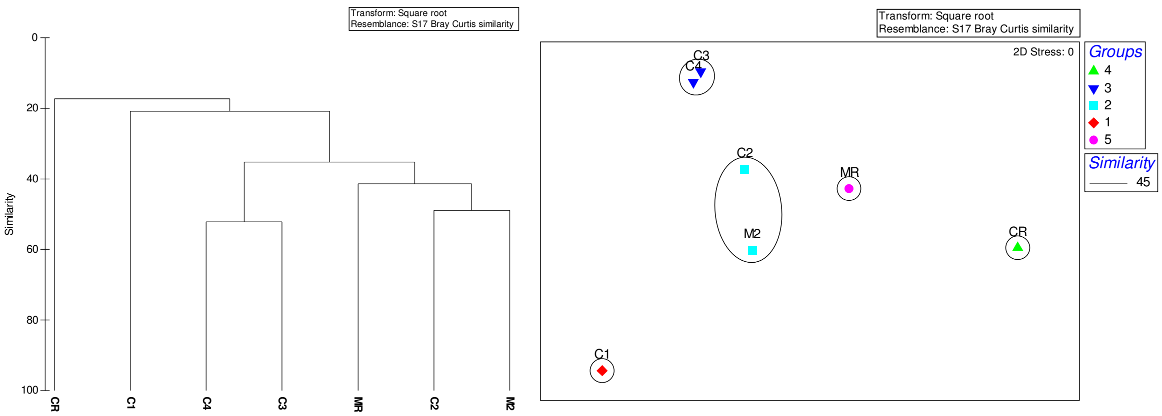


Figure 5.1: (a) Bray Curtis similarity matrix based on hierarchical cluster analysis indicating the similarity between samples in relation to the riparian vegetation community (represented by dominant species found in the riparian zone) at each site; and (b) 2 Dimensional NMDS for riparian community data indicating percentage similarity and groups.

5.3.2 Riparian Vegetation and Macroinvertebrate Community Structure.

Figure 5.2 shows the (dis)similarity between macroinvertebrate communities at sites on the Crocodile and Magalies Rivers in relation to riparian vegetation integrity. The RDA bi-plot describes 79.8% of the variation in data, where 51.5% is displayed on the first axis and 28.3% is displayed on the second axis. Families that ordinated to the lower left of the RDA graph showed positive correlations to marginal, non-marginal and overall riparian integrity. These families were mostly species with moderate/high or high water quality requirements based on SASS5 data (Gerber & Gabriel, 2002; Thirion 2007) that occurred at relative reference site MR.

5.3.3 Riparian Vegetation and FFGs

Figure 5.3 shows the (dis)similarity between FFGs at sites on the Crocodile and Magalies Rivers in relation to riparian vegetation integrity. The RDA bi-plot describes 67.2% of the variation in data, where 54.4% is displayed on the first axis and 12.8% is displayed on the second axis. There was a strong positive correlation between non-marginal riparian integrity and shredder FFGs (SHD and SHH), where overall VEGRAI scores were correlated with the increased presence of predators (PRS and PA).

Figure 5.2: Redundancy analysis ordination of riparian components at each site indicating macroinvertebrate family affinities to particular riparian components based on macroinvertebrate community structure data for high and low flow scenarios. [Marginal – Marginal Vegetation; Non-Marg – Non-Marginal Vegetation; VEGRAI – Riparian Vegetation Response Assessment Index].

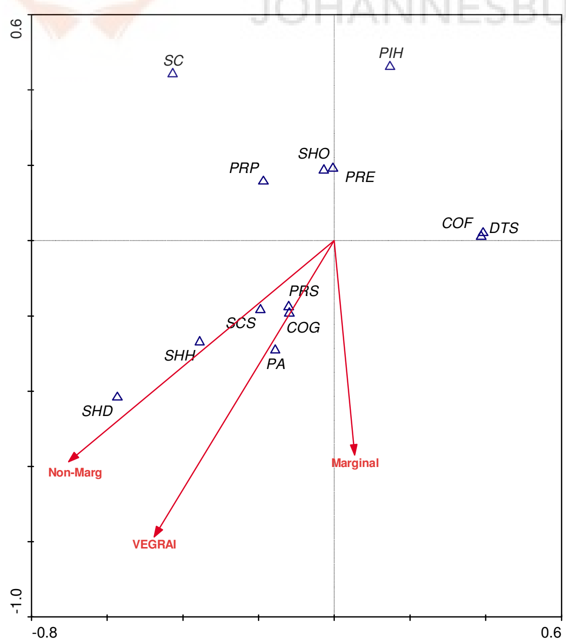


Figure 5.3: Redundancy analysis ordination of riparian components at each site indicating FFGs in relation to particular riparian integrity components. [Marginal – Marginal Vegetation; Non-Marg – Non-Marginal Vegetation; VEGRAI – Riparian Vegetation Response Assessment Index].

5.4 Discussion

5.4.1 Riparian Vegetation Integrity

The VEGRAI model shows the response of different riparian vegetation components to impacts regimes of varying land uses (Table 5.1). Sites initially selected as relative reference sites (MR and CR) showed the highest riparian integrity due to good marginal and non-marginal zone intactness. Figure 5.1a and 5.1b shows the separation of these sites into **Group 4** (CR) and **Group 5** (MR). This separation is based on riparian species composition at sites due to differing vegetation units and Ecoregional types. The MR site falls within the Western Bankenveld Ecoregion and Gold Reef Mountain Bushveld bordering on Moot Plain Bushveld vegetation unit (Savanna Biome) (Mucina & Rutherford, 2006; Kleynhans *et al.,* 2007). In contrast the CR falls within the Highveld Ecoregion, occurring in the Egoli Granite Grassland vegetation unit (Mesic Highveld Grassland Biome) (Mucina & Rutherford, 2006; Kleynhans *et al.,* 2007). These two vegetation units have relatively different species compositions as can be seen in Appendix C.

Riparian integrity at MR was excellent because of the natural surrounding land use. This is in agreement with the findings of Amis *et al.* (2007) who found that natural land use was a good predictor of riparian integrity, having a positive correlation at a number of scales. Site CR showed non-marginal zone intactness of 77.2%. The reason for the change from reference was due to change in the natural burning regime and the encroachment of a recreational area into the riparian zone. It is believed that the abundance of the woody species *Leucosidea sericea* in the non-marginal zone would be lower under natural conditions due to the occurrence of natural fires in the area. This would allow for recruitment of other riparian species, creating a higher riparian diversity. The change in the marginal zone at site CR was due to altered flows from the upstream urban area causing scouring and some removal of vegetation.

Agricultural sites were placed in **Group 1** (C1) and **Group 2** (C2 and M2). Group 1 was separated from the other agricultural sites in Group 2 due to species composition which is related to the variation of vegetation units (Mucina & Rutherford, 2006). The greatest concern in terms of agricultural impacts was the full/partial removal of the riparian zone up until the non-marginal zone at site C1 and C2. Streams are usually thermally altered due to removal of riparian vegetation and receive lower amount of leaf litter inputs (Allan, 2004). An important alteration in agricultural rivers due to riparian removal is the decline in woody debris (Johnson, Breneman & Richards, 2003 cited in Allan, 2004). Wood substrate creates habitat for fish and invertebrates and its absence can cause a decrease in local diversity within agricultural catchments (Johnson *et al.*, 2003 cited in Allan, 2004).

Urban sites were grouped in **Group 3** (C3 and C4) in terms of vegetation composition (Figure 5.1a and 5.1b). Both of these sites occur in Gold Reef Mountain Bushveld in the Savanna Biome (Mucina & Rutherford, 2006). Partial clearing of the non-marginal riparian zone occurred at these urban sites and is represented by a change from reference conditions (Table 5.1). Riparian impacts due to urban land use normally stems from vegetation removal. Impacts from removal in urban areas involves the entry of pollutants to rivers via runoff, unpredictable hydrology due to increased impervious surface area which impacts the marginal zone, increased water temperatures and bank destabilization (Paul & Meyer, 2001). Urban land use usually contributes a low percentage of total catchment area, however, it has a disproportionately large influence at a local and catchment scale due to impacts such as the ones mentioned above (Paul & Meyer, 2001).

The primary impacts related to riparian modification between urban and agricultural land use are very similar in the present study, and mostly stem from removal. Thus, there was no significant statistical difference noted between riparian zones of sites. Secondary impacts from removal that are related to land use are increased flows in urban rivers, whereas increased inorganic sediment input would affect agricultural rivers. Pollutants entering rivers, due to a decrease in filtering potential of the riparian zone, are also land use specific (Allan, 2004). This would infer that heavy metals and PCB pollutants would enter streams due to removal of riparian vegetation in urban areas, whereas the route of entry of biocides would be facilitated in agricultural rivers.

5.4.2 Riparian Vegetation, Macroinvertebrate Community Structure and FFGs

Figure 5.2 indicates that riparian vegetation integrity was associated with species occurring at site MR, which is a relative reference site that showed high species richness and diversity values (Figure 4.3), the highest SASS and APST scores (Table 4.10 and Figure 4.9) and the highest macroinvertebrate community integrity according to MIRAI (Table 4.11 and Figure 4.10). Taxon richness, EPT and diversity of macroinvertebrate communities all increased with increased woody vegetation cover in the riparian zone, and was dependant upon local land use features in a study by Rios & Bailey (2006). The presence of sensitive Ephemeroptera (Leptophlebiidae and Tricorythidae) and Trichoptera (Philopotamidae) are associated with natural land use at MR, and are thus linked with increased riparian integrity. The Plecopterans are, however, absent from this site. According to reference macroinvertebrate data for this Ecoregion, one should see the

Perlidae family in at least an A abundance at a 30% frequency of occurrence (Appendix C). This indicates that water quality impacts are present due to the loss of this important indicator taxa at the MR site. Tolerant taxa have been known to commonly occur at agricultural sites, whereas more diverse communities with sensitive families are related to patches of natural land (Rios & Bailey, 2006).

Shade, which is provided by intact, over-hanging riparian vegetation, is necessary for natural thermal regimes and improves stream ecosystem health (Bunn, Davies & Mosisch, 1999). Supporting this finding is the study by Vondracek, Blann, Nerbonne, Mumford, Nerbonne, Sovell & Zimmerman (2005) who showed that macroinvertebrate assemblages were affected by land use and the degree and nature of riparian vegetation and agricultural practices. A higher degree of shading was observed at site MR when compared to the other study sites. It must be noted that the reach that MR is situated in may contribute to differences in the riparian zone in comparison to other sites.

Figure 5.3 shows the contribution of riparian vegetation metric groups to FFG structure at sites on the Crocodile and Magalies Rivers. Comparing the length of the vectors in Figure 5.2 and Figure 5.3, the varying metrics were more strongly associated with FFGs than individual macroinvertebrate families. This indicates a stronger relationship between FFGs and riparian vegetation overall. The amount and type of particulate organic matter at sites is related primarily to input from primary riparian producers, and therefore the correlation between non-marginal riparian integrity and shredder FFGs (SHD and SHH) was expected. The correlation of overall VEGRAI scores with the presence of predators (PRS and PA) was interesting, indicating that overall riparian health is necessary for optimal trophic functioning, providing a sufficient number of primary consumers for secondary consumers to predate upon.

5.5 Summary and Conclusion

A difference in riparian integrity was noted between relative reference and test sites, but could not be easily distinguished between test sites with different land uses. Increasing riparian integrity was related to more intact macroinvertebrate communities at MR, and therefore riparian integrity is an important factor for macroinvertebrate integrity in this study. The primary riparian modifications between urban and agricultural land use stemmed from removal, however, secondary impacts differed. These impacts were more predictive of macroinvertebrate FFG structure than actual community structure. This indicates that riparian integrity and comparison with biological traits such as FFGs were most useful in showing impacts due to organic matter inputs.

Chapter 6 : Conclusions and Recommendations

Water conservation has become a major priority in South Africa because of the semi-arid category in which the country falls and ever increasing demands on water supplies. Because of the implications in terms of water shortage and quality, a number of strategies, monitoring programmes, concepts and initiatives have been formulated and implemented by the DWAF over the years. Of these national initiatives the adoption of the Bill of Rights and the National Water Act have paved the way in providing a holistic approach to water management through equitability and sustainability of water resources. An important concept for management comes in the form of the "Ecological Reserve" which relates to the quality, quantity and amount of flow that aquatic ecosystems need for long term sustainability of water resources. This concept acknowledges the importance of the role that biotic components play in healthy ecosystem functioning. It is thus important that a diverse approach be taken in aquatic ecosystem integrity assessments to provide data that not only generates management classes, but also provides more detailed information which allows for high confidence decisions to be made.

6.1 Can Changes in Aquatic Community Structure be noted in Relation to Land use?

The aim of the present study was firstly to elucidate whether the community structure of macroinvertebrates and diatoms adjacent to agricultural land differed taxonomically to communities associated with urban and natural land use. Referring to community structure and taxonomic make up; initially, changes in water quality per land use were noted, and differences in diatom taxonomic structure (primary producers) was a subsequent response to the specific changes in water quality (Figure 6.1). It appears that urban land use with related nutrient impacts affected the diatom primary producers less severely than salt and sediment impacts from agricultural practices. In contrast, the macroinvertebrate community structure (primary and secondary consumers) responded more severely to habitat and water quality changes due to urban land use, as opposed to impacts from agricultural land use (Figure 6.2).

