ESTABLISHMENT OF REFERENCE CONDITIONS AND COMPARISON OF TWO INVERTEBRATE-BASED BIOMONITORING TECHNIQUES, PALMIET RIVER CATCHMENT

ALISTAIR W. FYFE

Thesis submitted for the Degree of

MASTER OF SCIENCE

In the Department of Zoology

University of Cape Town

November 2012

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DECLARATION

This thesis reports original research carried out in the Freshwater Research Unit, Department of Zoology, University of Cape Town, between September 2008 and November 2012. It has not been submitted in whole or part for a degree at any other university. Data presented here are original, and any other sources of data acquired through collaborative activates are fully acknowledged.

Date: 20 MAY 2013

Signature: Signed

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ABSTRACT

The primary aims of this study were to assess reference (pristine water chemistry, hydrology and riparian habitat) conditions within the upper Palmiet River in the south-western Cape of South Africa, and to compare two common bioassessment protocols,SASS5 (South African Scoring System 5) and MIRAI (Macroinverte brate Response Assessment Index), both of which use invertebrates to assess ecosystem integrity of rivers.

In all, three pristine reference sites and five impacted (*a priori* impacted in the form of water chemistry, hydrology or riparian habitat) sites were sampled in three separate seasons (spring, summer and autumn), purely within riffle biotopes in the upper reaches of the river. Supplementary data for an additional four impacted sites, within the same catchment, were added from Ollis (2004). At the reference sites no spatial differences were found in water chemistry, although water temperature and discharge fluctuated highly significantly with season (p<0.01), which lead to significant temporal differences in conductivity (p<0.05), instantaneous water temperature (p<0.01), pH (p<0.05), water velocity (p<0.01) and percentage dissolved oxygen saturation (p<0.01). No spatial physico-chemical differences were found. Spatial differences in mean abundance (p<0.01) and number of taxa (p<0.05), and temporal differences in mean abundance (p<0.01) of macroinvertebrates at the three reference sites could also be attributed to differences in these variables.

The two bioassessment techniques used: SASS5, which pre-assigns sensitivity scores to macroinvertebrates to assess instream water quality, and MIRAI, which similarly to SASS pre-assigns sensitivity scores to macroinvertebrates, but assesses flow, habitat and water-quality modification. According to the SASS protocol, the Palmiet "reference" sites did indeed support macroinvertebrate assemblages of reference quality. The most sensitive taxa occurred in spring and autumn. These differences were reflected in assemblages collected in spring and autumn being assigned a higher health indicator symbol (ecological category) than those collected in summer.

Having established reference conditions for the Palmiet River, the usefulness of the SASS and MIRAI protocols were compared using the nine sites that had been identified *a priori* as being impacted by a varying degree of anthropogenic influences, such as stream diversion, water abstraction, stream damming, channelization, rural development and urbanisation.

Both SASS and MIRAI identified impairment where sites were expected to be impacted, signifying robustness for both approaches. SASS was found to assign an ecological category at least one symbol higher than that produced by the MIRAI. The applicability and sensitivity of each protocol in defining ecological categories was explored independently.

The SASS regional approach developed by Dallas and Day (2007) was compared to a SASS seasonally-distinct catchment-specific approach. Both procedures revealed their ability to differentiate between reference and impact sites. Where a site was found to be impacted by the Dallas and Day (2007) approach, the seasonal-catchment specific bands revealed a lesser degree of impairment. Difference in ecological output was attributed to the scope of each protocol. Dallas and Day (2007) biological bands were developed at an ecoregion scale, incorporating a vast degree of inter-catchment variability, and may lack the sensitivity of the actual condition of streams within a specific catchment.

MIRAI's reliance on expert-based rating was investigated by creating hypothetical scenarios in which rating scores were varied. Depending on the ratings, ecological categories could be made to differ by more than one symbol. Furthermore, when rating scores were altered, habitat and flow-modification metrics changed more than the water quality metric did. A greater emphasis is placed by MIRAI in habitat and flow metrics than of water quality.

Distance-based redundancy analysis (dbRDA) was used to regress macroinvertebrate assemblages against environmental predictor variables in order to ascertain whether flow or water quality variables governed biological assemblages. Percentage dissolved oxygen saturation ($p<0.01$) and pH ($p<0.05$) explained the two highest proportions of variation in macroinvertebrate assemblages. Thus, w ater quality variables were seen as driving the system more so than flow or habitat variables. With this in mind, SASS was seen as the most appropriate bioassessment technique to ascertain the current ecological state of streams within the Palmiet River catchment.

Caution is needed in applying either SASS or MIRAI. In the case of SASS, a seasonal, catchment-specific approach is required in defining reference conditions. With respect to the use of MIRAI, greater emphasis is required in the water quality metric, and importance needs to be placed in increasing the robustness of the protocol by limiting the nature of variability in the rating score approach.

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And of course, thanks to my parents, Rocky and Maggie, who have always believed in me, encouraged me and supported me in my ecological pursuits, and to my girlfriend Nina, for challenging me to conclude the final stages of this dissertation.

STRUCTURE OF THESIS

This thesis, which has been formatted according to the style of the *African Journal of Aquatic Science*, has been structured as follows:

- A general introduction to the initiation and importance in the assessment of ecological integrity, and use developments thereof to the use of aquatic macroinvertebrates for bioassessment of river ecosystems is provided in **Chapter 1**, with particular reference to South Africa.
- A detailed description of the study area, all study sites investigated and periods of sampling are provided in **Chapter 2**.
- The development of spatial and temporal reference conditions within the Palmiet River catchment, with specific examination of physico-chemical variables, macroinvertebrate assemblages and effects on SASS biotic indices, are provided and discussed in **Chapter 3**.
- In **Chapter 4**, the use of the MIRAI and its comparison with SASS with respect to its applicability and sensitivity in defining ecological categories for impacted sites within the Palmiet River catchment.
- Finally, **Chapter 5** discusses the significance of results found in Chapters 3 and 4, followed by a general discussion and conclusion.

CHAPTER 1

INTRODUCTION

"The present and future quality of South Africa's freshwater resources is fundamentally important if the continued existence of both the resource, and the populations reliant on the resource, is to be ensured."

(Dallas and Day 1993)

Water is one of our Earth's fundamental natural resources. It forms an integral part of life – without it nothing can survive. It plays a fundamental part, in maintaining the environment, food production, hygiene, industry and power generation. In a semi-arid country such as South Africa, water is highlighted as a scarce commodity (Malherbe 2006), unevenly distributed both spatially and through time (Ferreira 2008). Combined with vastly expanding populations, the demand for basic water needs increases exponentially, placing a significantly greater pressure on overall water demand, not only at a community level, but also at an individual level (Davies and Day 1998). Basson *et al.* (1997) stated that due to the burgeoning human population, and the "predictably unpredictable" rainfall regime in South Africa, water demands are expected to increase beyond supply within the next two decades. The effective management and sustainable utilization of water as a resource is becoming progressively ever more important. Understanding the natural structure and patterns of river systems is becoming vitally important in the management and use of water for all (Schael 2006).

1.1 Managing aquatic ecosystems

"to manage the quantity, quality and reliability of the nation's water resources in such a way as to achieve optimum, long-term, environmentally sustainable social and economic benefit for society from their use."

(DWAF 1997, cited by Roux 1999)

Odum (1971) defined an ecological system or ecosystem as "any unit that includes all the organisms (i.e. the community) in a given area interacting with the physical environment so that flow of energy leads to clearly defined trophic structure, biotic diversity and material cycles (i.e. exchange of material between living and non-living parts) within the system." Ecosystems thus include both physical and chemical (abiotic) environments in addition to biological (biotic) components. Roux (1999) went one step further and defined an aquatic ecosystem as those environments that provide a medium for habitation by aquatic organisms and sustain aquatic ecological processes, supplying drinking water for wildlife, and water for maintaining riparian biota and processes.

Not only do aquatic ecosystems provide water for aquatic species and processes, but also provide a basic resource which human society relies on for quality of life, including its health and recreation (Roux 1999). Water is the primary resource upon which social and economic developments are based and sustained (DWAF 1994). Aquatic ecosystems must, therefore, be effectively protected and managed to ensure they retain inherent vitality and remain fit for ecosystem, domestic, industrial, agricultural and recreational uses, now and in the future. To ensure this, social, economic and ecological factors must be considered in an inter-related manner when managing aquatic ecosystems.

The social element includes the concepts of beauty/aesthetics, value, history and relevance (Roux 1999). These concepts must be defined by the beholder, and are derived from cultural norms and expectations as they relate to natural systems (Steedman 2004). The economic element includes aspects such as resource use, manufacturing, distribution and consumption (Minns 1995). The ecological element of an ecosystem includes factors such as species distribution and abundance, the structure, stability and productivity of ecosystems and the ability of ecosystems to self-organise and evolve (Roux 1999). All three factors of ecosystems are inter-related and each is as important as the others (Figure 1.1).

The social element is dependent on the ecological element, and the economic element is dependent on the social and ecological elements. If focus was initially placed on either "social well being" or "economic development", the environment would experience devastating impacts, limiting any further future benefits. Therefore, the goals of societies must reflect the constraints and boundaries inherent to natural systems, and thus set resource management as a priority, not focussing on how the resource can be used, but on the ecological state in which the resource should be maintained and how it should be protected to allow sustainable utilization (Cocklin *et al.* 1992).

Effective decision-making, and resource management, are ultimately dependent on the information provided by appropriate and proper resource monitoring. For this reason, the development and application of various monitoring tools are important steps that need to be initially taken in the ongoing process of harmonising economic development, human welfare and environmental protection (Roux 1999, Ferreira 2008). The collection of appropriate and adequate data, of dependable quality, is essential for generating the kinds of information that will effectively guide decision-making in the ecosystem arena (Roux 1999). This basis in South Africa - adopted by DWAF, for measuring and assessing the ecological component of aquatic ecosystems, is ecological integrity.

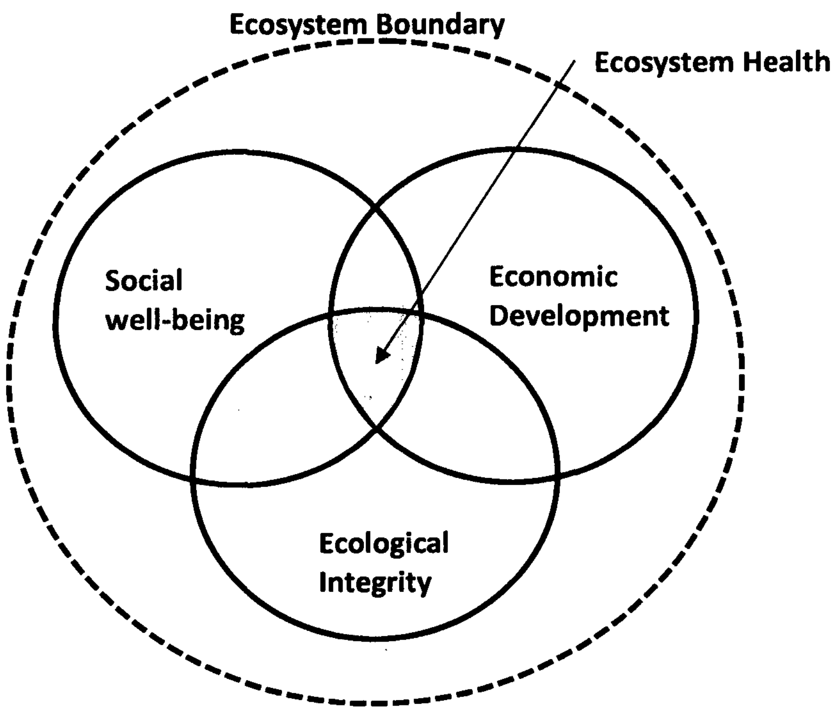


Figure 1.1: The inter-relatedness of the ecological, social and economic elements of an ecosystem (adapted from Roux 1999).