A subsequent aim was to show whether indicator species or families were present in the above mentioned communities that were specific to each adjacent land use. Referring to Figure 6.1 and Figure 6.2 indicator species and families were present and it was possible to separate relative reference land use from urban and agricultural land use based on these indicators. It was also possible to separate the relative reference land use from other test sites (urban and agricultural combined) by applying the different diatom, macroinvertebrate and riparian vegetation indices (Figure 6.1, Figure 6.2 and Figure 6.3). However, resolution between the test sites (urban vs. agricultural) was more vague with no more than half of an EC separating the two different land uses. Based on these results it appears that biotic indices mask the changes in the actual taxonomic components, erroneously suggesting that sites with different land uses are similar in terms of ecosystem integrity. When taxonomic structure is statistically refined, it tells a different story in the underlying taxonomic responses per different land use. It must be noted that integrity indices most certainly have their place in management of aquatic systems, but in research of this nature it appears to be more useful to utilise taxonomic make up and biological traits (in this case of FFGs) to show specific impacts, as these are factors can be compared across a relatively broad spatial scale

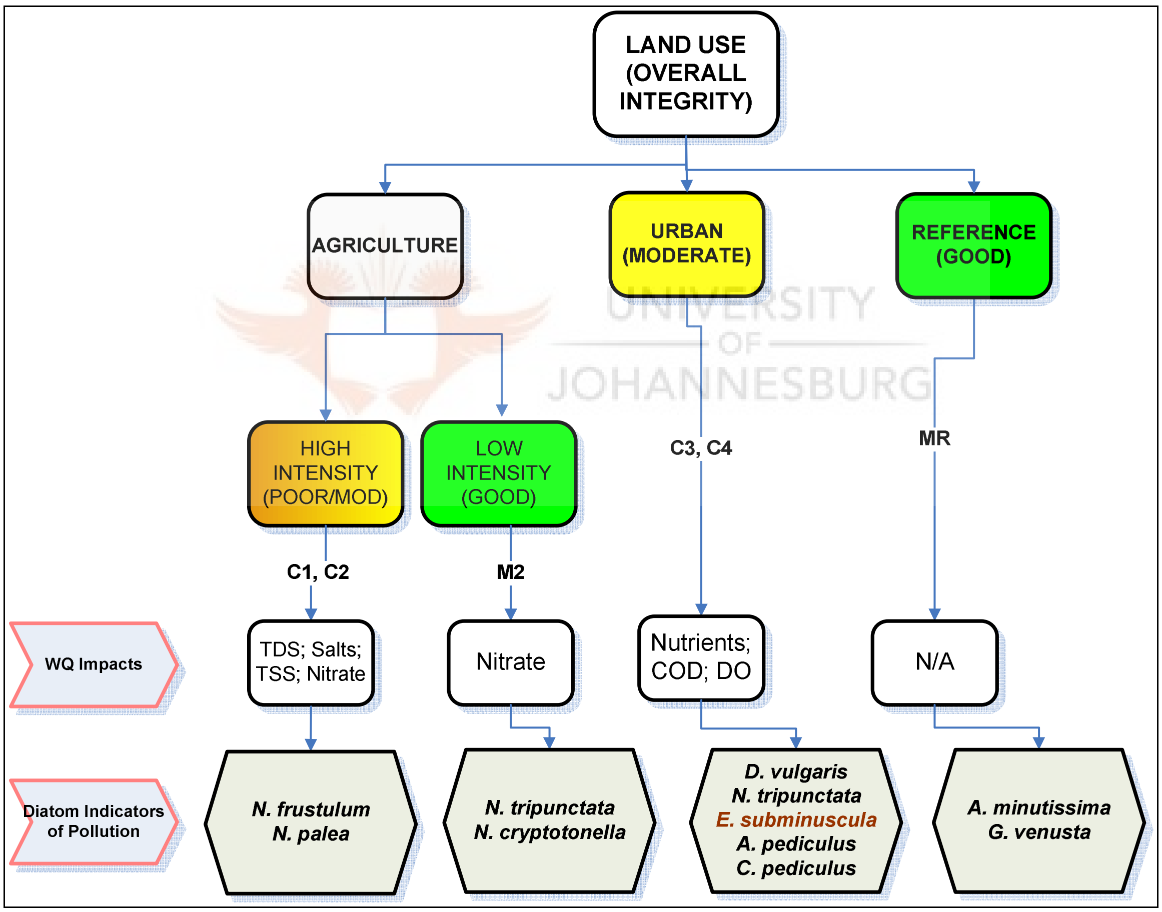


Figure 6.1: A summary of the results for water quality problems, water quality classes and indicator diatom species in relation to specific land use.

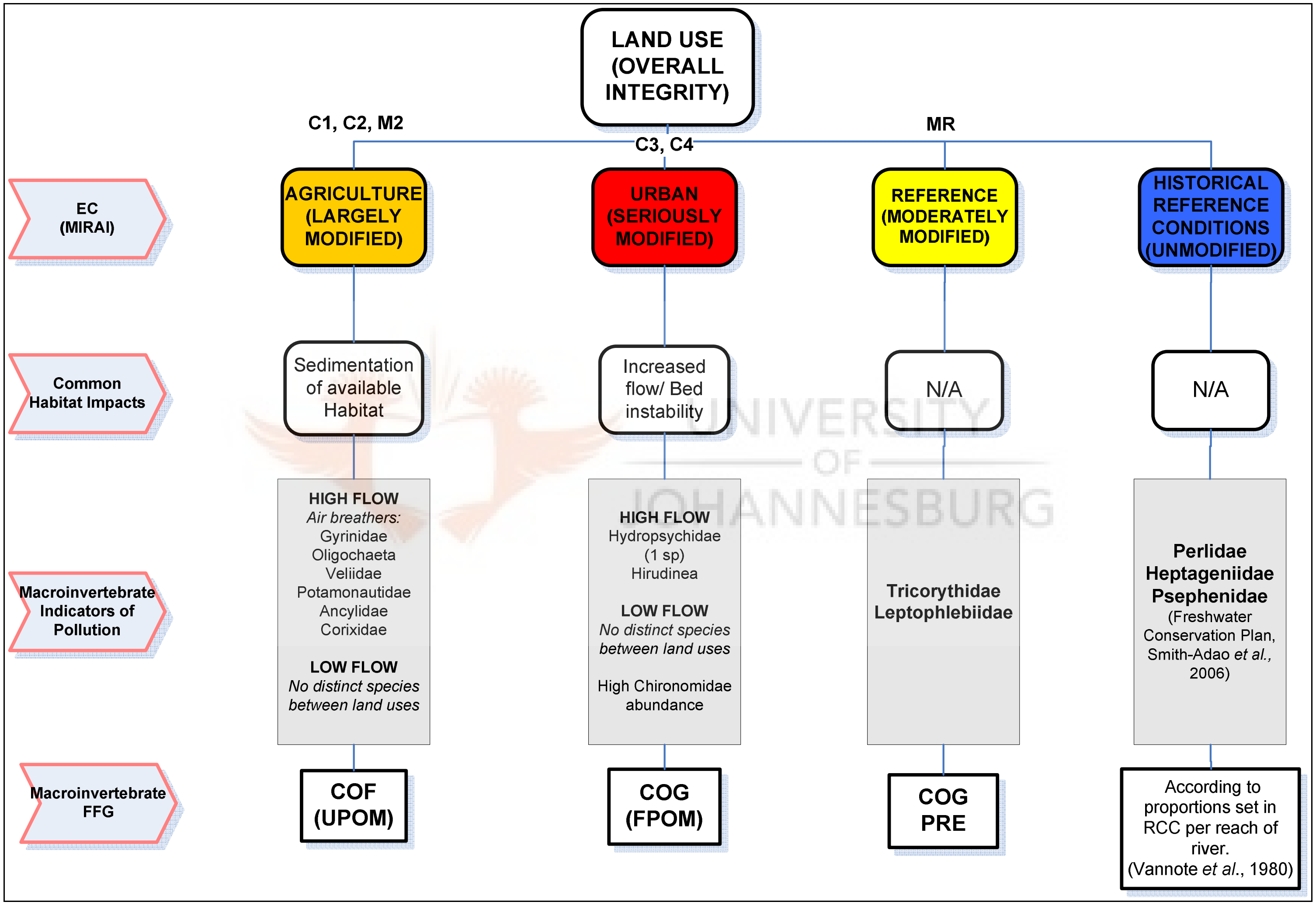


Figure 6.2: A summary of the results for habitat quality problems, macroinvertebrate ECs, indicator macroinvertebrate families, and macroinvertebrate FFGs in relation to specific land use

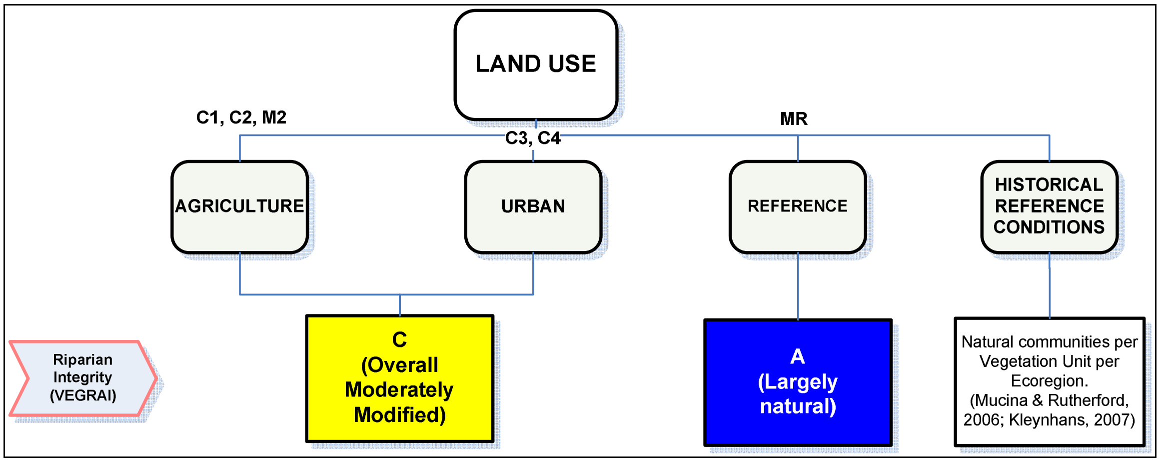


Figure 6.3: A summary of the results for riparian vegetation integrity relation to specific land uses.

The final aim was to ascertain whether aquatic macroinvertebrates at agriculturally impacted sites showed different FFG traits in comparison to sites with urban and natural land use activities. Referring to Figure 6.2, different FFG traits between urban and agricultural land uses were evident. The monitoring of biological traits such as FFGs proved useful in elucidating problems that were related to the input of organic particulate matter into these systems, and showed differences in the type of organic matter between land uses.

It can be concluded that sites could be separated according to land use based on community structure of diatoms and macroinvertebrates, and biological trait analysis of feeding groups (summarised in Figure 6.1, Figure 6.2 and Figure 6.3 above). It must be noted that diatom communities were more defined in their response to land use practices in comparison to macroinvertebrate communities. The original study hypotheses are thus supported by the results of this study and are accepted.

6.2 Research needs

The present study revealed several research needs and recommendations for further studies.

- Firstly, biotic index scores are indistinct and do not indicate much in the way of change in rivers due land use impacts, however taxonomic changes did occur between different land uses. Having mentioned this, there was a degree of overlap of macroinvertebrate families at different land uses. It is thus recommended that future studies include a higher taxonomic resolution of macroinvertebrate community structure, with identification of individuals to at least genus level in the case of more complex families such as the Chironomidae, and to species level in other families.

- It is also suggested that more biological traits of macroinvertebrates be included in future research to provide a clearer idea of what is going on in the aquatic ecosystem. Traits that are suggested are size, number of descendants per reproductive cycle, voltinism, life duration, body form, respiration and *r*K functionality.
- There is a gap in knowledge of species traits in South African macroinvertebrates, and a further recommendation would be to undertake an extensive literature review to identify biological traits to genus and species levels. This information may ultimately be used to create a biological trait database for South Africa and possibly formulate an index based on biological trait data. Finally, resolution of land use (the extent of land use) and stressors (pesticides and possibly heavy metal analysis) is imperative if further studies related to land use impacts are to be undertaken. Information on these drivers will allow the explanation of taxonomic response to be of a higher confidence.