1.2 Biological Integrity (Ecosystem Health)

"Biological integrity is required if an aquatic ecosystem is to support and maintain a balanced, integrated, adaptive assemblage of organisms having a species composition, diversity and functional organisation comparable to that of the natural habitats within a region."

(Karr and Dudley 1981).

To produce sensible guidelines for limiting the effects of economic or social impacts on aquatic biota, it is necessary to have some measure of biological integrity or ecosystem "health" (Dallas and Day 2004). Ecosystem health, whilst being a useful and widely understood concept, is difficult to describe in precise scientific terms (Schofield and Davies 1996).

The view taken in this study is similar to that of Karr (1999), where health or integrity as a word and concept in ecology, is useful precisely because it is a concept that all people are familiar with. It is not a huge leap from "my health" to "ecosystem health" (Karr 1999). There is no denying that ecosystem health resonates with the wider public, implying, as Hasket *et al.* 1992) stated, vitality, vigour, unimpaired function and feelings of good health. Throughout this study, ecological integrity of a river will be defined as the "ability of the river to support and maintain a balanced, integrated composition of physico-chemical habitat characteristics, as well as biotic components, on a temporal and spatial scale, that are comparable to the natural characteristics of ecosystems of the region" (Roux 1999).

Indicators for measuring ecosystem integrity

Ecosystem indicators are characteristics of the environment, both biotic and abiotic, that can provide information on the degree of ecosystem integrity (Thornton *et al.* 1994). Such indicators can be used for measuring and quantifying change in an ecosystem. Five major classes of environmental factors may affect the ecological condition or integrity of aquatic ecosystems (Roux 1999): physico-chemical variables, flow regime, habitat structure, biotic interactions and energy sources.

These components highlight the complexity and interactive nature of aquatic ecosystems (Dallas 2002). Alterations to the physical, chemical or biological processes associated with these factors can adversely affect the ecological integrity of the water body. Figure 1.2 illustrates how the alteration of the dynamic character of any of these factors, as a result of natural events or anthropogenic activities, can have an impact on the ecological integrity of an aquatic ecosystem. Ideally a suite of indicators therefore needs to be considered in the assessment of overall ecological integrity.

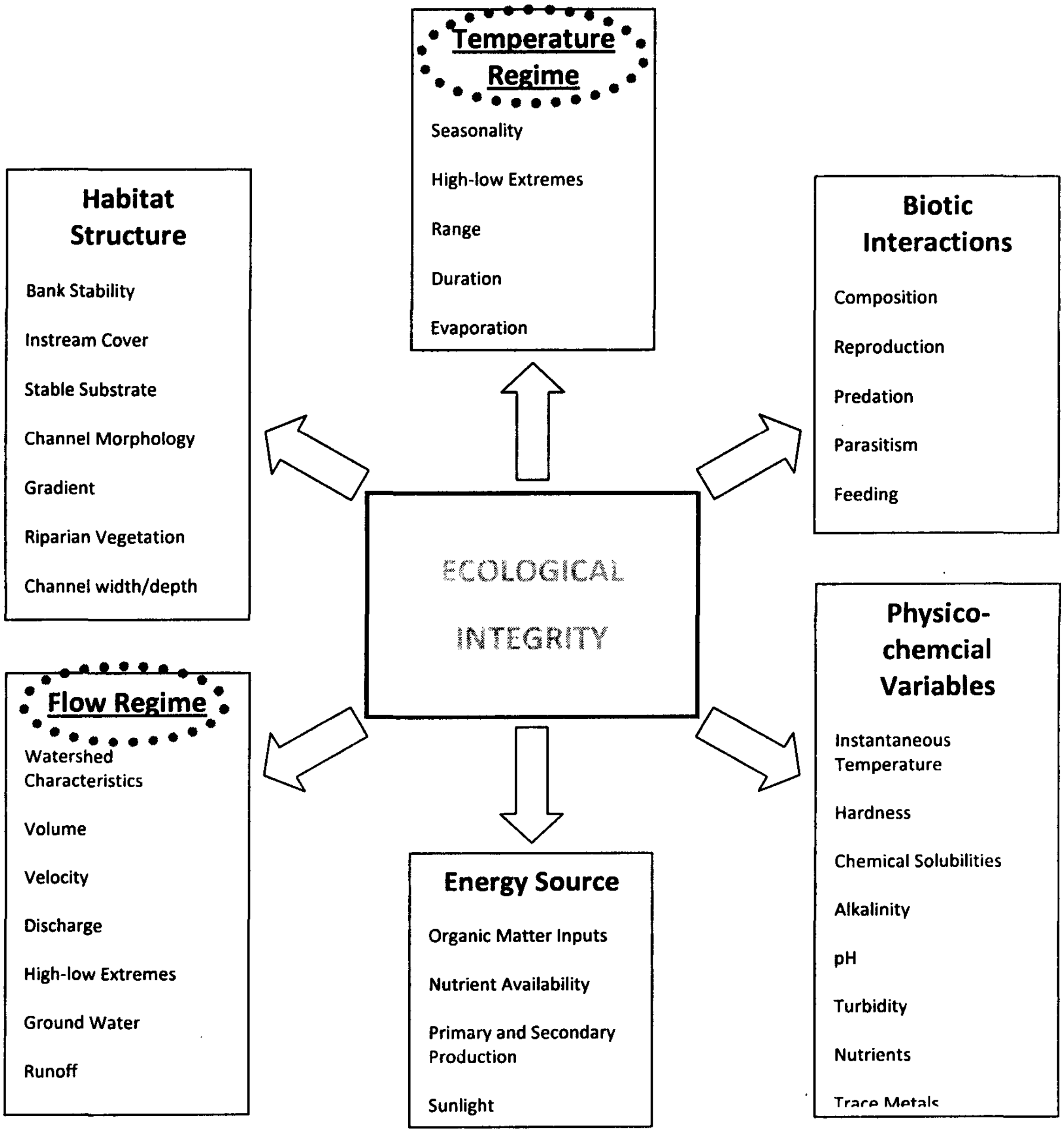


Figure 1.2: Factors affecting ecological integrity, adapted from Roux (1999), with the addition of Temperature Regime. Flow and Temperature Regimes highlighted by dashed circles, indicating that these two components of ecological integrity will be explored.

Biological assessment (bioassessment) is normally achieved using a series of tools that integrate the effects of these indicators. Its utility in assessing environmental condition, in such as water quality and general river condition, in spatially and temporally variable lotic ecosystems, is investigated in this study.

1.3 Origins of Biomonitoring

'The complexity of biological systems and the diverse influences of human activities require a multimetric approach that reflects all important aspects of stream biology ranging from individual to assemblage level, and responds to anthropogenic activities in a detectable manner."

(Kerans and Karr 1994)

Disturbance

A disturbance is defined by White and Pickett (1985) as "any relatively discrete event in time that disrupts ecosystem, assemblage or population structure and changes resources, substratum availability, or the physical environment."

Physico-chemical monitoring

Historically, data gathered to assist the management of water resources was non-biological in nature (Barbour *et al.* 1996). Monitoring actions focussed largely on chemical and physical water quality variables, and regulatory efforts were aimed at controlling individual physico-chemical stressors (Roux 1999). The presumption was that measureable improvements in water quality would result in an improvement in ecosystem condition.

The measurement of only physical and chemical water quality variables cannot provide an accurate account of the overall condition of an aquatic ecosystem, however (Roux 1999). Assessment of the common physical attributes and chemical constituents of water, although essential for determining the type and concentration of pollutants entering a river, is limited to the period of sample collection and to the physical and chemical analyses performed. Widely recognised limitations of physico-chemical monitoring include the intermittent nature of the measurements, in that unless samples are collected continuously, pulsed releases of effluents that result in an alteration of water quality may not be recorded. Furthermore, the potential number of constituents that could be present is vast, while routine analyses are usually limited to non-toxic determinants such as temperature, conductivity, total alkalinity and nutrient concentrations. The number and variety of potentially toxic compounds (e.g. trace metals, biocides) that could affect water quality is considerable, as is the cost of analysing the full range of these compounds, so routine testing for all possible toxins is thus unrealistic. The sensitivity of chemical analytical methods when measuring very low concentrations of pollutants may also be inadequate, particularly for substances that are characteristically present in these low concentrations but which are persistent and tend to accumulate in the environment (Dallas and Day 2004).

A further complicating factor, when assessing the effect of altered water quality by means of physical and/or chemical data, is that of synergism and antagonism (Jackson and Davis 1994). Although each water quality variable has an effect on aquatic organisms (beneficial or detrimental), the overall effects of changes in the magnitude of more than one variable, may be greater or less than the effect of each in isolation (Dallas and Day 2004). For example, changes in pH are particularly significant in altering the toxicity of a variety of chemical constituents, including trace metals (Dallas and Day 2004). These subtle magnifying and reducing effects would not necessarily be revealed by routine physico-chemical monitoring. In addition, while focussing on physico-chemical monitoring, other structural impacts that have led to alterations of river flow, loss of habitat area, loss of habitat diversity, obstructions to passage through streams and riparian degradation, may be overlooked (Harris 1995, Roux 1999).

Bioassessment using riverine macroinvertebrates

A development worldwide to address the cumulative effects of changes within aquatic ecosystems has been the introduction of in-stream biological-effect or response monitoring in water resource management (Roux 1999). Bioassessment or "biomonitoring" is increasingly being recognised as an important component in, not only aquatic ecosystem assessments, but overall ecological monitoring and assessment of water resources, and may be defined as the utilisation of one or more components of the biota to assess the effect of a change in another component such as water quality (Dallas 2002). Simply put, it is the process of determining whether instream processes have altered the biological properties of an ecosystem (Hawkins and Norris 2000). Since organisms are dependent on the medium in which they live, they are sensitive to all alterations to the water body by, for example, pollution or habitat alteration. It is reasonable to assume that such alterations will be reflected in the composition of the biotic assemblages found in the system. Elements of the biota can therefore be seen as a series of indicators of the overall ecological condition of the aquatic ecosystem (Hawkes 1979), thereby providing reflection of the effect of changes in water quality on the ecosystem by enabling long-term analysis of both regular and intermittent discharges, varying concentrations of pollutants, single and multiple pollutants, and synergistic or antagonistic effects (Dallas and Day 2004).

Bioassessment provides a time- and constituent-integrated assessment of the ecological or biological integrity of the system under consideration (Dallas 2002). It has been acclaimed as a more sensitive and reliable measure of environmental conditions than both physical and chemical measurements (Warren 1971), and using living organisms as indicators of disturbance in an ecosystem has proven successful (Rosenberg and Resh 1993).