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Appendix A - Site Maps and Aerial Photos

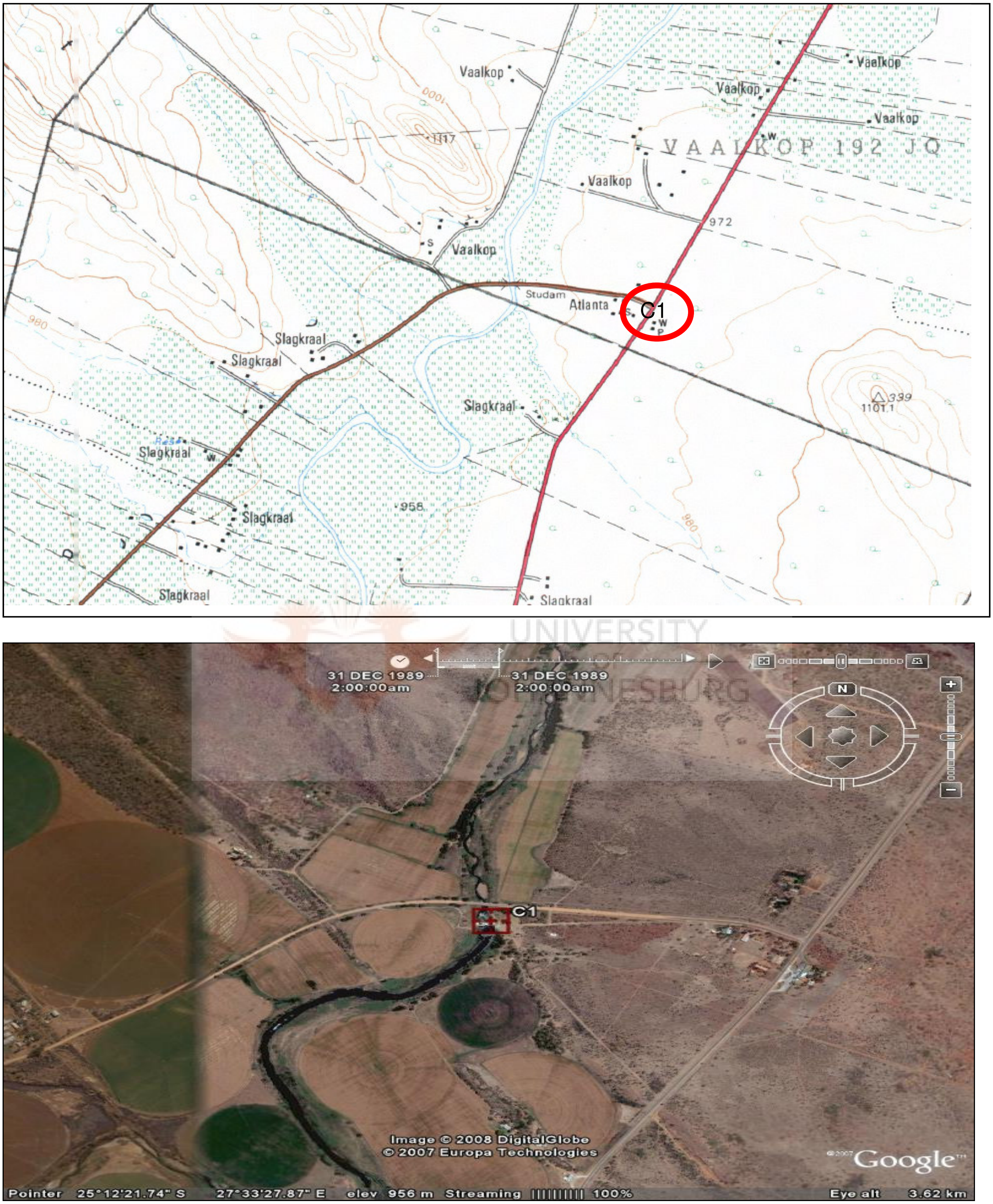

Figure A1: SA quarter degree topographical (QDS) 1:50,000 map 2527BA (Assen) and aerial photo for site C1 (Europe Technologies Image, *Google Earth* 2007).

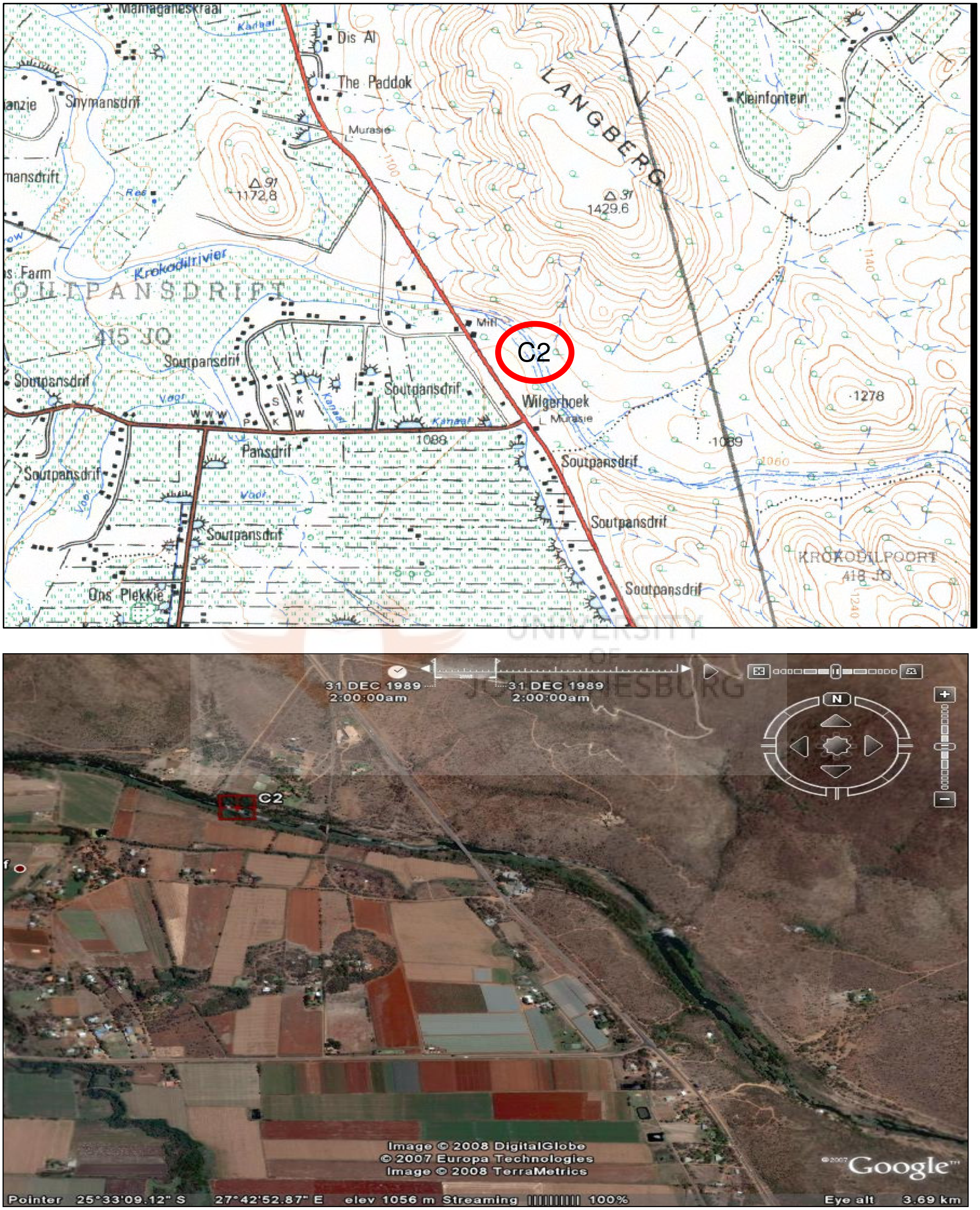

Figure A2: SA quarter degree topographical (QDS) 1:50,000 map 2527DA and aerial photo for site C2 (Europe Technologies Image, *Google Earth* 2007).

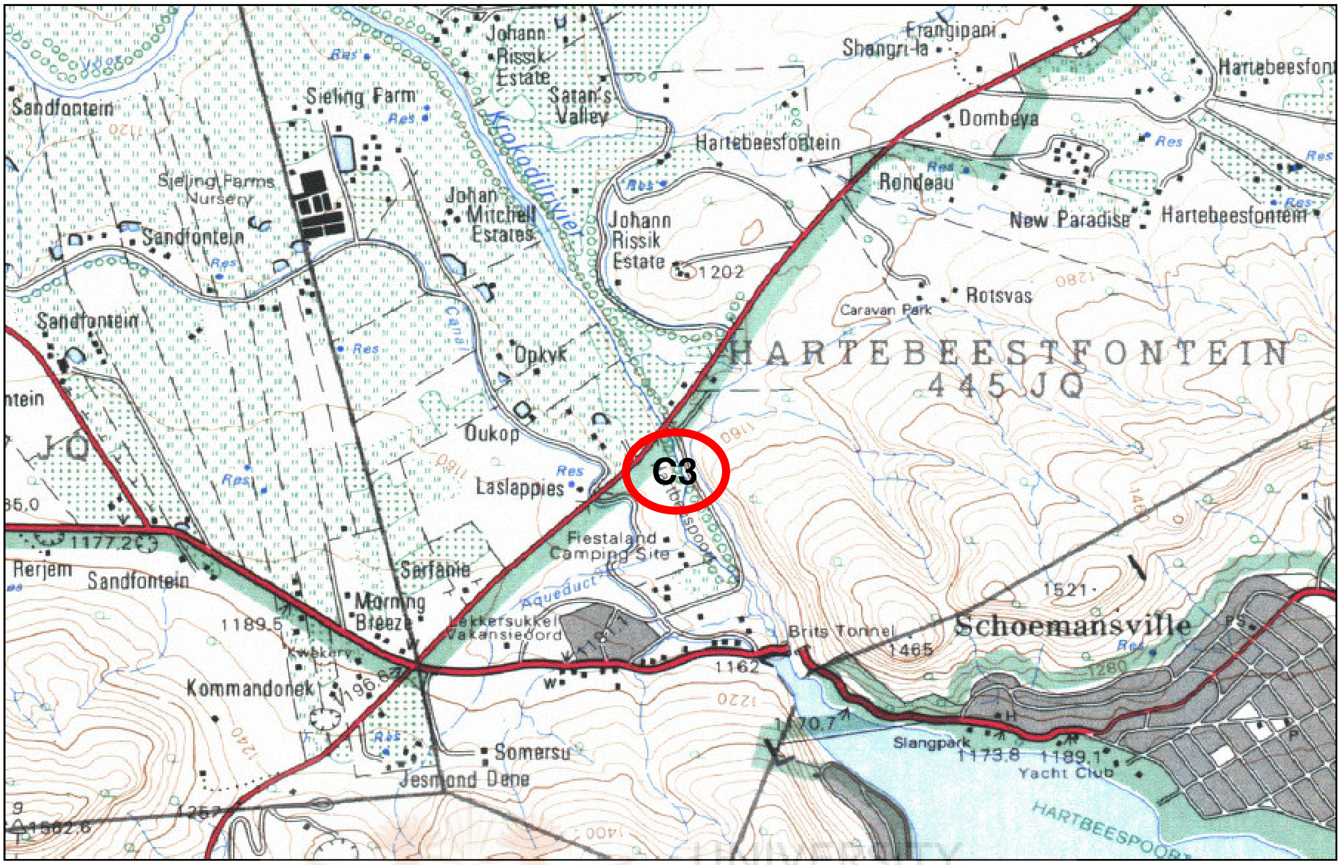

Figure A3: SA quarter degree topographical (QDS) 1:50,000 map 2527DB and aerial photo for site C3 (Europe Technologies Image, *Google Earth* 2007).

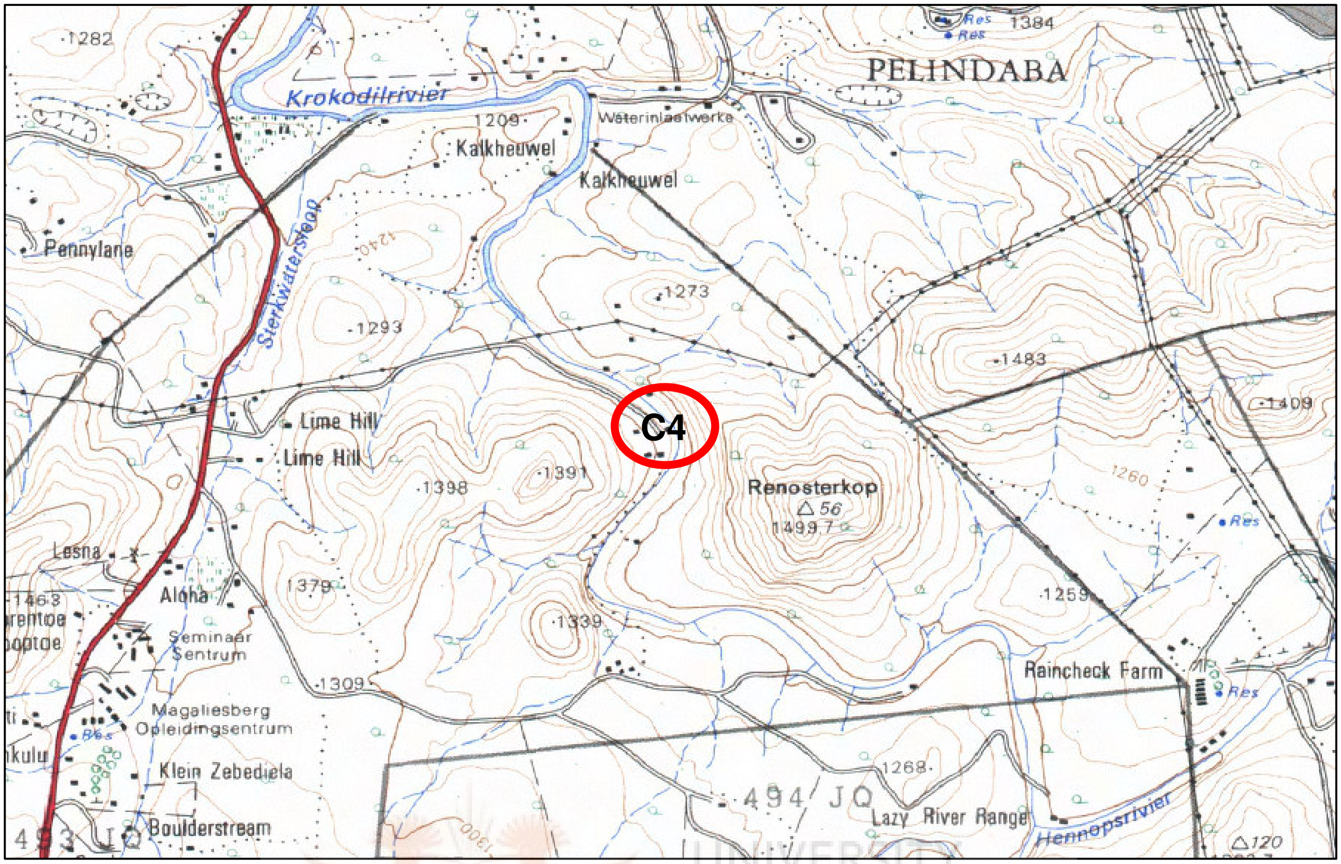

Figure A4: SA quarter degree topographical (QDS) 1:50,000 map 2527DD and aerial photo for site C4 (Europe Technologies Image, *Google Earth* 2007).

Figure A5: SA quarter degree topographical (QDS) 1:50,000 map 2627BB and aerial photo for site CR (Europe Technologies Image, *Google Earth* 2007).

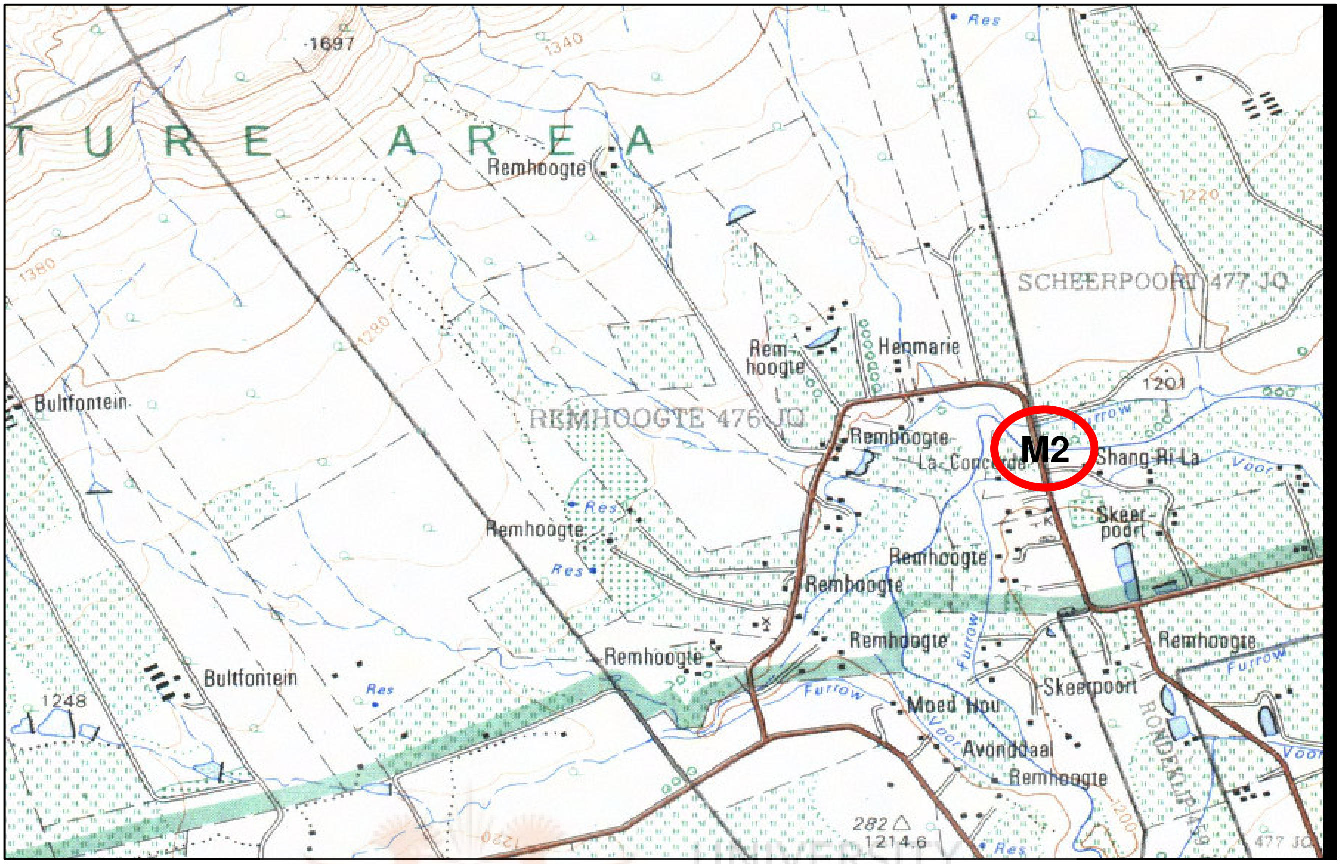

Figure A6: SA quarter degree topographical (QDS) 1:50,000 map 2527DC and aerial photo for site M2 (Europe Technologies Image, *Google Earth* 2007).

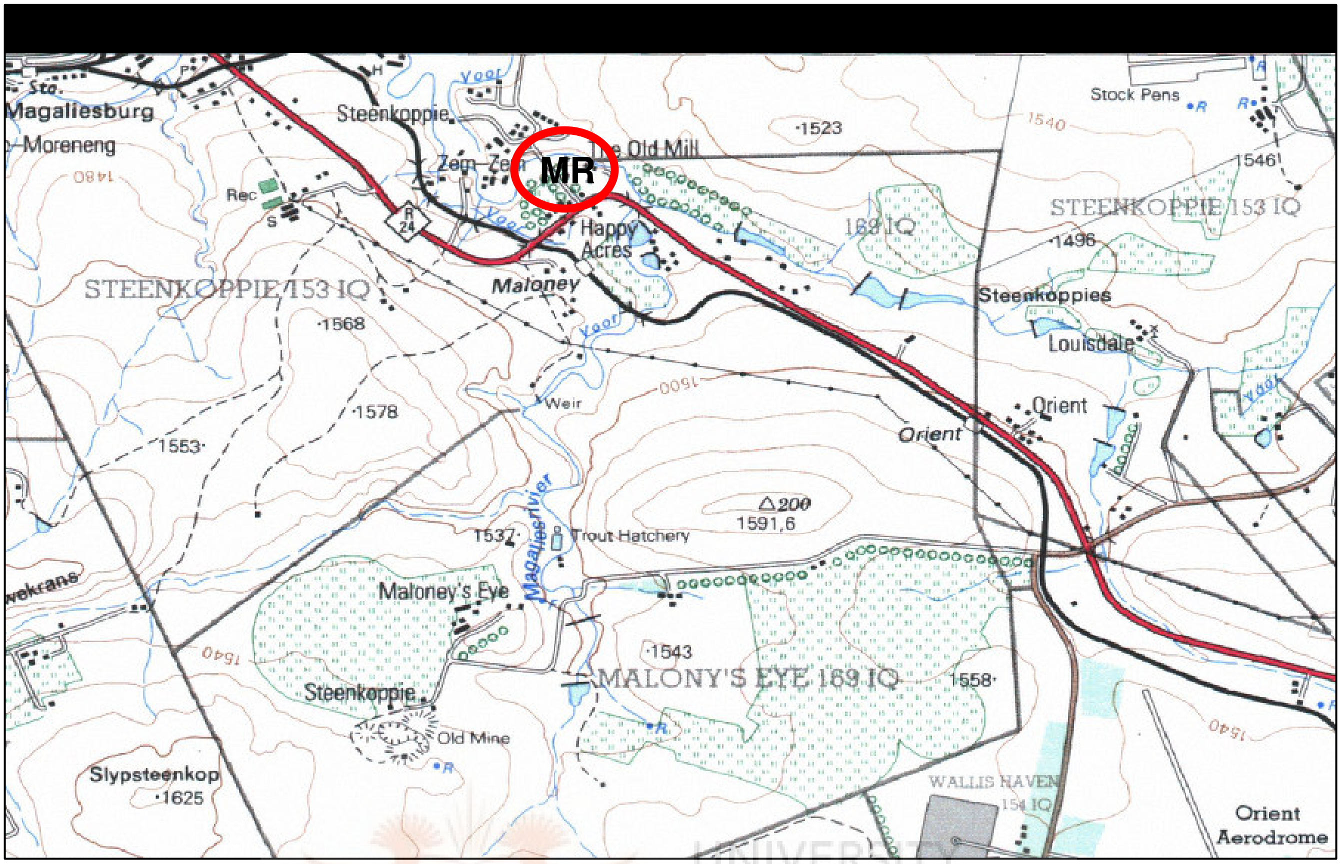

Figure A7: SA quarter degree topographical (QDS) 1:50,000 map 2627BA and aerial photo for site MR (Europe Technologies Image, *Google Earth* 2007).

Appendix B - Water Quality and Diatoms

Figure B1: Water quality graphs for. (a) temperature, (b) turbidity, (c) oxygen concentration, (d) oxygen saturation, (e) conductivity, (f) pH, (g) TDS and (h) nitrite for variables measured over the high and low flow periods in 2006.

Figure B1 (Cont): Water quality graphs for(i) nitrate, (j) chloride, (k) calcium, (l)COD, (m) sulphates, (n)ammonia and (o) ammonium measured over the high and low flow periods in 2006.

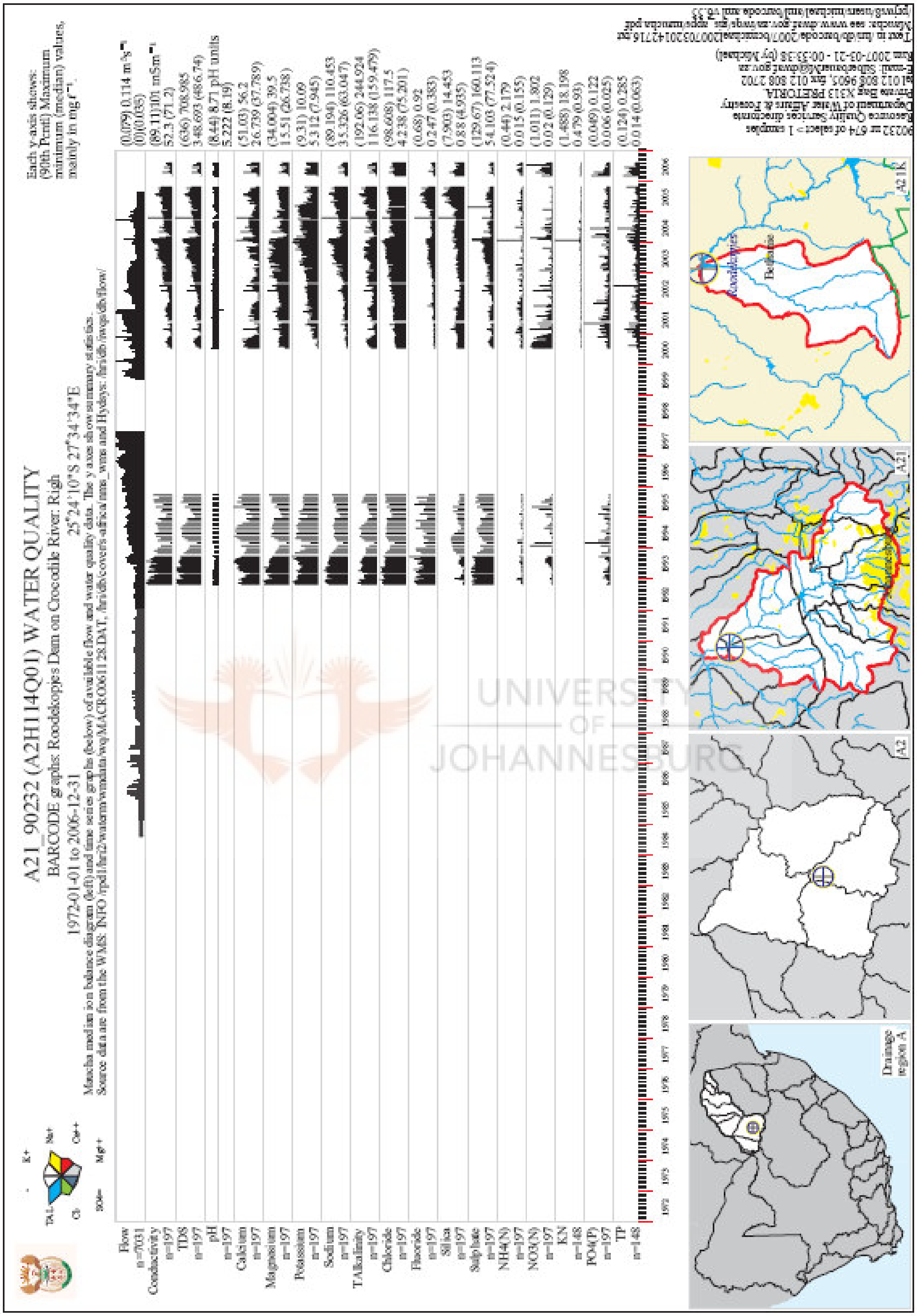

Figure B2: Historical water quality time series graphs of available flow and water quality data for Roodekopjes Dam on Crocodile River near agricultural site C1 (DWAF,2008).