Origins of biomonitoring systems and indices

More than a century ago, people recognised that human activities produced pollution harmful to the biota (for a review see Davis 1995). The Saprobien System, which stems from the research work of Kolkwitz and Marsson in German rivers in the early 1900s, is generally considered to be the first biological scoring system for the assessment of water quality in river ecosystems (Ollis *et al.* 2006). Within the same period, Kofoid (1903) made an effort to track the extent of biological degradation; biological degradation was even considered an indicator of the presence of human activities. So began biological monitoring (Kofoid 1903 and 1908).

Since then, biotic indices, defined as being numerical indices that use one or more components of biota to provide measure of biological condition, based on macroinvertebrate assemblages, have proven to be useful measures of stream or ecosystem "health" and are widely applied today (e.g. Hellawell 1986, Rosenberg and Resh 1993, Ollis *et al.* 2006), with many countries beginning to rely on biological assessments as their primary measure of the ecological health of surface waters (Gerritsen *et al.* 2000, Ollis *et al.* 2006).

One of the advantages of biotic indices, as reported by Ollis *et al.* (2006) in a detailed overview of macroinvertebrate-based biotic indices used for river assessment worldwide, is that biological monitoring data can be summarised and presented as simple, numeric or categorised indices. These indices allow the results of ecological assessments to be communicated in a way that is understandable to natural resource managers, decision-makers, politicians and the general public (Fore *et al.* 1996), providing a scientific basis for management decisions that affect aquatic resources. Historically, biotic indices have often been calculated *a posteriori* from quantitative macroinvertebrate sampling (e.g. Chutter 1972, Hilsenhoff 1988). However, limited time and resources available associated with such rigorous and intensive sampling prompted the development of rapid qualitative bioassessment methods such as the Australian SIGNAL biotic index (Chessman 1995), the BMWP system (Biological Monitoring Working Party) (e.g. Wright 1995) and the BMWP-based SASS (South African Scoring System) (Chutter 1998).

Biotic effects on riverine macroinvertebrate assemblages that have been effectively assessed using biotic indices worldwide include the effects on receiving water bodies of organic pollution in Wisconsin, USA (Hilsenhoff 1988), discharges from sewage treatment works in Rome, Italy (Solimini *et al.* 2000) and in the rivers Esk (Scotland), Ivel (England) and Taf (Wales) (Balloch *et al.* 1976), wastewater discharges and mixed effects of runoff such as storm water runoff in New South Wales, Australia (Chessman *et al.* 1997), the effects of agriculture and fertilisation in Alaska (Smith *et al.* 2007), mining-derived impacts in Spain (Garcia-Criado *et al.* 1999) and in south-eastern Australia, which includes damming (Chessman and McEvoy 1998) and metal pollution in the Tennessee Valley, USA (Kerans and Karr 1994).

Advantages of using aquatic macroinvertebrates

Various organisms have been used in the bioassessment of water quality and ecological integrity of aquatic ecosystems. These include bacteria, protozoans, diatoms, algae, macrophytes, macroinvertebrates and fish (Barbour *et al.* 1999, Brown 2001, Chessman 1995, Chutter 1998, Dallas 1995, Dallas and Day 1993, Dallas and Day 2004, Friedrich *et al.* 1996, Hawkes 1979, 1982, Hellawell 1986, Hynes 1960, Manly 1995, Milner and Oswood 2000, Norris *et al.* 2001, Rosenberg and Resh 1993, Plafkin *et al.* 1989, Wright 1995). There is a general consensus that benthic macroinvertebrates are amongst the most sensitive components of aquatic ecosystems and are the most widely used group (Cairns and Dickinson 1971, Hellawell 1986, Norris 1994, Resh *et al.* 1995, Dallas 2002), for lotic systems (Metacalfe 1989, Metacalfe-Smith 1994, Knoben *et al.* 1995, Resh *et al.* 1996, Norris and Thoms 1999, Milner and Oswood 2000, Sandin *et al.* 2001), because they reflect the cumulative effects of factors affecting an aquatic ecosystem over time (Balloch *et al.* 1976, Cullen 1990, Roux and Everett 1994, Chutter 1995, Dallas 1995, Barbour *et al.* 1999, Karr and Chu 2000). Consequently, these biological indicators are a key to environmental and water resources management, and are used in policy making and implementation (Chessman 1995, Norris and Norris 1995, Noss 1995, Moog and Chovanec 2000).

There are several advantages to using benthic macroinvertebrates in bioassessment (summarised in Hellawell 1986, Metcalfe 1989, Reynoldson and Metcalfe-Smith 1992, Rosenberg and Resh 1993, Metacalfe-Smith 1994, Dallas 1995). Briefly, benthic macroinvertebrates are largely non-mobile (when in their aquatic phase), and relatively abundant inhabitants of rivers, occupying most habitats (Ollis *et al.* 2006). Thus macroinvertebrates are representative of the location being sampled, which allows effective spatial analyses of disturbance (Dallas and Day 2004). There are often many species within a community with varying sensitivity to stresses and relatively quick reaction times, resulting in a spectrum of graded, recognisable responses to environmental changes. Macroinvertebrates have life cycles that are long enough (compared to other groups such as planktonic organisms) for temporal changes caused by disturbances to be detected, but short enough to enable the observation of recolonisation patterns following disturbance. They are relatively easy and inexpensive to collect, particularly if qualitative sampling is undertaken, and are well-suited to experimental approaches to biomonitoring.

Whilst indicating that a water body is impacted, living organisms (including macroinvertebrates) seldom provide insight into the cause of the problem (Dallas and Day 2004). Reynoldson and Metcalfe-Smith (1992) state that biological systems should be the standard for monitoring assessment, and that the role of chemical and physical analyses is most important in the identification of factors causing impairment, and the selection of appropriate remedial actions. For this reason, Hawkes (1997) concluded that bioassessment and physico-chemical monitoring are complementary.

The South African approach

In South Africa, riverine macroinvertebrates are some of the most commonly assessed components of the biota and SASS is used as the routine rapid bioassessment tool to assess water quality and general river condition. It forms the backbone of the River Health Programme (RHP), a national programme aimed at assessing the ecological state based on macroinvertebrates, whereby each macroinvertebrate taxon is allocated a sensitivity/tolerance score according to the water quality conditions it is known to tolerate (Dallas 1997). Data interpretation is based on two calculated values, namely SASS5 score, which is the sum of the sensitivity/tolerance scores for taxa present at a site, and average score per taxon (ASPT), which is SASS5 score divided by the number of taxa. SASS has proved to be an efficient and effective means of assessing water quality impairment and general river health (Dallas 1997).

Tools for interpreting bioassessment data, such that an observed effect is in some way quantified, vary from comparatively simple tables that provide values for different categories of impact (e.g. SASS: Chutter 1998) to complex predictive models, which relate environmental variables to biotic communities (e.g. NBI: Smith *et al.* 2007). Whatever level of complexity is adopted in data interpretation, it is necessary to know the "expected" condition, either as an expected index value or as an expected macroinvertebrate assemblage or both (Dallas 2000). This "expected" condition is referred to as a reference condition. Bioassessment is generally applied within the context of ecological reference conditions, which represent an expected, realistic and scientifically authentic ecological benchmark against which bioassessment information is compared (Dallas and Day 2004).

1.4 Ecological Reference conditions

"An ecological reference condition is the condition that is representative of a group of minimally-disturbed or 'least-impacted' sites organised by selected physical, chemical and biological characteristics."

(Reynoldson *et al.* 1997)

Reference conditions define what is expected, or what is found naturally, at a site or in a particular area or region, and provide a means of comparing observed conditions with expected conditions so that the degree of impairment or deviation from natural conditions can be ascertained This serves as a foundation for developing criteria for the protection of aquatic ecosystems. Unfortunately, generally a historical lack of attention to the conservation of pristine areas has resulted in the scarcity of non-impacted sites in most regions, especially in lowland areas. Consequently, minimally-disturbed or least-impacted sites are generally used to determine the best attainable reference condition (Roux and Everett 1994, Hughes 1995, Omernik 1995, Reynoldson *et al.* 1997, Norris and Thoms 1999).

As Dallas and Day (2004) reported, a reference condition is usually derived from conditions measured at a group of similar reference sites, although single site-specific reference conditions are sometimes also used. Conditions are typically used in an upstream/downstream or "paired" scenario where a monitoring site is compared to the condition at a single reference site, where there are concerns with specific point sources of impact (Day and Dallas 2004). A typical example would be sites upstream and downstream of a dam, increasing water depth upstream, but reducing water-flow downstream, or any other potential anthropogenic impact.

Once the best attainable reference conditions have been decided upon for the aquatic ecosystems of a region, these can be used as benchmarks to classify the degree of impairment at monitoring sites (Hughes *et al.* 1986, Norris 1994, Hughes 1995, Resh *et al.* 1995, Gerristen *et al.* 2000, Dallas 2002). However, before the reference conditions for a region can be defined, a classification system is required to group similar reference sites, where biological attributes from sites within a homogenous entity are more similar to one another than to sites within different homogenous entities. In this way, classification systems attempt to partition spatially variable characteristics of lotic systems, providing a more efficient monitoring and assessment programme (Ollis *et al.* 2006).

Defining reference conditions with the use of macroinvertebrates

The two approaches for classifying reference sites are fundamentally different even though they begin with the same premise and require the same data (see Ollis *et al.* 2006). The regional approach (i.e. ecoregions), which is widely used in the United States (e.g. Gerritsen *et al.* 2000), classifies reference sites *a priori*, based on geographic and physical attributes. This approach assumes that monitoring site characteristics match the chosen regional reference sites (Reynoldson *et al.* 1997), predefined largely using mapped landscape characteristics such as climate, physiography, geology, soils and vegetation (Omernik 1987). Within the regional perspective, additional qualifiers such as stream size, hydrologic regime, elevation and natural riparian vegetation need to be considered for further partitioning variability of macroinvertebrate assemblages (Barbour *et al.* 1999). Metric measures such as richness, composition, tolerance/intolerance, feeding (e.g. functional feeding groups) or indices such as SASS5 Scores and Average Score per Taxon (ASPT) are then interpreted within the homogenous regions.

The multivariate approach been adopted by both the United Kingdom – RIVPACS (River Invertebrate Prediction and Classification System) (Wright *et al.* 1993) and Australia - AusRivAS (Australian River Assessment System) (Smith *et al.* 1999) within their respective bioassessment programmes. These programmes classify reference sites *a posteriori,* using multivariate analysis of macroinvertebrate fauna (Reynoldson *et al.* 1997). Faunal data are used to group sites *a posteriori* that have similar taxonomic composition, providing an objective way of grouping reference sites with similar macroinvertebrate assemblages. Groups thus do not conform necessarily to geographic stratification (Gerritsen *et al.* 2000). As Reynoldson *et al.* (1997) reported, the multivariate approach does not assume that monitoring sites exactly match reference site groups, but instead calculates the probability of belonging to each of the groups (e.g. a predicted or "expected" macroinvertebrate assemblage is compared with the actual assemblage and the ratio of observed/expected

(O/E) taxa is used as a measure of ecological condition (Wright *et al.* 1993). The expected scores for a monitoring site may then be calculated based on the expected taxa.