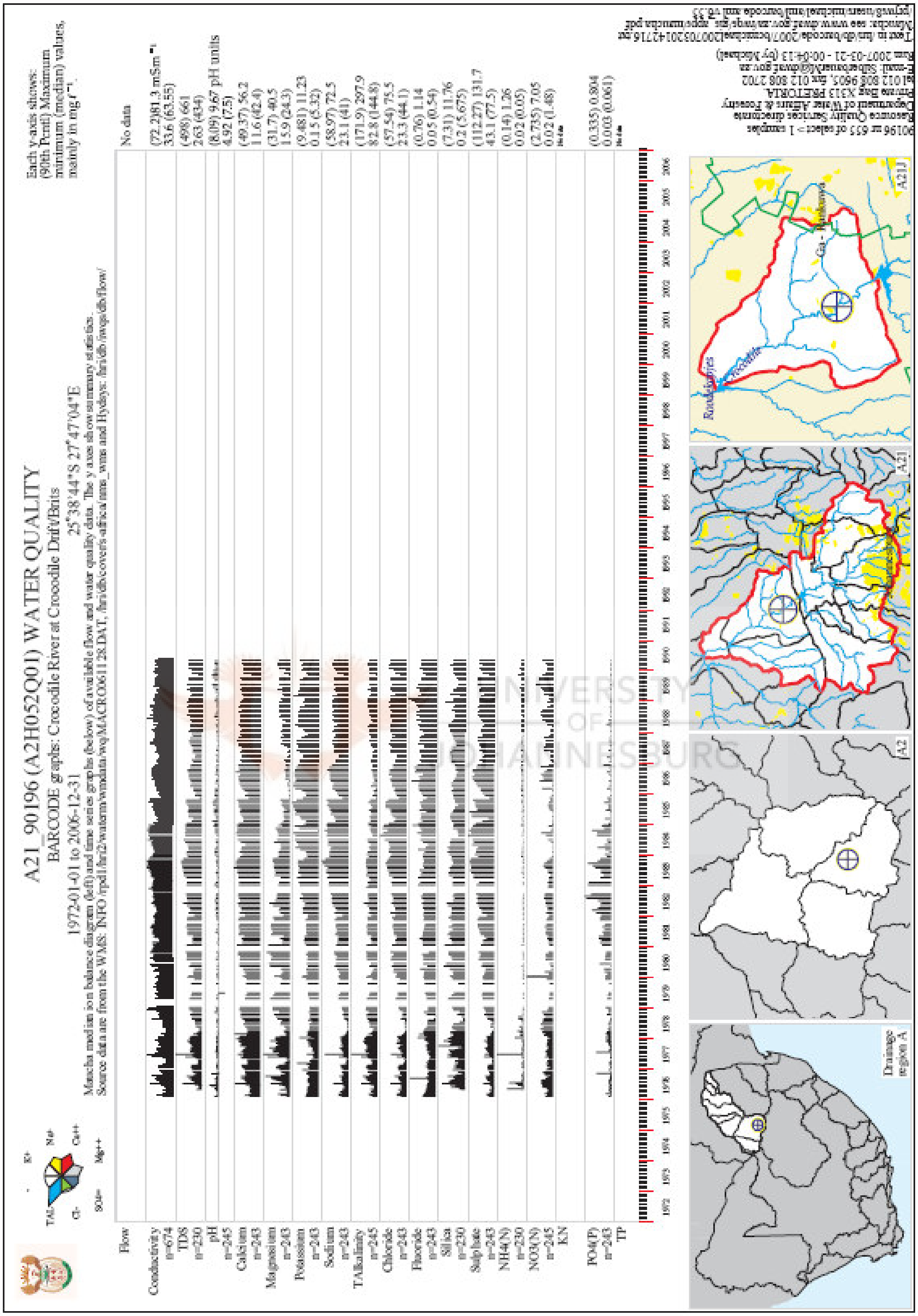

Figure B3: Historical water quality time series graphs of available flow and water quality data for Crocodile River at Brits near agricultural site C2 (DWAF,2008).

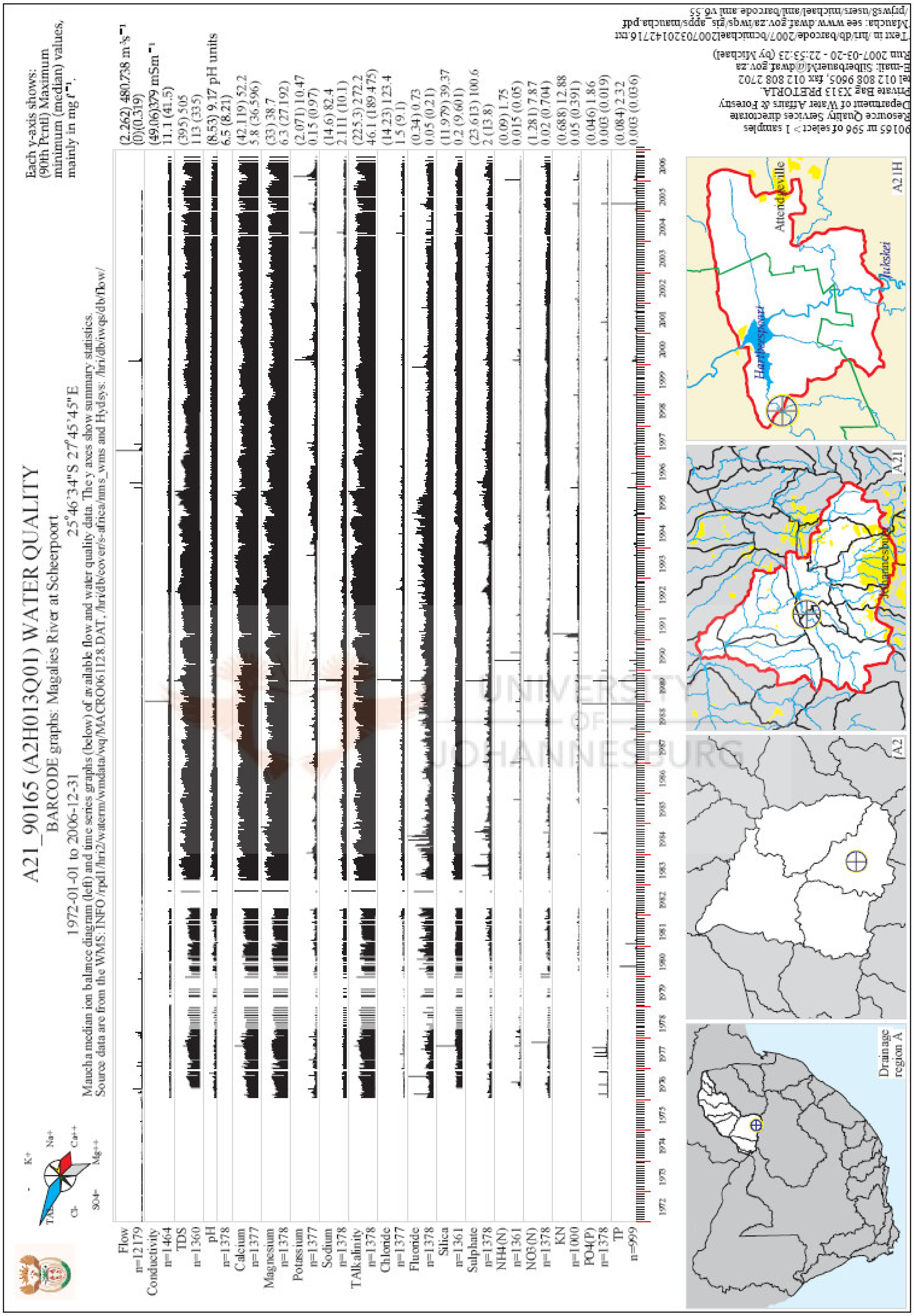

Figure B4: Historical water quality time series graphs of available flow and water quality data for the Magalies River at Scheerpoort near agricultural site M2 (DWAF,2008).

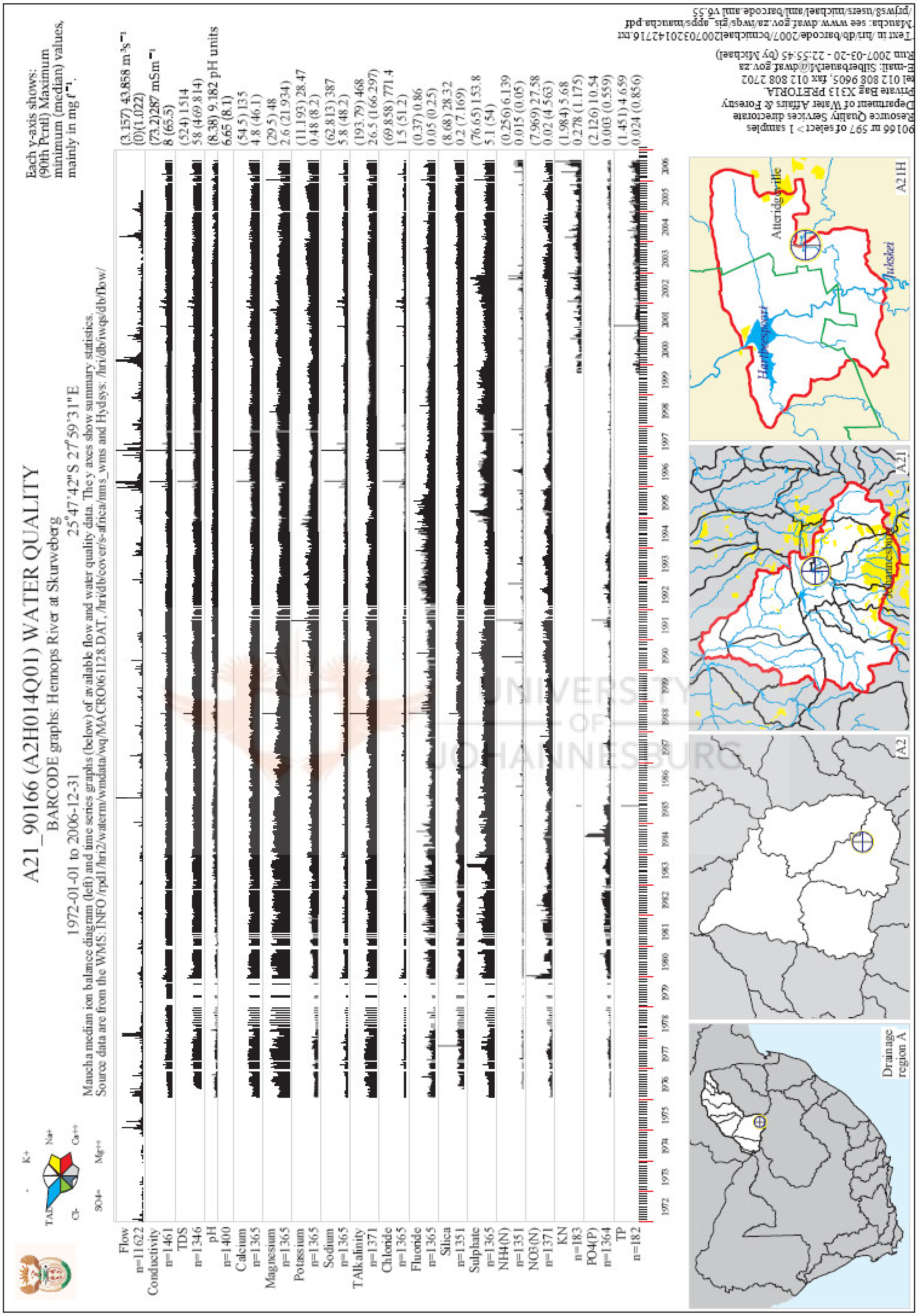

Figure B5: Historical water quality time series graphs of available flow and water quality data for the Hennops River at Skurweberg near urban site C4 (DWAF,2008).