There has been much debate on the relative scientific validity of each approach and studies give support to both the ecoregions approach (e.g. Feminella 2000) and the multivariate approach (e.g. Marchant *et al.* 2000, Sanden and Johnson 2000). Yet others propose an intermediate option which utilises a geographic framework for initially partitioning reference sites, but which is validated and refined by subsequent analysis of the biological data (e.g. Gerristen *et al.* 2000, Johnson 2000).

A major limitation of using macroinvertebrates in bioassessment is their variable distribution and patchiness that result in spatial and temporal variability in macroinvertebrate assemblages (e.g. Marchant 1988, Palmer *et al.* 1991). Although the causes of spatial and temporal variability in lotic systems are not always known, it is important that this variability be taken into account when macroinvertebrates are used in bioassessment.

1.5 Spatial and Temporal Variability in riverine ecosystems: flow and thermal regimes and response of macroinvertebrate community assemblages

Riverine ecosystems are extremely complex systems, naturally heterogeneous over space and time (Palmer and Poff 1997). Natural variability may occur at multiple spatial and temporal scales, driving and affecting a multitude of abiotic and biotic factors, influencing and generating both patterns and process in ecological systems (Townsend 1989). Ecosystems are shaped by a number of environmental forces that impart to them a specific structure, species composition and fluctuations in the abundance and distribution of organisms (James and King 2010). These forces are ecological drivers – factors that exercise an overriding influence on the fitness and survival of individuals and populations (Poff and Ward 1990). In rivers, the primary drivers are climate, geology and topography, manifested in stream discharges (flow), stream size, temperature, substrate and resource availability (Wohl *et al.* 2007). Variations in any of these drivers may lead to spatial and/or temporal variability in the physico-chemical and macroinvertebrate community make-ups within a specific stream. Understanding both the spatial and temporal scale of changes in abiotic variables, and the impact these may have on the biotic assemblages is important in the context of bioassessment.

Spatial Variability

Spatial variability of environmental variables and macroinvertebrate assemblages are widely studied characteristics of lotic systems (Hawkes 1975, Statzner and Higler 1986, Hawkins *et al.* 1997) and , in context with bioassessment, spatial heterogeneity is often taken into account by partitioning areas into relatively homogenous regions. The underlying assumptions are that natural variation is predictable among systems within the same region (e.g. in this study at catchment level) where environmental features and macroinvertebrate assemblages are similar, and that by stratifying natural variation into spatially distinct, homogenous catchments, one can detect responses to disturbance at one site by comparing it to a reference site in the same catchment.

The ecoregion approach adopted by South Africa is one that was developed by Dallas and Day (2007), which classifies reference sites based characteristics such as climate, vegetation, physiography, geology and soils. Several studies have shown that ecoregions adequately correlate with water chemistry (Ravichandran *et al.* 1996) and macroinvertebrate assemblages (Harding *et al.* 1997, Gerristen *et al.* 2000, Feminella 2000). By contrast, others have shown that ecoregional differences do not adequately explain patterns in water chemistry (Harding et al 1997), and in macroinvertebrate assemblages (Hawkins and Vinson 2000, Marchant *et al.* 2000). With the discrepancies in spatial scale, studies have highlighted the need for a sub-regional level (Rabeni and Doisy 2000, Sandin and Johnson 2000) or ecosystem-type (Johnson 2000) classification below that of ecoregions (e.g. to catchment scale) for further reducing spatial variability. This approach provides a better understanding of the ranges in physico-chemical variables and composition of macroinvertebrate assemblages. The sub-regional (or termed catchment) approach was taken in this investigation, limiting sites solely to the Palmiet River catchment.

Dallas and Day (2007) remarked that understanding factors contributing to spatial variability in environmental variables and macroinvertebrate assemblages in riverine ecosystems is a complex task, since potential influences act at several scales. River systems reflect the characteristics of the catchment, site and water chemistry. Many variables within each of these components interact with one another and with biotic components of aquatic systems to create spatially complex assemblages.

The reduction from ecoregion to catchment approach however, does not eliminate outside catchment variation. In a statement by Hynes (1975), "In every respect, the valley rules the stream", he highlighted that characteristics within a particular stream may be unique to the the specific system. Altitude (Wright 1995), longitude/latitude (Marchant *et al.* 1997) and channel slope (Collier 1995) are all (been shown to be) catchment-scale environmental variables that affect biotic distributions. At a scale of site, factors such as stream width (Linke *et al.* 1999), and flow pattern (Smith *et al.* 1999) have been strongly associated with macroinvertebrate structure. At a scale of habitat, variables such as the nature of t he substratum, including substrate diversity (Marchant *et al.* 1997), type (Wohl *et al.* 1995) and texture (Downes *et al.* 1998) are considered to exert a strong influence on biotic community structure.

Stream water chemistry, which is influenced by, for example, geology (Day and King 1995) may strongly influence macroinvertebrate assemblages (Poff and Allan 1995). Stream temperature (Hawkins *et al.* 1997), conductivity (Collier 1995), pH (Reynoldson *et al.* 1997), dissolved oxygen (Dallas and Day 1993), turbidity (Dallas and Day 2003) and stream velocity (Allan and Castillo 2007) are physico-chemical variables known to influence biotic assemblages.

Temporal variability

Lotic systems exhibit daily and seasonal periodicity in factors such as discharge and temperature, affecting instream biological communities, with macroinvertebrate assemblages being been linked to precipitation events and stream hydrographs (McElravy *et al.* 1989). In the south-western Cape, with its Mediterranean climate, river systems often show evidence of seasonal differences. Many aquatic organisms are known to have specific water chemistry and habitat requirements, and seasonal differences in stream factors such as discharge (McElravy *et al.* 1989) and temperature (Hawkins *et al.* 1997) may lead to variation in instream water chemistry variables and the distribution and abundance of macroinvertebrate assemblages.

The flow and temperature regimes within the Mediterranean-climate region of the Western Cape, generally follow that of rainfall, which has been termed by Davies and Day (1998) as being "predictably unpredictable", and consequently exhibit both strong seasonal and inter-annual variability. In this region, high flows and low temperatures abruptly commence in autumn or early winter (April-June), and floods occur during late autumn, winter and early spring (May-September). Warming and drying, and declining flow, are gradual over several months in summer (December-March), ending abruptly in autumn, when the next year's rains commence. Although the separate occurrence of flooding and drying, cooling and heating up, is often predictable within certain periods of the year, the intensity and frequency of these periods vary greatly from year to year, depending on the frequency and intensity of rainfall and temperature.

The Flow regime

"Flow, arguably the most characteristic physical attribute of stream ecosystems, plays a central role in stream ecology."

(Hynes 1970)

The varying nature of flow, spatially and temporally, may have a variety of effects on the stream ecosystem, depending on the magnitude, frequency, duration and timing of hydrologic conditions (Poff *et al.* 1997, Gasith and Resh 1999). These effects include scouring of accumulated sediment and debris, and redistribution of streambed substrate and organic matter in the channel; changing channel morphology and forming new erosional (riffles) and depositional (pools) zones; washing away in-channel and encroaching riparian vegetation; restoring channel connectivity; and homogenizing water quality conditions along the stream channel (Keller 1971, King *et al.* 1988).

The role of flow in determining the distribution and abundance of aquatic organisms has been of interest to ecologists since early this century (Gasith and Resh 1999). For the biotas of streams, the processes of flooding and drying are key environmental factors influencing distribution: finding refugia (Boulton 1989, Harrison 2000) and recolonisation (Resh 1982, 1992), abundance: reduction in population densities (Bunn *et al.* 1986, King *et al.* 1988, McElravy *et al.* 1989), and life histories: evolutionary adaptations (Stanley *et al.* 1994), flexible life cycles (Bunn *et al.* 1989) and optimal hatching periods (Williams 1996). Numerous examples of behavioural (Statzner *et al.* 1988) and morphological (Resh and Solem 1996) adaptations and responses of stream organisms to increased flow have been described. The survival of many organisms depends on the ability to adapt to extreme variations in flow (flooding and drying), and those organisms unable to adapt would be absent from that river ecosystem.

The Temperature regime

> "Temperature is probably the most important, but least discussed, parameter in determining water quality."
>
> Blakey (1966)

The importance of water temperature in river ecosystems has been recognised for some time (e.g. Ward 1985, Cassie 2006, Dallas 2007). Scientific research between the 1960s and the 1980s mainly focussed on the effects of thermal pollution resulting from the installation of power stations and changes in the thermal regime below impoundments. More recently the potential impact of climate change on aquatic ecosystems has been drawing in research (Dallas 2007). Studies have largely focused on reporting and understanding the thermal regime, including water temperature modelling. Documenting anthropogenic causes of thermal changes and the ecological consequences of these changes; and developing methods for estimating thermal tolerance ranges *via* both field experimentation and laboratory studies are now common (Dallas 2007).

In the northern hemisphere (Europe, North America and Japan) the thermal characteristics of lotic habitats have been reasonably well documented (Ward 1985) and are considered important in influencing life histories of aquatic organisms (e.g. Brittain 1975, Vannote and Sweeney 1980). Thermal data for southern hemisphere rivers is limited, however, with most information for Africa and Australia derived from ecological and hydrological studies (e.g. Harrison and Elsworth 1958, Oliff 1960, Appleton 1976), with a few focussed studies on water temperature (e.g. Brittain 1991, Rivers-Moore and Jewett 2004, Rivers-Moore *et al.* 2004, 2005).

Lotic systems in regions of seasonal climates, such as South Africa, exhibit patterns of diel and annual temperature periodicity (Ward 1985). Temporal patterns of thermal change may vary both within and among rivers, and at any given time a river has different temperatures at different locations. The annual (seasonal) cycle is a sinusoidal one, with temperatures highest in the summer and lowest in the winter. The amplitude, as revealed by minimum and maximum daily temperatures, may vary on the basis of regional factors such as altitude and latitude (Dallas 2007).

Understanding temporal trends and variation in water temperature is necessary if the ecological responses of aquatic organisms to changes in water temperature are to be determined. Except for birds and mammals, almost all organisms associated with fresh water are poikilothermic (i.e. they are unable to control their body temperatures, which are therefore the same as that of the ambient water temperature). Aquatic organisms are very susceptible to changes in water temperature, thus temperature is recognised as a key environmental variable structuring aquatic invertebrate assemblages (Arscott *et al.* 2001).

Temperature effects may be evident at the individual level through physiological and behavioural effects; at the population level through development of the individuals, fecundity and survival; and at the community level by favouring temperature tolerant taxa over temperature intolerant ones leading to a shift in community structure (Mitchell 1999). All organisms have a range of temperatures at which optimal growth (adult size), reproduction and general fitness occur. This is often termed the "optimum thermal regime" (Vannote and Sweeny 1980). Temperatures outside the optimum thermal regime may affect geographical distribution and community structure (Reid and Woods 1976, Vannote and Sweeney 1980, Hart 1985, Hawkins *et al.* 1997, Kishi *et al.* 2005, Bell 2006), growth (Harper 1973, Thorup 1973, Lavery and Costa 1976, Markarian 1980, Hury n 1996), metabolism

(Hellawell 1986), food and feeding habits (Kishi *et al.* 2005), reproduction and life histories (Nebeker 1971a, b, c, 1972, Campbell 1986, King *et al.* 1988, Brittain 1991, Huryn 1996) and movements and migrations (Elliot 2000, Gardener *et al.* 2003, Bell 2006) of aquatic organisms.