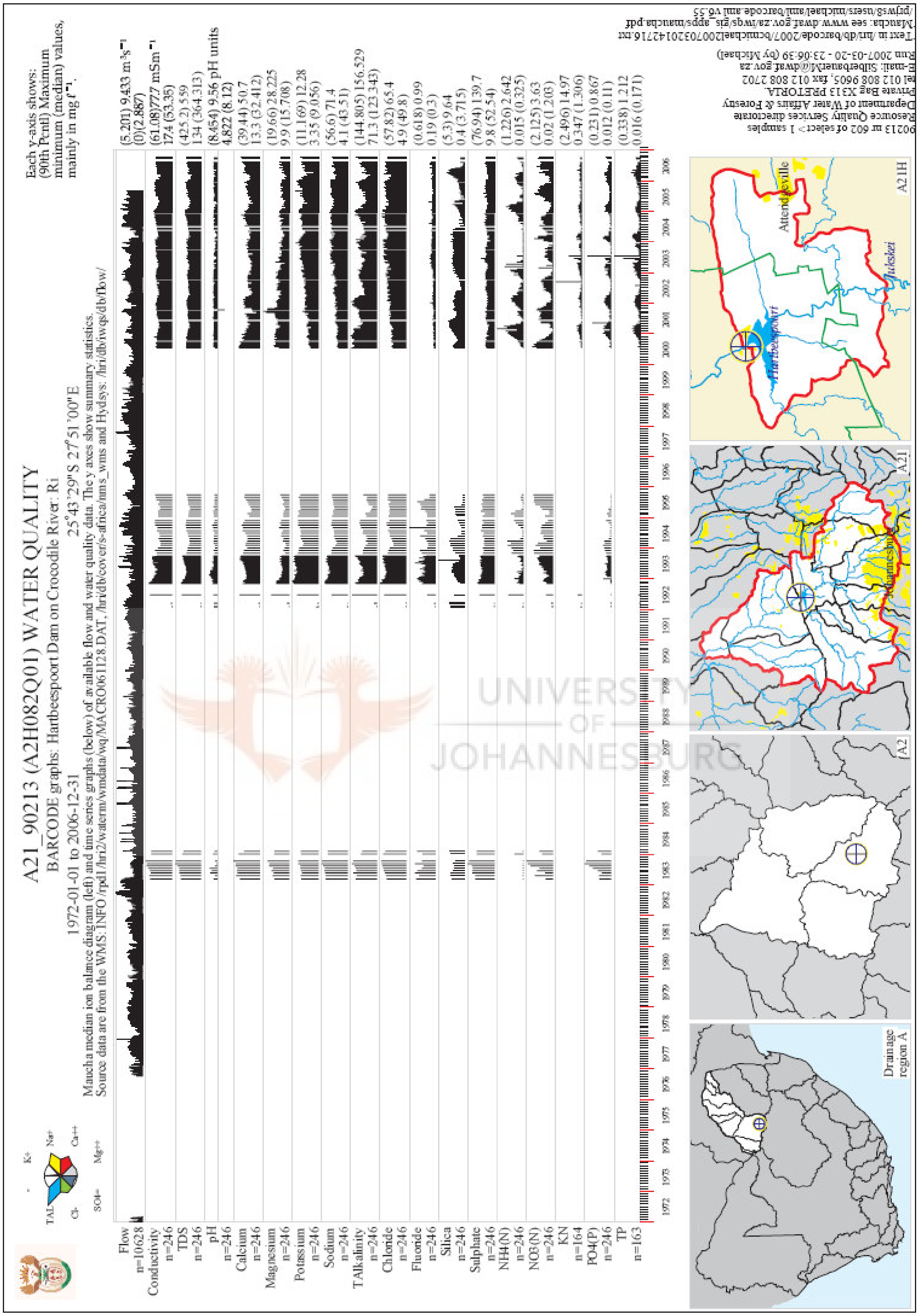

Figure B6: Historical water quality time series graphs of available flow and water quality data for the Hartbeespoort Dam (Right Canal) on Crocodile River: near urban site C3 (DWAF,2008)..

Table B1: Species list for high flow indicating species abundances, names and acronyms taken from OMNIDIA species list database (Lecointe *et al*., 1993).

Species	Abbreviation	CR H	C1 H	C2 H	C3 H	C4 H	MR H	M2 H
Achnanthes minutissima Kutzing v.minutissima Kutzing (Achnanthidium)	AMIN	0	0	0	0	0	230	9
Achnanthidium exiguum (Grunow) Czarnecki	ADEG	0	2	0	0	4	0	0
Achnanthidium pyrenaicum (Hustedt) Kobayasi	ADPY	0	0	0	0	0	0	9
Achnanthidium saprophila (Kob.& Mayama) Round & Bukhtiyarova f.teratogene	ADSG	17	0	0	0	0	0	0
Achnanthidium eutrophilum (Lange-Bertalot)Lange-Bertalot	ADEU	0	0	0	0	0	0	0
Amphora pediculus (Kutzing) Grunow	APED	0	45	11	50	10	19	8
Amphora veneta Kutzing	AVEN	0	0	0	0	0	0	0
Aulacoseira granulata (Ehr.) Simonsen	AUGR	0	9	18	43	8	0	1
Aulacoseira muzzanensis (Meister) Krammer	AMUZ	0	0	0	0	0	0	0
Cocconeis pediculus Ehrenberg	CPED	0	12	0	34	2	43	1
Cocconeis placentula Ehrenberg fo. teratogene	CPTG	0	0	0	23	130	67	0
Cocconeis placentula Ehrenberg var.euglypta(Ehr.)Grunow	CPLE	264	0	9	0	84	0	19
Cocconeis placentula Ehrenberg var.lineata (Ehr.)Van Heurck	CPLI	0	0	14	0	0	0	0
Craticula cuspidata (Kutzing) Mann	CRCU	0	0	0	0	0	0	0
Craticula molestiformis (Hustedt) Lange-Bertalot	CMLF	0	0	27	0	0	0	0
Cyclostephanos invisitatus(Hohn & Hellerman)Theriot Stoermer & Hakansson	CINV	0	0	0	0	0	0	1
Cyclotella meduanae Germain	CMED	0	0	0	0	0	0	0
Cyclotella meneghiniana Kutzing	CMEN	0	9	4	17	6	0	0
Cymbella affinis Kutzing var.affinis	CAFF	0	0	0	0	0	0	0
Cymbella bengalensis Grunow	CBEN	0	2	0	0	0	0	0
Cymbella kappii(Cholnoky) Cholnoky	CKPP	0	0	0	0	0	3	0
Cymbella tumida (Brebisson)Van Heurck	CTUM	0	0	0	2	2	0	0
Cymbella turgidula Grunow 1875 in A.Schmidt & al. var. turgidula	CTGL	0	1	0	0	0	0	0
Diatoma vulgaris Bory 1824	DVUL	0	5	2	168	0	2	0
Discostella woltereckii (Hustedt) Houk & Klee	DWOL	0	0	0	0	0	0	3
Denticula kuetzingii Grunow var.kuetzingii	DKUE	0	0	0	0	0	0	0
Encyonopsis microcephala (Grunow) Krammer	ENCM	0	0	0	0	0	14	0
Encyonopsis leei Krammer var. leei	ENLE	0	0	0	0	0	42	0
Eolimna minima(Grunow) Lange-Bertalot	EOMI	0	2	0	0	12	0	0
Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin	ESBM	0	7	11	12	44	0	26
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	FSAP	5	2	0	7	6	0	2
Fragilaria biceps (Kutzing) Lange-Bertalot	FBCP	0	0	2	1	0	0	30
Fragilaria capucina Desmazieres fo.teratogene	FCAT	0	0	0	0	0	0	0
Fragilaria capucina Desmazieres var.vaucheriae(Kutzing)Lange-Bertalot	FCVA	0	0	0	0	0	0	0
Fragilaria ulna (Nitzsch.) Lange-Bert. var. biceps (Kutzing) Lange-Bertal.	FUBI	4	10	0	3	0	6	0
Fragilaria tenera (W.Smith) Lange-Bertalot	FTEN	0	0	0	0	0	0	1
Gomphonema acuminatum Ehrenberg	GACU	0	0	0	0	0	0	0
Gomphonema italicum Kützing	GITA	0	0	0	18	0	0	0
Gomphonema parvulum Kutzing fo. teratogene	GPAT	21	4	6	6	10	0	15
Gomphonema parvulum var.parvulum f.saprophilum Lange-Bert.&Reichardt	GPAS	0	0	0	0	26	0	0
Gomphonema venusta Passy. Kociolek & Lowe	GVNU	0	0	0	0	0	78	0
Gomphonema pseudoaugur Lange-Bertalot	GPSA	0	0	0	0	14	0	0
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	GPUM	24	0	0	3	0	0	11
Gyrosigma attenuatum (Kützing) Rabenhorst	GYAT	0	12	0	0	0	12	1
Gyrosigma acuminatum (Kutzing)Rabenhorst	GYAC	0	0	0	0	0	0	0
Gyrosigma scalproides (Rabenhorst)Cleve	GSCA	0	0	0	0	0	0	10
Luticola mutica (Kützing) D.G. Mann	LMUT	0	0	0	0	2	0	0
Melosira varians Agardh	MVAR	0	0	0	34	0	0	9
MICROCOSTATUS Johansen & Sray	MCCT	0	0	0	0	0	0	1
Mayamaea atomus var. permitis (Hustedt) Lange-Bertalot	MAPE	0	0	0	0	10	0	0
Navicula sp.	NASP	0	0	0	0	0	0	2
Navicula agrestis Hustedt	NAGR	0	0	0	0	0	0	1
Navicula antonii Lange-Bertalot	NANT	0	0	0	0	0	0	2
Navicula arvensis Hustedt	NARV	0	0	0	0	0	0	1
Navicula capitatoradiata Germain	NCPR	0	0	0	0	0	0	3
Navicula cryptocephala Kutzing	NCRY	0	0	0	0	0	0	0
Navicula cryptotenella Lange-Bertalot	NCTE	0	0	48	3	18	0	111
Navicula cryptotenelloides Lange-Bertalot	NCTO	0	0	0	22	0	0	0
Navicula erifuga Lange-Bertalot	NERI	0	0	0	0	0	0	2
Navicula gregaria Donkin	NGRE	29	0	0	0	0	9	0
Navicula recens (Lange-Bertalot) Lange-Bertalot	NRCS	0	127	0	3	6	0	0
Navicula reichardtiana Lange-Bertalot var. reichardtiana	NRCH	0	0	0	0	0	0	1
Navicula rostellata Kutzing	NROS	0	20	0	0	0	0	0
Navicula schroeteri Meister var. schroeteri	NSHG	0	0	0	0	0	0	32
Navicula symmetrica Patrick	NSYM	0	0	0	0	12	9	15
Navicula tripunctata (O.F.Müller) Bory	NTPT	0	0	4	125	14	15	29
Navicula veneta Kutzing	NVEN	0	0	0	1	0	0	1
Navicula zanoni Hustedt	NZAN	0	0	0	0	0	0	0
Nitzschia sp.	NITZ	0	126	0	23	4	0	10
Nitzschia amphibia Grunow f.amphibia	NAMP	0	0	0	0	0	0	2
Nitzschia dissipata(Kutzing)Grunow var.dissipata	NDIS	0	0	0	0	0	0	1
Nitzschia frustulum(Kutzing)Grunow var.frustulum	NIFR	0	42	40	0	18	0	26
Nitzschia intermedia Hantzsch ex Cleve & Grunow	NINT	0	18	0	0	0	0	0
Nitzschia linearis(Agardh) W.M.Smith var.linearis	NLIN	0	0	0	0	0	0	1
Nitzschia liebetruthii Rabenhorst var.liebetruthii	NLBT	0	0	156	12	0	0	0
Nitzschia palea (Kutzing) W.Smith	NPAL	0	31	24	35	10	0	3
Nitzschia recta Hantzsch in Rabenhorst	NREC	0	0	0	0	0	0	0
Nitzschia supralitorea Lange-Bertalot	NZSU	0	0	0	0	0	0	1
Nitzschia tsarenkoi	NTSK	0	0	4	0	0	0	0
Placoneis elginensis (Greg) Cox	PELG	0	0	0	0	0	4	0
Pinnularia divergens W.M.Smith var. divergens	PDIV	0	0	0	0	0	0	0
Pinnularia subbrevistriata Krammer	PSBV	0	0	0	0	0	0	0
Planothidium frequentissimum(Lange-Bertalot)Lange-Bertalot	PLFR	0	0	0	0	0	0	1
Planothidium lanceolatum(Brebisson ex Kützing) Lange-Bertalot	PTLA	24	0	0	0	0	0	0
Pleurosigma salinarum (Grunow) Cleve & Grunow	PSAL	2	12	6	0	0	0	0
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	RABB	0	0	0	0	0	0	0
Reimeria sinuata (Gregory) Kociolek & Stoermer	RSIN	9	4	0	3	4	0	0
Reimeria uniseriata Sala Guerrero & Ferrario	RUNI	0	0	0	0	2	0	1
Sellaphora seminulum (Grunow) D.G. Mann	SSEM	0	7	0	0	0	0	0
Staurosira construens Ehrenberg	SCON	0	2	0	0	0	0	0
Staurosira elliptica (Schumann) Williams & Round	SELI	0	6	14	5	0	6	0
Staurosirella pinnata (Ehr.) Williams & Round	SPIN	0	0	0	0	0	1	0
Stephanodiscus agassizensis Hakansson & Kling	SAGA	0	0	0	0	0	0	0
Surirella angusta Kutzing	SANG	4	0	0	0	0	0	1
Thalassiosira pseudonana Hasle et Heimdal	TPSN	0	0	0	0	0	0	3
Thalassiosira weissflogii (Grunow) Fryxell & Hasle	TWEI	0	4	0	0	0	0	0
Tryblionella apiculata Gregory	TAPI	0	0	0	0	4	0	0
Tryblionella hungarica (Grunow) D.G. Mann	THUN	0	0	0	0	0	0	0
Tryblionella levidensis Wm. Smith	TLEV	0	0	0	0	0	0	1

Table B2: Species list for low flow indicating species abundances, names and acronyms taken from OMNIDIA species list database (Lecointe *et al*., 1993).