The importance of flow and temperature

There can be no question that the natural flow and temperature regime has a profound influence on the biodiversity of rivers. Both river flow and temperature are interrelated and are thus arguably two of the most important parameters which determine many aquatic habitat attributes and the general health of river ecosystems. The flow and temperature regimes influence biodiversity via several interrelated mechanisms that operate over different temporal scales. The relationship between biodiversity and the physical nature of the aquatic habitat is likely to be driven primarily by large events that influence channel form and shape by limiting overall habitat suitability. Many features of both flow and temperature influence life history patterns, especially the seasonally and predictability of the overall pattern, but also the timing of particular flow and temperature events. Slight temporal changes in flow or temperature conditions, if maintained for a period of time, may lead to an alteration of community assemblages. The structure and function of a riverine ecosystem and the many responses and adaptations of its biota are all dictated by these biotic patterns of temporal variation in river flow and temperature. For effective freshwater ecosystem management, it is essential that we have a better understanding of both these abiotic processes, ultimately resulting in and providing a better protection of our riverine ecosystems.

Necessity for Temporal Studies in Pristine Sites

Very few rivers in South Africa and even in other parts of the developed world, still have natural flow regimes and most are impacted by water-resource developments and changes in land use (King et al 2000). Boulton (2003) stated that "investigations exploring the direct and indirect effects of temporal changes in flow and temperature on aquatic ecosystems are hampered, thus virtually impossible, by the lack of adequate pre-impact data or suitable reference sites." There is now general agreement among scientists and many managers that to protect freshwater biodiversity and maintain the essential goods and services provided by rivers, we need to mimic components of natural flow variability, taking into consideration the magnitude, frequency, timing, duration, rate of change and predictability of flow events (e.g., floods and droughts), and the sequencing of such conditions (Arthington *et al.* 2006).

1.6 South African Macroinvertebrate-based Biomonitoring for Rivers

South Africa has recognised the scarcity of fresh water and the importance of protecting river ecosystems through the National Water Act (NWA, No. 36 of 1998). As custodian of the water resources in South Africa, the Department of Water Affairs (DWA) is the primary agency responsible for the ongoing management of aquatic resources, viewing aquatic ecosystems as pillars on which social and economic developments are based and sustained. DWA ensures that water bodies retain their ecological characteristics in order to remain fit to provide basic needs of humans as well as to support economic growth (Roux 1999). According to DWA (2003) a water resource is an ecosystem that includes the physical and structural habitats (both instream and riparian), the water and aquatic biota and all the processes which link habitat, water and the aquatic habitat. This definition stresses the fact that water resources are linked with other features and processes of nature (Ferreira 2008). It is thus important to manage them in such a way that takes into account their dynamic character.

DWA seeks to both maintain existing healthy ecosystems, and to improve or restore ecosystems which are impaired beyond their desired state. To address the need for information on the state of aquatic ecosystems in South Africa, as well as to reach the above set goals and to fulfil these promises, the DWA launched the River Health Programme in 1994, primarily focussing on gathering information concerning the ecological state of riverine ecosystems (Roux 1999).

SASS5 (South African Scoring System, Version 5)

SASS5 is one of the primary indices that have been used in the RHP to date, and makes use of a biotic index, SASS, in which each macroinvertebrate taxon is pre-assigned a sensitivity weighting based on its tolerance to water quality impairment. The index is applied within a spatial framework that takes into account potential natural variation in macroinvertebrate assemblages that respond to geographic and/or habitat differences. Monitoring data are interpreted relative to a derived reference condition that takes into account the natural variation at a suite of reference sites, normally established *via* classification and ordination techniques (Dallas *et al.* 2010).

Origins of SASS (Dickens and Graham 2002)

South Africa has experienced a surge of interest in river assessment, since Chutter (1972) developed a Biotic Index, which was excessively labour-intensive within the field. In the 1990s Chutter set out to develop an index that would be faster and easier, basing it on the BMWP method (refer to page 29) developed earlier in the UK. His index, called SASS (South African Scoring System), evolved through several iterations (e.g. Chutter 1994, 1998; Dallas 1995, 1997, 2000a, b, 2001, 2005, 2007a, 2010). Over recent years the method has become the standard for the rapid bioassessment of rivers in South Africa and, indeed, southern Africa. It now forms the backbone of the National River Health Programme (RHP) (Uys *et al.* 1996) and is increasingly being included in the quantification of the Ecological Reserve as required by the South African National Water Act (1998).

1.7 EcoClassification and EcoStatus

An ecological classification approach termed EcoClassification, adopted by South Africa, aids in the quantification of the Ecological Reserve process by ascertaining and categorising the Present Ecological State (PES) of rivers compared to their natural state. The EcoClassification procedure allows for the grouping of rivers according to similarities based on a form of a directional (either bottom-up or top-down) nested hierarchy. The principle of river classification is that rivers grouped together as a particular level of the "typing" will be more similar to one another than rivers in other groups, providing a spatial framework for selecting reference sites.

In previous RHP approaches, as in Figure 1.3, individual characteristics within a studied system were analysed separately (Malherbe 2006). The Ecological Category (EC) for each individual component of the ecosystem was analysed, assuming that there was no relationship between different ecological characteristics within that system. Then, based on expert knowledge and judgement, these components were integrated. No formalised method existed and this made reproducibility of results nearby impossible (DWAF 1999). Thus, EcoStatus was developed (DWAF 2001, DWAF 2004) as a rule-based method that integrates the biophysical components of a river to provide a realistic and reproducible result about the EcoStatus of a river (Kleynhans *et al.* 2005).

EcoStatus consists of six indices, which are set out for ecosystem "responders" (fish, macroinvertebrates and riparian vegetation) and "drivers" (hydrology, geomorphology and water quality). The EcoStatus software integrates values for each index to provide the user with one value for the current EC (Malherbe 2006). The index developed for assessing the response of macroinvertebrates is the Macro-Invertebrate Response Assessment Index (MIRAI) (Kleynhans *et al.* 2005). MIRAI integrates the habitat requirements of the macroinvertebrate community with the macroinvertebrate assemblage at a site, and compares the current community structure with a reference condition, to determine the EC for macroinvertebrates (Malherbe 2006). The South African EcoStatus determination procedure has its origins in projects such as the Olifants River Reserve Study (DWAF 2001) and the Thukela River Reserve Study (DWAF 2004a). If no appropriate reference site exists, historical data from rivers in the same ecoregion can be used (Kleynhans *et al.* 2005).

The RHP has moved from instead of using SASS as the only protocol for macroinvertebrates, to the more holistic MIRAI approach. Chutter (1998) developed the SASS protocol as an indicator of water quality. Extensive literature has investigated SASS and its capabilities (Chutter 1972, 1995; Dallas 1995, 1997, 2004a, 2004b and 2007). It has since become clear that SASS gives an indication of more than mere water quality, but rather a general indication of the present state of the invertebrate community. SASS, however, has shortcomings, because the protocol was developed for application in broad synoptic assessments. Dallas (1995) highlighted that for this reason, SASS does not have a strong cause effect basis. Although MIRAI can be applied by using information collected during a standard SASS survey (Kleynhans *et al.* 2005), the MIRAI protocol was developed to overcome SASS shortcomings by providing a habitat-based cause-and-effect foundation to interpret the deviation of the aquatic macroinvertebrate assemblage from the reference condition. MIRAI has never been tested, or compared with the SASS protocol.

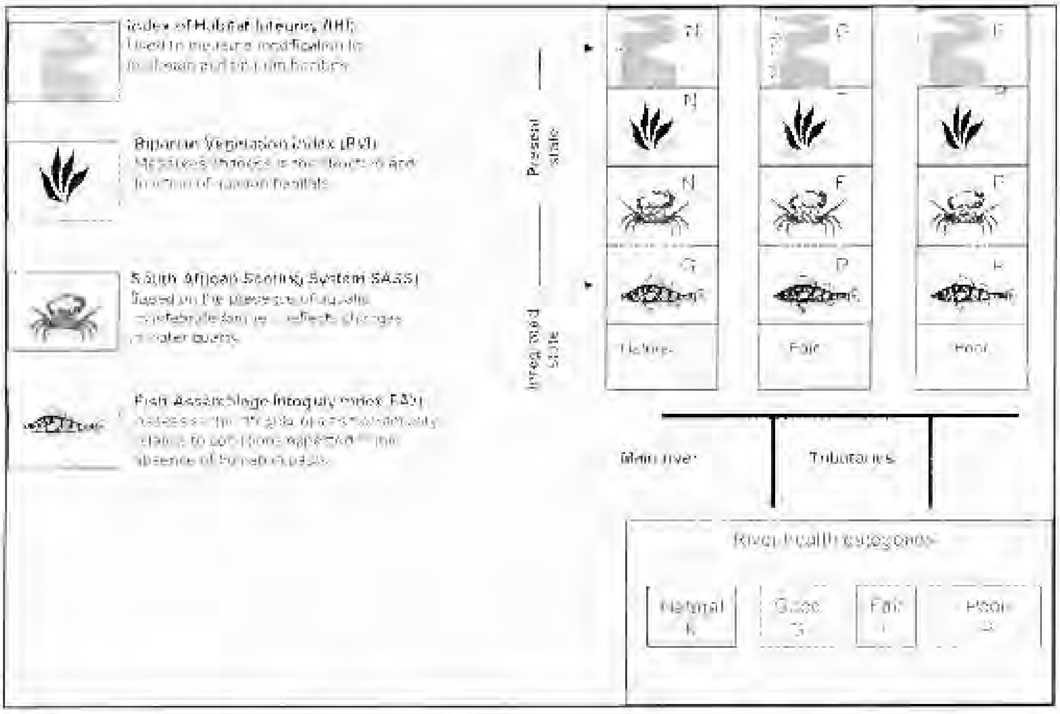


Figure 1.3: An example of the categorisation system used to indicate the health of river ecosystems in the River Health Programme (RHP) (taken from Roux 2004). The biological responses, as indicators of an ecosystem, are recorded in terms of a river health classification scheme that allocates a specific category of health to each river reach (Wepener 2008). The health categories used by the RHP are simply termed natural, good, fair and poor (Roux 2004).

To gain a full understanding of riverine ecosystem and function, knowledge and the development of reference conditions are required. These reference conditions, in turn, can be used as guides to ascertain differences from impacted sites. Importantly however, is the bioassessment protocol and relevant biotic indices used to ascertain such comparisons. A robust, scientific monitoring protocol that has been scientifically investigated, examining potential strengths and weaknesses of each is required if any strength is to be taken from reference versus impact results.

1.8 Study Aims

The aims of this study were to:

1. **develop spatial and temporal baseline data at reference sites within the Palmiet River catchment,** by examining physico-chemical variables, macroinvertebrate assemblages and effects on SASS5 biotic indices.

2. **investigate the use of MIRAI, and to compare it with SASS at the same set of sampling sites,** in order to ascertain which of the two bioassessment protocols provides the more accurate ecological categories (health index score) for impacted sites within the Palmiet River catchment.

Structure of thesis:

Chapter 2 describes the study sites .

Chapter 3 specifically explores the development of spatial and temporal baseline monitoring data at reference sites. To gain a proper understanding of naturally occurring spatial and temporal variability in stream community assemblages, spatial and seasonal variations in flow and temperature regimes, and the related biotic responses of aquatic macroinvertebrates were explored, at three close-to-pristine sites in the Palmiet River catchment.

Chapter 4 focuses on the use of MIRAI, and its comparison with SASS, with respect to the applicability and sensitivity in defining ecological categories for impacted sites, within the Palmiet River catchment.

Chapter 5 discusses the significance of results in Chapters 3 and 4, followed by a synopsis whereby the importance of such a study is highlighted.

CHAPTER 2

STUDY SITES

2.1 Study Area

Importance of Study Area (Palmiet River catchment)

The Palmiet River catchment falls within the Cape Floristic Region (CFR), one of the world's five Mediterranean hotspots. Home to the greatest non-tropical concentration of higher plant species in the world, the region is the only hotspot that encompasses an entire floral kingdom, and holds five of South Africa's 12 endemic plant families and 160 endemic genera (Rouget *et al.* 2003).

The Department of Environmental Affairs (DEA), DWA and the South African National Biodiversity Institute (SANBI) realised that urgent attention was needed to ensure the conservation of freshwater ecosystems within the Cape Floristic hotspot Region (Nel *et al.* 2011). These three organisations created the National Freshwater Ecosystem Priority Areas project (NFEPA), providing strategic spatial priorities for conserving freshwater ecosystems &and supporting sustainable water resources, in regions such as the CFR. NFEPA provides guidance on how many rivers, wetlands and estuaries, and which ones, should remain in natural or near-natural condition to support the water protection goals of the NWA (Act 36 of 1998) for conservation purposes. These strategic spatial priorities are known as Freshwater Ecosystem Priority Areas, or "FEPA".

The NFEPA project (Nel *et al.* 2011) split the Palmiet River catchment into two FEPAs (Freshwater Ecosystem Priority Areas): within the Kogelberg Reserve as a river FEPA, and above the Kogelberg Nature Reserve as a Phase 2 FEPA, designated for future conservation. In both cases, river conditions should not be degraded any further, and should be maintained in a good condition in order to contribute to specific regional biodiversity goals and support sustainable use of water resources.

General Description

The Palmiet catchment (Figure 2.1) is one of the biggest in the south-western Cape, with an area of 539km^2 (Branch and Day 1984).The Palmiet River rises in the vicinity of Landdroskop (1133m) in the Hottentots Holland Mountain Range. Initially it flows in an easterly direction, dropping rapidly in altitude over the first few kilometres. About 4km from its source, the river leaves the steep slopes of the Hottentots Holland Range and moves south towards Grabouw, approximately 12km away. From here to the Eikenhof Dam, the river flows down the less steep foothills between the Hottentots Holland Range and the Groenlandberge. The gradient in this region is still steep, but not nearly as steep as in the upper regions. Just before the Eikenhof Dam, the river enters the Elgin Valley. From here to the sea, the slope of the land transversed by the river is gentle. For the next 35km the river flows close to the western boundary of the Elgin Valley, flanked by the foothills of the Kogelberg Biosphere Reserve mountains on its west bank and the cultivated lands of the valley on its east bank. It flows in a southerly direction until its junction with the Klein Palmiet where it moves northeast for about 6km before swinging south again. About 15km from the mouth, the river leaves the Elgin Valley and enters the deep valley between the Dwarsrivierberg and Perdeberg ranges. It flows predominantly in a south-westerly direction until the junction with the Louws and Dwars Rivers were it moves southeast and heads for the sea. Near the coastal road which traverses the river, the river begins to broaden out into an estuary about 1.67km long. The coastal plain is extremely narrow at the mouth so that the river changes from a foothills stream to an estuary with no intervening stretches typical of a lower river.

Climate

The Palmiet River and its catchment lie within a Mediterranean climate zone, receiving most of its rainfall in winter from May to September. Summers (November to February) are warm to hot and dry. The winter rainfall in the Palmiet catchment area is associated with westerly cyclones which move over the south-eastern Cape. Orographic rainfall also occurs, the mountainous regions receiving more rain than the valleys (Clarke 1989). Rainfall varies from about 700mm per year in the low-lying central eastern and coastal region to *ca.* 1500mm inland. Highest average monthly rainfall generally occurs between June and August, whereas lowest monthly averages occur in December and January. The mean annual rainfall for the entire catchment area is 1139mm (Clarke 1989). In the Palmiet River catchment the lowest average daily minimum and maximum temperatures occur during July while the highest averages occur during January and February. Due to the large differences in elevation, coastal and inland temperatures differ considerably (Clarke 1989).

Geology

The geology of the Palmiet River catchment (Figure 2.2) is dominated by sandstones, quartzites and s hales of the Table Mountain Group, and shal es and sandstones of the Bokkeveld Series (Lambrechts 1979). Witteberg Series quartzites and shales occur to a lesser extent (Nel 1980). The greater resistance to weathering has left the Table Mountain Sandstone as the most prominent feature of the landscape, forming high ground and mountain ranges, while the less resistant shales now occur only at lower elevations.

Vegetation

The Palmiet Catchment is situated in the Cape Floristic Kingdom, and largely within the Fynbos biome, which is one of six flora and biogeographical kingdoms. The vegetation of the upper catchment of the Palmiet River (Nuweberg catchment) is dominated by mesic mountain fynbos (Figure 2.3). Mountain fynbos is a vegetation type which is characterised by ericoid shrubs and restioi d herbs, with the frequent occurrence of proteoid shrubs (Rebelo *et al.* 2006, Manning 2007). This vegetation grows on acidic, strongly leached soils where not only are nitrogen and phosphorus in short supply, but where one or more of the other nutrient elements (potassium, sulphur, copper, zinc, and molybdenum) may also be limiting (Specht and Moll 1983). This type of vegetation occurs on foothills, slopes and summits of mountains of the Cape Fold Belt, on soils that are well-leached and often sandy. The water of the upper reaches of the Palmiet River is very pure, and the waters are darkly stained by humic acids, which is typical of rivers which drain south-facing, fynbos-covered slopes (Snaddon 1998). Humic acids originate as polyphenols, which are secondary plant compounds found in fynbos plants (Midgley and Schafer 1992). The polyphenols are leached into the soils of the catchment, as a result of the death and decay of the vegetation (Snaddon 1998). These compounds are transformed into humic acids, which are then washed into the groundwater and move into the river (Snaddon 1998). The Palmiet River and tributaries are especially darkened in winter, when the increased runoff carries large quantities of humic acids into the river (Snaddon 1998).

Land-use

The area between the Palmiet and Kromme Rivers is intensively cultivated (Figure 2.4). Apples form the main crop. Other deciduous fruits such as pears and peaches are also grown but are of minor importance (Clarke 1989). The rest of the catchment area lies within boundaries of State "Forests" (managed by the South African Department of Forestry).These are Jonkershoek and Nuweberg State Forests in the north, Lebanon State Forest in the east, Highlands State Forest in the southeast, Kogelberg State Forest in the southwest and Grabouw State Forest in the west. Several pine plantations have been established within these State Forests. The largest occur to the east and west of Grabouw in the Grabouw and Lebanon State Forests, respectively. Cultivated lands and plantations comprise approximately 41% of the catchment area (RHP 2003). Of the remaining 59%, 56% is covered by natural fynbos vegetation, 1% by urban areas (i.e. towns of Grabouw and Betty's Bay), and 2% to other minor land-uses (RHP 2003). Other than a few saw-mills, a carton factory and a large fruit juice factory, there is very little industry in the Palmiet River catchment (Clarke 1989). Sewage is discharged into the Palmiet River from wastewater treatment works near the town of Grabouw (RHP 2003).

Impoundment

The Palmiet River is impounded by five major in-stream dams (Clarke 1989) (see Figure 2.1): Nuweberg Dam (built in 1971, capacity $3.9\times10^6 m^3$), Eikenhof Dam (built in 1977, capacity $22.7\times10^6 m^3$), Applethwaite Dam (built in 1952, capacity $3.3\times10^6 m^3$), Kogelberg Dam (built in 1987, capacity $19\times10^6 m^3$) and Arieskraal Dam (built in 1967, capacity $5.9\times10^6 m^3$) (Ollis 2005). All these dams are located in the upper and middle reaches of the river, within 3.5-

4km from the source. The Nuweberg Dam is used for domestic supply, while the Eikenhof, Applethwaite and Arieskraal Dams are used for irrigation (Gale 1992). The Kogelberg Dam and the off-stream Rockview Dam together comprise the Palmiet Pumped Storage Scheme, which is used to generate electricity (Gale 1992). Releases of water from the five major in-stream dams vary from none except when overtopping (Applethwaite Dam), to controlled release (Nuweberg, Eikenhof and Kogelberg Dams), to constant bottom-release (termed hypolimnetic release) (Arieskraal Dam) (Ollis 2005).