Species	Abbreviation	CR L	C4 L	C3 L	C2 L	C1 L	MR L	M2 L
Achnanthes minutissima Kutzing v.minutissima Kutzing (Achnanthidium)	AMIN	0	0	0	0	0	216	241
Achnanthidium exiguum (Grunow) Czarnecki	ADEG	0	2	0	0	0	0	0
Achnanthidium pyrenaicum (Hustedt) Kobayasi	ADPY	0	0	0	0	0	0	85
Achnanthidium saprophila (Kob.& Mayama) Round & Bukhtiyarova f.teratogene	ADSG	0	0	0	0	0	0	0
Achnanthidium eutrophilum (Lange-Bertalot)Lange-Bertalot	ADEU	48	0	0	0	0	0	10
Amphora pediculus (Kutzing) Grunow	APED	0	23	62	90	13	6	2
Amphora veneta Kutzing	AVEN	0	7	0	0	0	0	0
Aulacoseira granulata (Ehr.) Simonsen	AUGR	0	0	32	8	0	0	0
Aulacoseira muzzanensis (Meister) Krammer	AMUZ	0	0	4	0	0	0	0
Cocconeis pediculus Ehrenberg	CPED	0	13	40	34	17	64	0
Cocconeis placentula Ehrenberg fo. teratogene	CPTG	1	0	18	6	0	44	1
Cocconeis placentula Ehrenberg var.euglypta(Ehr.)Grunow	CPLE	42	21	8	32	16	0	0
Cocconeis placentula Ehrenberg var.lineata (Ehr.)Van Heurck	CPLI	15	0	0	0	0	0	0
Craticula cuspidata (Kutzing) Mann	CRCU	0	3	0	0	0	0	0
Craticula molestiformis (Hustedt) Lange-Bertalot	CMLF	0	0	0	7	0	0	0
Cyclostephanos invisitatus(Hohn & Hellerman)Theriot Stoermer & Hakansson	CINV	0	0	0	0	0	0	0
Cyclotella meduanae Germain	CMED	0	0	2	0	0	0	0
Cyclotella meneghiniana Kutzing	CMEN	0	2	10	5	0	0	1
Cymbella affinis Kutzing var.affinis	CAFF	0	0	0	0	0	0	2
Cymbella bengalensis Grunow	CBEN	0	0	0	0	0	0	0
Cymbella kappii(Cholnoky) Cholnoky	CKPP	0	0	0	0	0	8	0
Cymbella tumida (Brebisson)Van Heurck	CTUM	0	0	4	0	0	0	1
Cymbella turgidula Grunow 1875 in A.Schmidt & al. var. turgidula	CTGL	2	0	0	0	0	0	1
Diatoma vulgaris Bory 1824	DVUL	0	99	138	54	342	4	0
Discostella woltereckii (Hustedt) Houk & Klee	DWOL	0	0	0	0	0	0	0
Denticula kuetzingii Grunow var.kuetzingii	DKUE	1	0	0	0	0	0	0
Encyonopsis microcephala (Grunow) Krammer	ENCM	0	0	0	0	0	8	3
Encyonopsis leei Krammer var. leei	ENLE	0	0	0	0	0	40	0
Eolimna minima(Grunow) Lange-Bertalot	EOMI	0	6	0	0	0	0	0
Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin	ESBM	0	24	10	32	0	0	0
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot	FSAP	8	0	4	0	0	0	0
Fragilaria biceps (Kutzing) Lange-Bertalot	FBCP	1	0	2	0	2	0	2
Fragilaria capucina Desmazieres fo.teratogene	FCAT	6	0	0	0	0	0	0
Fragilaria capucina Desmazieres var.vaucheriae(Kutzing)Lange-Bertalot	FCVA	21	0	0	0	0	0	0
Fragilaria ulna (Nitzsch.) Lange-Bert. var. biceps (Kutzing) Lange-Bertal.	FUBI	9	0	16	23	22	4	0
Fragilaria tenera (W.Smith) Lange-Bertalot	FTEN	0	0	0	0	0	0	0
Gomphonema acuminatum Ehrenberg	GACU	1	0	0	0	2	0	0
Gomphonema italicum Kützing	GITA	0	0	14	0	0	0	0
Gomphonema parvulum Kutzing fo. teratogene	GPAT	14	6	4	12	0	0	4
Gomphonema parvulum var.parvulum f.saprophilum Lange-Bert.&Reichardt	GPAS	0	0	0	0	0	0	0
Gomphonema venusta Passy. Kociolek & Lowe	GVNU	0	0	0	0	0	60	0
Gomphonema pseudoaugur Lange-Bertalot	GPSA	0	0	0	0	0	0	0
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	GPUM	28	0	4	0	0	0	3
Gyrosigma attenuatum (Kützing) Rabenhorst	GYAT	0	0	0	0	0	16	0
Gyrosigma acuminatum (Kutzing)Rabenhorst	GYAC	1	0	0	0	0	0	0
Gyrosigma scalproides (Rabenhorst)Cleve	GSCA	7	0	0	0	0	0	0
Luticola mutica (Kützing) D.G. Mann	LMUT	0	0	0	0	0	0	0
Melosira varians Agardh	MVAR	6	2	22	0	0	0	1
MICROCOSTATUS Johansen & Sray	MCCT	0	0	0	0	0	0	0
Mayamaea atomus var. permitis (Hustedt) Lange-Bertalot	MAPE	0	0	0	0	0	0	0
Navicula sp.	NASP	0	0	0	0	0	0	0
Navicula agrestis Hustedt	NAGR	0	0	0	0	0	0	0
Navicula antonii Lange-Bertalot	NANT	0	0	0	0	0	0	0
Navicula arvensis Hustedt	NARV	0	0	0	0	0	0	0
Navicula capitatoradiata Germain	NCPR	0	6	2	9	0	0	7
Navicula cryptocephala Kutzing	NCRY	0	0	0	20	0	0	0
Navicula cryptotenella Lange-Bertalot	NCTE	0	9	6	20	0	0	13
Navicula cryptotenelloides Lange-Bertalot	NCTO	0	0	16	0	0	0	0
Navicula erifuga Lange-Bertalot	NERI	0	0	0	0	0	0	0
Navicula gregaria Donkin	NGRE	68	27	0	0	19	6	0
Navicula recens (Lange-Bertalot) Lange-Bertalot	NRCS	0	0	8	0	0	0	0
Navicula reichardtiana Lange-Bertalot var. reichardtiana	NRCH	0	0	0	0	0	0	0
Navicula rostellata Kutzing	NROS	0	0	0	0	0	0	0
Navicula schroeteri Meister var. schroeteri	NSHG	0	0	0	0	0	0	0
Navicula symmetrica Patrick	NSYM	0	91	0	0	0	6	0
Navicula tripunctata (O.F.Müller) Bory	NTPT	0	8	114	76	0	8	20
Navicula veneta Kutzing	NVEN	0	11	2	0	0	0	0
Navicula zanoni Hustedt	NZAN	0	0	0	0	0	0	1
Nitzschia sp.	NITZ	19	21	16	0	2	0	2
Nitzschia amphibia Grunow f.amphibia	NAMP	0	9	0	28	0	0	0
Nitzschia dissipata(Kutzing)Grunow var.dissipata	NDIS	0	0	0	10	0	0	5
Nitzschia frustulum(Kutzing)Grunow var.frustulum	NIFR	0	19	0	41	0	0	1
Nitzschia intermedia Hantzsch ex Cleve & Grunow	NINT	0	0	0	0	0	0	0
Nitzschia linearis(Agardh) W.M.Smith var.linearis	NLIN	6	17	0	0	0	0	4
Nitzschia liebetruthii Rabenhorst var.liebetruthii	NLBT	0	9	18	0	0	0	0
Nitzschia palea (Kutzing) W.Smith	NPAL	0	17	4	2	0	0	0
Nitzschia recta Hantzsch in Rabenhorst	NREC	0	0	0	0	0	8	0
Nitzschia supralitorea Lange-Bertalot	NZSU	0	0	0	0	0	0	0
Nitzschia tsarenkoi	NTSK	0	0	0	0	0	0	0
Placoneis elginensis (Greg) Cox	PELG	0	0	0	0	0	2	0
Pinnularia divergens W.M.Smith var. divergens	PDIV	8	0	0	0	0	0	0
Pinnularia subbrevistriata Krammer	PSBV	5	0	0	0	0	0	0
Planothidium frequentissimum(Lange-Bertalot)Lange-Bertalot	PLFR	0	9	0	0	0	0	0
Planothidium lanceolatum(Brebisson ex Kützing) Lange-Bertalot	PTLA	65	0	0	0	0	0	0
Pleurosigma salinarum (Grunow) Cleve & Grunow	PSAL	0	0	0	0	0	0	0
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	RABB	30	0	0	0	3	0	0
Reimeria sinuata (Gregory) Kociolek & Stoermer	RSIN	8	0	0	0	4	0	0
Reimeria uniseriata Sala Guerrero & Ferrario	RUNI	0	0	0	0	0	0	0
Sellaphora seminulum (Grunow) D.G. Mann	SSEM	0	0	0	0	0	0	0
Staurosira construens Ehrenberg	SCON	0	0	0	0	0	0	0
Staurosira elliptica (Schumann) Williams & Round	SELI	0	5	6	11	0	2	0
Staurosirella pinnata (Ehr.) Williams & Round	SPIN	0	0	0	0	0	4	0
Stephanodiscus agassizensis Hakansson & Kling	SAGA	0	0	0	18	0	0	0
Surirella angusta Kutzing	SANG	1	0	0	0	0	0	0
Thalassiosira pseudonana Hasle et Heimdal	TPSN	0	0	0	0	0	0	3
Thalassiosira weissflogii (Grunow) Fryxell & Hasle	TWEI	0	0	0	0	0	0	0
Tryblionella apiculata Gregory	TAPI	0	0	0	0	0	0	0
Tryblionella hungarica (Grunow) D.G. Mann	THUN	0	5	0	0	0	0	0
Tryblionella levidensis Wm. Smith	TLEV	0	0	0	0	0	0	0

Table B3: Descriptions for ecological parameters discussed in Chapter 3 taken from Van Dam *et al.* (1994).

Classification of Ecological Indicators			
pH			
Acidobiontic	optimal occurrence at pH <5.5		
Acidophilous	mainly occuring at pH <7		
Circumneutral	mainly occuring at pH values about 7		
Alkaliphilous	mainly occuring at pH >7		
Alkalibiontic	exclusively occuring at pH >7		
Indifferent	no apparent optimum		
Salinity			
	Cl^- (mg l^{-1})	**Salinity (‰)**	**Cond. mS/m**
Fresh	<100	<0.2	<3
fresh-brackish	<500	<0.9	<139
brackish-fresh	500-1000	0.9-1.8	139-277
Brackish	1000-5000	1.8-9.0	277-1385
Nitrogen uptake mechanism			
Nitrogen autotrophic – sensitive	tolerating very small concentrations of organically bound nitrogen		
Nitrogen autotrophic – tolerant	tolerating elevated concentrations of organically bound nitrogen		
Nitrogen heterotrophic – facultative	needing periodically elevated concentrations of organically bound nitrogen		
Nitrogen heterotrophic – obligatory	needing continuously elevated concentrations of organically bound nitrogen		
Oxygen requirements			
continuously high	~100% saturation		
fairly high	>75% saturation		
Moderate	>50% saturation		
Low	>30% saturation		
very low	~10% saturation		
Saprobity			
	Pollution	**Oxygen saturation**	**BOD_5 (mg l^{-1})**
Oligosaprobous	Unpolluted to slightly polluted	>85	<2
β-mesosaprobous	Moderately polluted	70 - 85	2 - 4
α-mesosaprobous	Strongly polluted	25 - 70	4 - 13
α-meso-polysaprobous	Very heavily polluted	10 - 25	13 - 22
Polysaprobous	Extremely polluted	<10	>22

Appendix C - Habitat, Macroinvertebrate and Riparian Vegetation Data

Table C1: Macroinvertebrate abundance data for the Crocodile (C) and Magalies (M) rivers at high (H) flow.