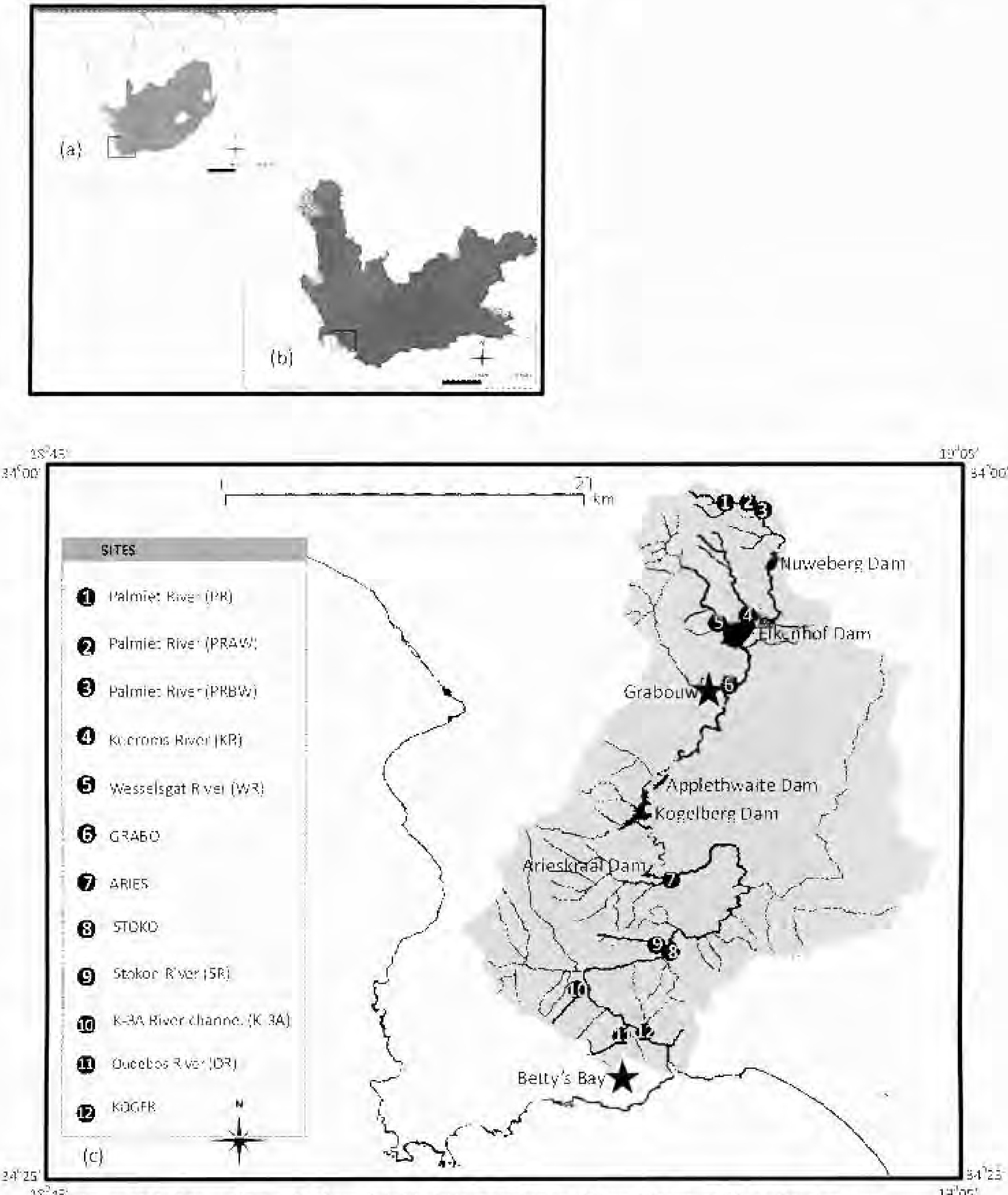


Figure 2.1: Map of South Africa (a), the Western Cape (b) and Palmiet River Catchment showing the location of sampling sites (c).

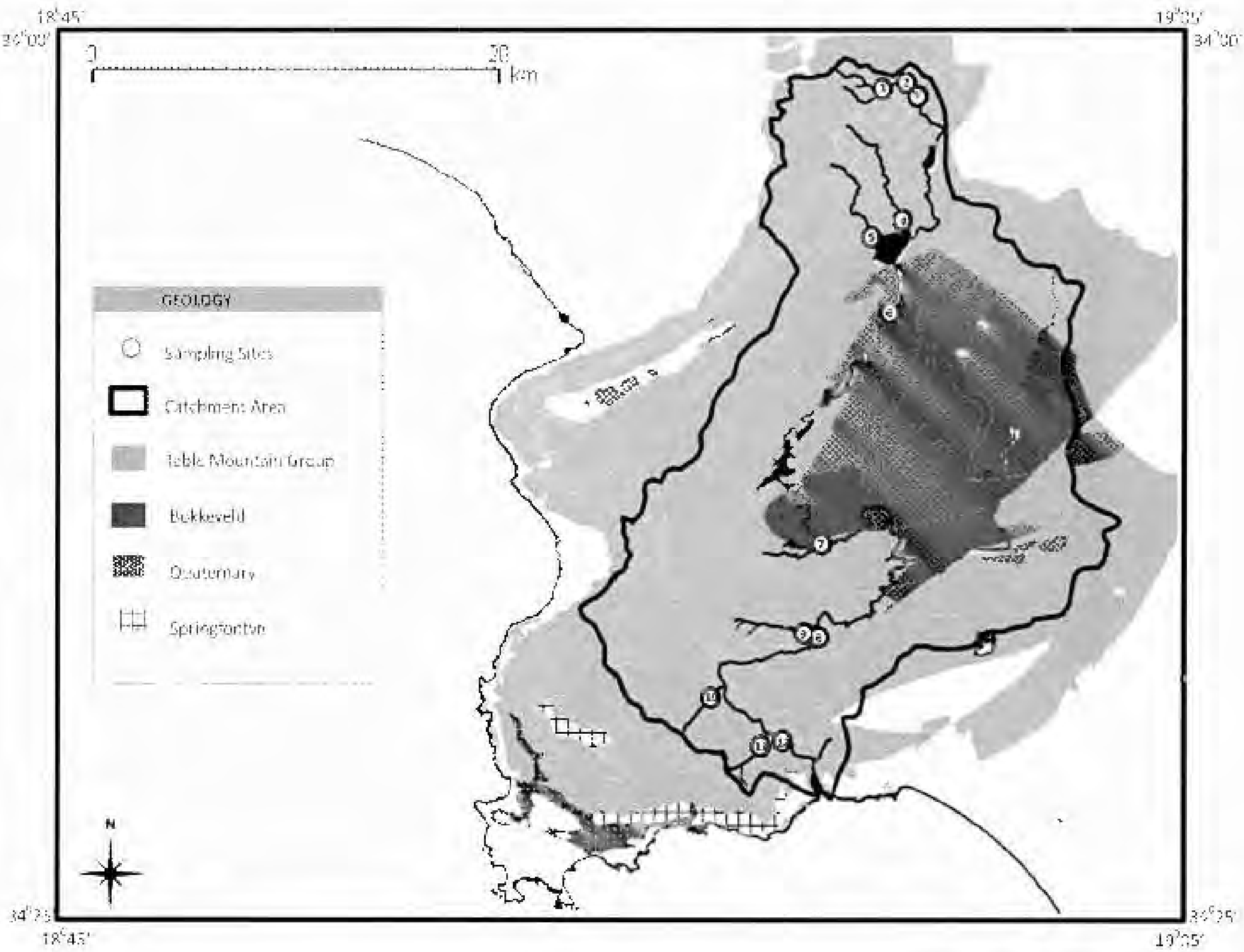


Figure 2.2: Underlying geology of the Palmiet River catchment (City of Cape Town, GIS Department).

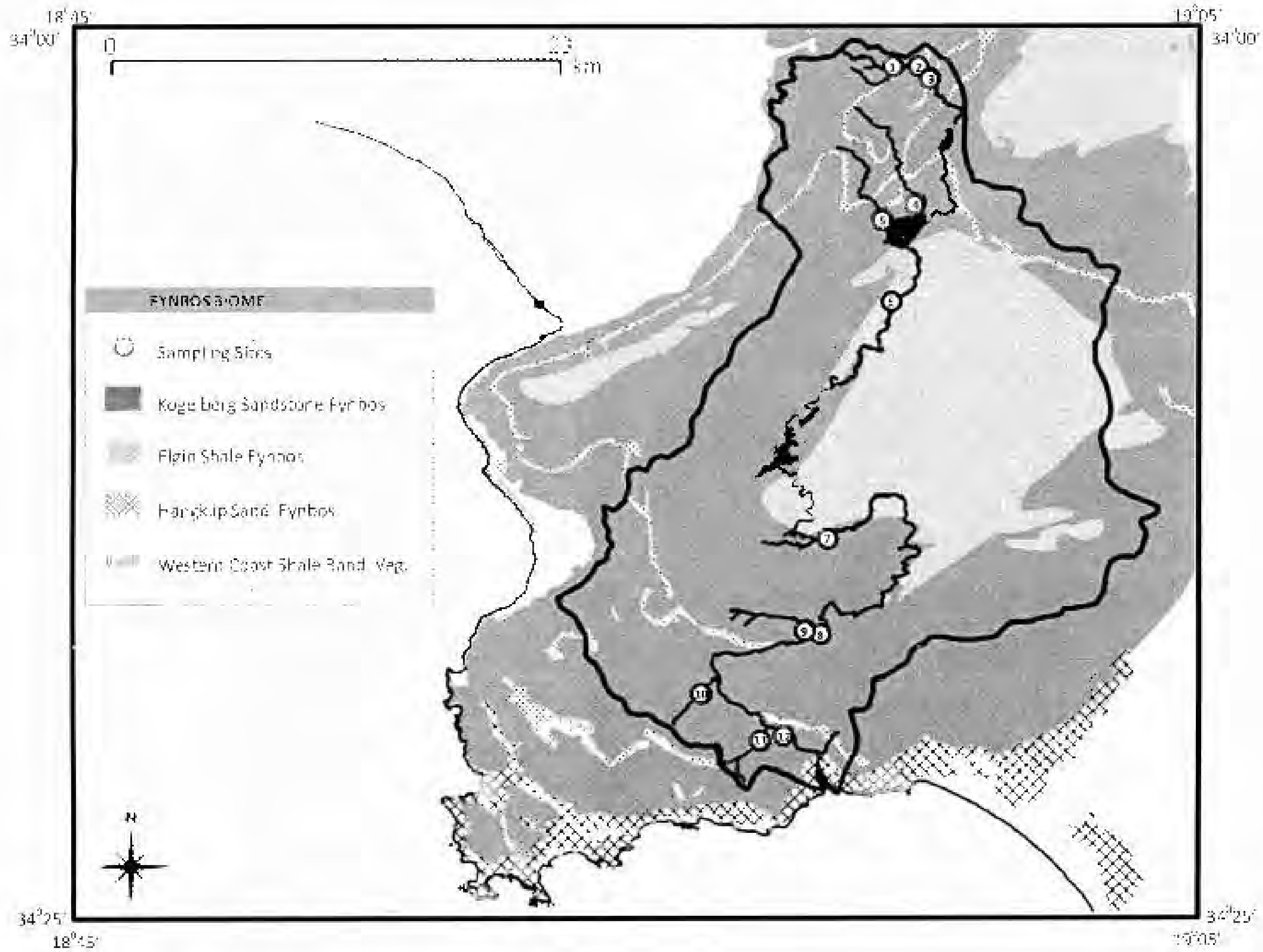


Figure 2.3: The vegetation types within the Palmiet River catchment (Rebelo *et al.* 2006).