	CR H	C4 H	C3 H	C2 H	C1 H	MR H	M2 H
Porifera	0	0	0	0	0	0	1
Turbellaria	5	0	0	0	0	15	32
Annelida							
Oligochaeta	7	15	10	6	26	0	20
Hirudinea	0	13	62	2	12	0	0
Crustacea							
Amphipoda	2	0	0	0	0	1	0
Potamaninautidae	4	2	5	0	7	11	8
Hydracarina	0	0	0	0	0	1	0
Ephemeroptera							
Baetidae 2sp	175	0	0	237	80	0	150
Baetidae >2sp	0	150	85	0	0	65	0
Caenidae	75	30	0	3	25	10	0
Leptophlebiidae	0	0	0	0	0	10	1
Tricorythidae	0	0	0	32	3	167	0
Odonata							
Chlorolestidae	0	0	0	5	10	0	0
Coenagrionidae	5	15	10	14	5	5	7
Aeshnidae	10	0	0	0	0	15	0
Cordulidae	1	0	0	0	0	10	0
Gomphidae	4	2	0	1	5	15	0
Hemiptera							
Belostomatidae	0	0	6	4	0	0	0
Corixidae	2	2	3	0	2	0	15
Gerridae	0	0	15	0	0	20	0
Naucoridae	0	0	0	0	3	0	0
Notectidae	1	0	0	0	0	0	0
Veliidae	15	0	0	11	3	5	2
Trichoptera							
Ecnomidae	0	0	0	0	0	0	0
Hydropsychidae 1 sp	0	27	133	0	0	0	0
Hydropsychidae 2s	30	0	0	0	15	0	0
Hydropsychidae >2sp	0	0	0	11	0	55	25
Philopotamidae	0	0	0	0	0	15	0
Coleoptera							
Dytisidae	14	0	0	0	1	0	0
Elmidae/Dryopidae	0	5	0	2	0	0	0
Gyrinidae	40	0	10	77	10	11	22
Helodidae	0	0	0	0	0	1	0
Psephenidae	0	0	0	0	0	1	0
Diptera							
Athericidae	1	0	0	0	0	2	0
Ceratopgonidae	0	0	1	1	3	20	0
Chironomidae	41	90	60	26	27	25	20
Culicidae	5	5	0	0	0	2	0
Dixidae	0	0	0	0	0	15	0
Psychodidae	0	5	0	0	0	0	0
Simuliidae	30	35	45	23	190	35	75
Syrphidae	0	0	1	0	0	0	0
Tabanidae	0	1	5	3	1	13	0
Tipulidae	2	0	0	0	0	13	0
Gastropoda							
Ancylidae	5	0	0	2	0	20	15
Lymnaeidae	0	0	3	0	4	0	0
Physidae	0	0	0	2	0	0	0
Planorbinae	0	0	0	0	2	0	0
Thiaridae	0	0	23	1	0	0	0
Pelecypoda							
Corbiculidae	0	0	0	0	13	0	5

Table C2: Macroinvertebrate abundance data for the Crocodile (C) and Magalies (M) rivers at low (L) flow.

	CR L	C4 L	C3 L	C2 L	C1 L	MR L	M2 L
Porifera	0	5	5	50	0	1	5
Turbellaria	9	135	183	26	22	26	321
Annelida							
Oligochaeta	25	7359	680	5050	8013	17	200
Hirudinea	0	2	104	0	31	0	4
Crustacea							
Atyidae	0	0	3	0	5	0	0
Potamonautidae	1	1	0	0	0	0	1
Ephemeroptera							
Baetidae 1sp	1764	1343	666	0	1	0	0
Baetidae 2sp	0	0	0	352	0	0	0
Baetidae >2sp	0	0	0	0	0	187	676
Caenidae	1818	511	1	117	0	71	9
Leptophlebiidae	0	0	0	3	0	42	2
Heptageniidae	0	0	0	0	0	1	0
Tricorythidae	10	8	0	0	0	622	0
Zygoptera juvenilles	0	0	0	0	0	1	1
Odonata							
Coenagrionidae	2	1	0	24	0	1	14
Aeshnidae	3	0	0	0	0	13	0
Gomphidae	0	0	0	5	0	1	0
Hemiptera							
Belostomatidae	0	0	0	0	0	0	25
Gerridae	0	0	0	0	0	1	0
Naucoridae	0	0	0	0	0	0	2
Nepidae	0	0	0	0	0	1	0
Notectidae	0	0	0	0	0	0	1
Veliidae	0	0	0	0	0	1	0
Trichoptera							
Ecnomidae	0	0	0	0	0	4	1
Hydropsychidae 1 sp	24	309	502	48	17	0	0
Hydropsychidae >2sp	0	0	0	0	0	47	148
Leptoceridae	0	0	0	1	0	3	0
Philopotamidae	0	0	0	0	0	75	0
Coleoptera							
Dytisidae	0	0	0	0	0	0	6
Elmidae/Dryopidae	2	0	0	3	0	3	0
Gyrinidae	0	0	0	3	0	4	1
Diptera							
Athericidae	4	0	0	0	0	13	0
Ceratopgonidae	0	0	0	16	56	24	4
Chironomidae	448	3937	1106	419	27	52	352
Culicidae	2	0	0	2	0	0	0
Ephydridae	0	0	0	0	0	1	0
Muscidae	0	0	1	0	3	5	0
Simuliidae	64	723	3	81	0	39	360
Tabanidae	0	3	0	4	26	5	2
Tipulidae	3	0	0	0	0	6	0
Gastropoda							
Ancylidae	5	18	7	2	0	1	45
Lymnaeidae	0	0	0	0	1	0	0
Physidae	0	0	10	21	0	0	0
Planorbinae	0	1	0	0	1	0	0
Thiaridae	0	0	49	0	0	0	0
Pelecypoda							
Corbiculidae	0	0	0	0	12	0	32
Sphaeriidae	0	0	0	0	3	0	1

Table C3: EC spreadsheet for MIRAI for site CR.

INVERTEBRATE EC METRIC GROUP		METRIC GROUP CALCULATED SCORE	CALCULATED WEIGHT	WEIGHTED SCORE OF GROUP	RANK OF METRIC	%WEIGHT FOR METRIC
FLOW MODIFICATION	FM	36.0	0.370	13.3461	1	100
HABITAT	H	34.2	0.259	8.8547	2	70
WATER QUALITY	WQ	38.8	0.370	14.3842	1	100
CONNECTIVITY & SEASONALITY	CS	55.0	0.000	0	3	0
						270
INVERTEBRATE EC				36.585		
INVERTEBRATE EC CATEGORY				E		

Table C4: EC spreadsheet for MIRAI for site C4.

INVERTEBRATE EC METRIC GROUP		METRIC GROUP CALCULATED SCORE	CALCULATED WEIGHT	WEIGHTED SCORE OF GROUP	RANK OF METRIC	%WEIGHT FOR METRIC
FLOW MODIFICATION	FM	40.0	0.286	11.4286	1	100
HABITAT	H	52.8	0.286	15.0714	1	100
WATER QUALITY	WQ	32.2	0.229	7.36364	2	80
CONNECTIVITY & SEASONALITY	CS	50.0	0.200	10	3	70
						350
INVERTEBRATE EC				43.8636		
INVERTEBRATE EC CATEGORY				D		

Table C5: EC spreadsheet for MIRAI for site C3.

INVERTEBRATE EC METRIC GROUP		METRIC GROUP CALCULATED SCORE	CALCULATED WEIGHT	WEIGHTED SCORE OF GROUP	RANK OF METRIC	%WEIGHT FOR METRIC
FLOW MODIFICATION	FM	47.3	0.263	12.4436	1	100
HABITAT	H	42.2	0.211	8.88617	2	80
WATER QUALITY	WQ	34.2	0.263	9.00585	1	100
CONNECTIVITY & SEASONALITY	CS	15.0	0.263	3.94737	1	100
						380
INVERTEBRATE EC				34.283		
INVERTEBRATE EC CATEGORY				E		

Table C6: EC spreadsheet for MIRAI for site C2.

INVERTEBRATE EC METRIC GROUP		METRIC GROUP CALCULATED SCORE	CALCULATED WEIGHT	WEIGHTED SCORE OF GROUP	RANK OF METRIC	%WEIGHT FOR METRIC
FLOW MODIFICATION	FM	45.6	*0.370*	16.8981	1	100
HABITAT	H	42.8	*0.259*	11.0963	2	70
WATER QUALITY	WQ	42.8	*0.370*	15.8642	1	100
CONNECTIVITY & SEASONALITY	CS	80.0	*0.000*	0	4	0
						270
INVERTEBRATE EC				43.8586		
INVERTEBRATE EC CATEGORY				D		

Table C7: EC spreadsheet for MIRAI for site C1.

INVERTEBRATE EC METRIC GROUP		METRIC GROUP CALCULATED SCORE	CALCULATED WEIGHT	WEIGHTED SCORE OF GROUP	RANK OF METRIC	%WEIGHT FOR METRIC
FLOW MODIFICATION	FM	49.3	*0.278*	13.696	1	100
HABITAT	H	43.2	*0.222*	9.60317	2	80
WATER QUALITY	WQ	42.4	*0.278*	11.7725	1	100
CONNECTIVITY & SEASONALITY	CS	60.0	*0.222*	13.3333	2	80
						360
INVERTEBRATE EC				48.405		
INVERTEBRATE EC CATEGORY				D		

Table C8: EC spreadsheet for MIRAI for site MR.

INVERTEBRATE EC METRIC GROUP		METRIC GROUP CALCULATED SCORE	CALCULATED WEIGHT	WEIGHTED SCORE OF GROUP	RANK OF METRIC	%WEIGHT FOR METRIC
FLOW MODIFICATION	FM	71.1	*0.310*	22.0636	1	90
HABITAT	H	67.3	*0.345*	23.2095	1	100
WATER QUALITY	WQ	82.4	*0.345*	28.4199	1	100
CONNECTIVITY & SEASONALITY	CS	60.0	*0.000*	0	2	0
						290
INVERTEBRATE EC				73.693		
INVERTEBRATE EC CATEGORY				C		

Table C9: EC spreadsheet for MIRAI for site M2.

INVERTEBRATE EC METRIC GROUP		METRIC GROUP CALCULATED SCORE	CALCULATED WEIGHT	WEIGHTED SCORE OF GROUP	RANK OF METRIC	%WEIGHT FOR METRIC
FLOW MODIFICATION	**FM**	44.1	*0.250*	11.0357	1	100
HABITAT	**H**	61.3	*0.250*	15.3125	1	100
WATER QUALITY	**WQ**	48.8	*0.250*	12.1944	1	100
CONNECTIVITY & SEASONALITY	**CS**	20.0	*0.250*	5	1	100
						400
INVERTEBRATE EC				43.5427		
INVERTEBRATE EC CATEGORY				**D**		

Table C10: Dominant plant species found in the riparian zone at each site on the Crocodile and Magalies rivers at the high flow sampling survey. [W] Weed; [I] Invader; [R] Riparian plant; [A] Aquatic plant; [E] Exotic.

Species	Status	CR	C4	C3	C2	C1	MR	M2
Abutilon sonneratianum		x						
Acacia ataxacantha			x	x				
Acacia dealbata	**W**		x	x				
Acacia karoo	**R**		x	x				
Acacia mearnsii	**I**			x			x	
Acacia sieberana				x				
Acacia tortilis						x		
Acacia xanthophloea				x				
Aloe branddraaiensis							x	
Amaranthus hybridus	**E**	x						
Arundo donax	**E/W**		x	x	x			x
Asparagus laricinus					x		x	x
Bidens pilosa	**E**	x						
Buddleja salviifolia	**R**	x					x	x
Capsella bursa-pastoris	**E**	x						
Carissa bispinosa	**R**				x			
Carissa edulis					x			
Cassia didymobotrya	**E/W**			x				
Celtis africana	**R**	x			x		x	x
Cirsium vulgare	**E/W**				x	x		
Cladium mariscus subsp. jamaicense	**A**						x	
Combretum erythrophyllum	**R**	x	x	x	x		x	x
Cotoneaster pannosa						x	x	
Cussonia paniculata				x				
Cynodon dactylon	**E**					x		
Cyperus dives	**A**				x	x		
Cyperus esculentus	**E/A**							x
Cyperus sp.	**A**			x				
Cyperus textilis	**A**		x			x	x	
Datura stramonium	**E/W**	x						
Diospyros lycioides	**R**				x		x	

Species	Status	CR	C4	C3	C2	C1	MR	M2
Dombeya rotundifolia				x				
Eichhornia crassipes	**E/W/A**				x	x		
Euclea crispa			x	x	x		x	
Gymnosporia buxifolia			x	x	x		x	x
Gymnosporia senegalensis					x			x
Halleria lucida		x					x	
Heteromorpha trifoliata							x	
Imperata cylindrica						x		
Juncus effusus	**A**				x		x	
Lantana camara	**E/W**		x					
Leucosidea sericea		x					x	
Ligustrum lucidum	**E/I**					x	x	x
Melia azederach	**E/I**		x	x	x	x	x	x
Morus alba	**I**		x		x			x
Nasturtium officinale	**E**	x	x		x			
Olea europea susp. africana				x	x		x	
Pellaea calomelanos							x	
Pennisetum clandestinum	**E**		x	x	x			
Pennisetum macrourum	**A**						x	
Persicaria senegalensis	**A**		x	x				
Persicaria lapathifolia	**A**		x					
Phragmites australis	**A**					x		x
Phragmites mauritanus	**A**	x		x	x			
Pinus pinaster	**E/I**		x					
Plantago longissima	**A**						x	
Poa annua	**E**				x			
Populus x canescens	**E/I**							x
Rhamnus prinoides		x					x	
Rhus dentata		x					x	
Rhus lancea	**R**		x	x	x	x		x
Rhus pyroides	**R**				x			x
Salix babylonica	**E**		x					x

Species	Status	CR	C4	C3	C2	C1	MR	M2
Salix mucronata					x			
Schoenoplectus brachyceras	**A**		x		x		x	
Sesbania punicea	**E/W**			x		x		
Setaria megaphylla	**R**	x		x	x		x	x
Solanum mauritianum	**E/W**	x						
Solanum nigrocapsicum							x	x
Tagetes minuta	**E**	x						
Typha capensis	**A**			x				
Typha latifolia	**A**	x						
Verbena bonariensis	**E**			x				
Ziziphus mucronata	**R**		x	x	x		x	