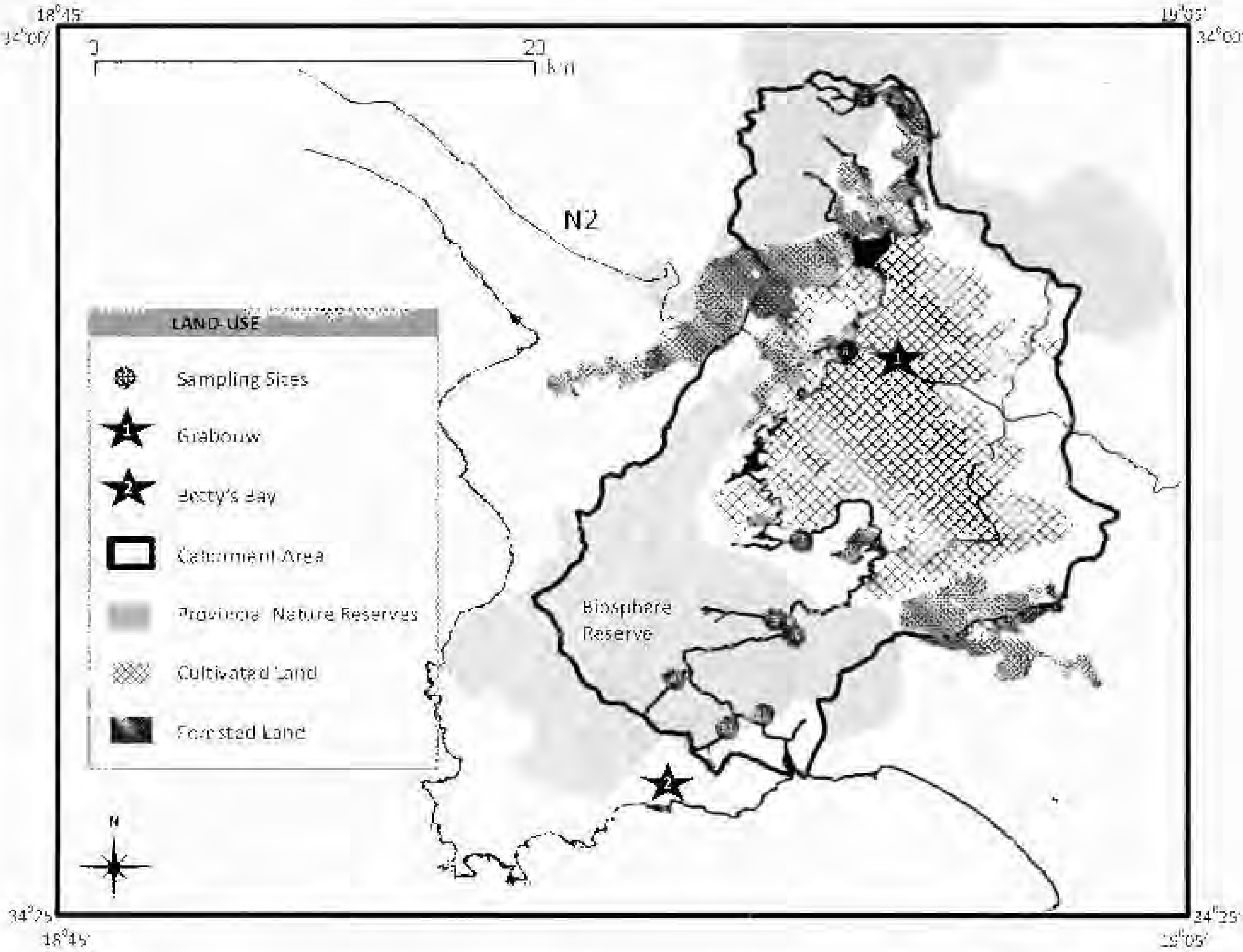


Figure 2.4: Land-use practices within the Palmiet river catchment, including important towns and national roads (City of Cape Town, Department of Land Affairs).

2.2 Sampling Sites

Twelve river sites were chosen within the Palmiet River catchment (Figure 2.1 and Table 2.1) all of which fall into the same Southern Folded Mountains ecoregion. All sites sampled are located within the upper-reaches (Mountain Stream and Rejuvenated-Foothills zones)(refer to Table 2.2 for definitions in river longitudinal zonations). Riffles (refer to Table 2.3 for morphological mesohabitat characteristics), stones-out-of-current, marginal and aquatic vegetation were sampled as per SASS protocol. Approximately 20m of river length was sampled at each site. Data obtained for sites were primary (self-collected) and secondary (from Ollis 2005 and River Database).

Reference sites

Site PR (Plate 1 and 2) is situated in the upper reaches of the Palmiet River, within the Nuweberg Reserve catchment. Sites K-3A (Plate 13 and 14) and OR (Plate 15 and 16) are found on tributaries of the Palmiet River (Table 2.1), situated within the Kogelberg Biosphere Reserve. These three sites were chosen due to the relatively unimpacted nature of the streams, and for the sake of this study are referred to as being "natural" or "reference condition" sites. The remaining nine sites were chosen as potentially impacted sites with varying water quality, flow and habitat impacts due to the close proximity of each site to anthropogenic disturbance.

Impacted sites

Sites PRAW (Plate 3 and 4) and PRBW (Plate 5 and 6) are found in the upper-reaches of the Palmiet River, situated directly up- and downstream respectively of a concrete-weir and low causeway bridge, within the Nuweberg Reserve catchment. Sites KR (Plate 7 and 8) and WR (Plate 9 and 10) are located on the Keeroms and Wesselsgat Rivers respectively, both tributaries of the Palmiet River, roughly 50m above the Eikenhof Dam. Site GRABO is located alongside the town of Grabouw, directly below a rural development and tarred bridge (no plates obtainable for secondary data). Site ARIES is roughly 5 km's downstream of the Arieskraal Dam, in a region surrounded by agriculture. Site STOKO is situated within the upper-end of the Kogelberg Biosphere Reserve, roughly 9km's downstream of the Arieskraal Dam. Site SR (Plate 11 and 12) is found on the Stokoe River, a tributary of the Palmiet River, within the Kogelberg Biosphere Reserve, and site KOGFR is located downstream of where the Oudebos tributary meets the Palmiet River, in the Kogelberg Reserve.

Limited nature of sampling sites

Sampling sites were limited to three reference-condition sites, and nine impacted sites. The limitation in number of reference sites was due to the lack of adequate comparable sites within the Palmiet River catchment. The high percentage of anthropogenic activities within the catchment limited the locality of reference sites to protected reserves, and most of the "possible" reference sites within reserves are located in different longitudinal zones, hampering the aim of analysing macroinvertebrate communities within upper-reaches of the stream. Only three suitable sites were identified, and thus explored within the study.

However, as already mentioned, with the large proportion of land-use activities within the Palmiet River catchment, one would expect a large number of potentially impacted sites. Most of the impacted sites within the catchment occurred in cultivated land and plantations where access was not granted, sites were often found not to contain suitable riffle mesohabitats. Only nine potentially impacted sites were deemed adequate for this study, within the Palmiet River catchment, being found in the upper-reaches, with sufficient riffle habitat for sampling.

Table 2.1: Site characteristics, including GPS co-ordinates, presence/lack of temperature gauges and seasonal sampling. Abbreviations: "NR" – Nature Reserve.

Site No.	Site Code	River (location)	Reference Condition Site	Coordinates (decimal degrees)		Type of Data	Seasonal Sampling	Temperature Gauge
				Latitude	Longitude			
1	PR	Palmiet (in Nuweberg NR)	Yes	-34.0645	19.0473	Primary	Yes	Yes
2	PRAW	Palmiet (above weir in Nuweberg NR)	No	-34.0685	19.0492	Primary	No	No
3	PRBW	Palmiet (below weir in Nuweberg NR)	No	-34.0685	19.0492	Primary	No	No
4	KR	Keeroms (above Eikenhof Dam)	No	-34.1121	19.0409	Primary	No	No
5	WR	Wesselsgat (above Eikenhof Dam)	No	-34.1171	19.0207	Primary	No	No
6	GRABO	Palmiet (along side town of Grabouw, below bridge)	No	-34.1503	19.0181	Secondary	No	No
7	ARIES	Palmiet (below Arieskraal Dam)	No	-34.235	18,9853	Secondary	No	No
8	STOKO	Palmiet (9km's downstream of Arieskraal Dam)	No	-34.2839	18.9686	Secondary	No	No
9	SR	Stokoe (below road in Kogelberg NR)	No	-34.2824	18.9899	Primary	No	No
10	K-3A	K-3a-river (tributary of Palmiet in Kogelberg NR)	Yes	-34.3029	18.9376	Primary	Yes	Yes
11	OR	Oudebos (tributary of Palmiet in Kogelberg NR)	Yes	-34.3279	18.9603	Primary	Yes	Yes
12	KOGFR	Palmiet (lower end of Kogelberg NR)	No	-34.3172	18.9672	Secondary	No	No

Table 2.2: Ecological definitions of longitudinal zones for South African rivers (after Rowntree and Wadeson 1999).

ZONE	DEFINITION
Mountain Headwater Stream	Very steep-gradient (>0.1) in V-notched canyons, dominated by vertical flow over bedrock and boulders, with waterfalls and plunge pools. Approximately equal vertical and horizontal flow components.
Mountain Stream	Steep-gradient (0.04-0.0.99) in steep-sided valley, dominated by cobbles and boulders, with local coarse gravel in quiet areas. Confined valley floor and low sinuosity. Second order stream. Reach type: plane-bed.
Transitional	Moderately steep-gradient (0.02-0.039) dominated by boulders and cobbles, with bedrock intrusions. Middle order river. Reach types: planar bedrock, regime. Pools much shorter than riffle/rapids.
Upper Foothills	Moderately steep (0.005-0.019), cobble-bed river in gentle-gradient valleys with confined valley floor and moderate sinuosity. Narrow floodplains of sand and gravel. Second to third order river. Reach type: run-riffle. Riffles/rapids about the same length as pools.
Lower Foothills	Lower gradient (0.001-0.005), mixed bed alluvial channel with sand and gravel dominating the bed. Reach types: pool-riffle or pool-rapid, sand bars are common in pools. Pools significantly greater extent than rapids of riffles. Flood plain often present.
Lowland	Low-gradient (0.0001-0.001), pool-like, sand-bed river in very broad valley associated with extensive floodplains and meanders. High sinuosity, fully-developed meandering channel pattern, with large silt deposits. Reach type: regime.

Table 2.3: Definitions of some common morphological units (after Rowntree and Wadeson 1999; King and Schael 2001)

MESOHABITAT	DEFINITION
Step	Free-falling water over slabs of bedrock or boulders, in step-like arrangements. Average water depth and velocity not distinguishing features.
Pool	Channel feature with slow through-flow. Deep relative to channel size with low to zero velocity. All kinds of substratum. Scoured at high flows.
Rapid	Tumbling, turbulent flow over bedrock or boulders. Variable water depth, with high to very high velocities, and white water.
Run	Moderately fast, fairly smooth flow over any substratum. Water surface rippled, not choppy. High water depth to substratum size ration. No obvious gradient in water surface.
Riffle	Rapid, turbulent flow over cobbles, gravel and small boulders. Water depth shallow relative to bed particle size. Distinct gradient in water surface. Flickering white water.
Backwater	Hydraulically detached alcove with no through-flow of water. If connected, water tends to enter and leave via same route. Velocity usually close to zero. Substratum usually sand, silt and debris.

2.3 Sampling Period

Primary sampling took place between November 2009 and December 2010. Reference sites (PR, K-3a and OR) were sampled once in every season, on the 4th-5th November 2009 (Spring), 22nd-23rd March 2010 (Summer), and 27th-28th May 2010 (Autumn). Secondary data was obtained from Ollis (2005) and the River Database which was collected between 23rd to 26th February 2003. All secondary data was collected according to SASS protocol. No sampling was undertaken during winter, when macroinvertebrates are naturally sparse and difficult to collect due to high water flows. Both biotic and abiotic data were collected within each of the three seasonal sampling periods, to satisfy requires for SASS and MIRAI. Antecedent conditions within each of the reference streams were obtained from installed piezometer and water-level loggers, from the Greater Table Mountain Group Aquifer Project undertaken by the City of Cape Town (City of Cape Town 2008) . The instruments were set to record every 30 minutes, and were programmed to collect data from the 6th August 2008, through to 14th April 2010. Impacted sites (PRAW, PRBW, KR, WR and SR) were sampled only once, as required by SASS and MIRAI, on the 30th-1st December 2010. The reference sites PR, K-3a and OR were sampled within the same period.

2.4 Level of Taxonomic Identification

Macroinvertebrate identification procedures followed that of the specific SASS and MIRAI protocols. Each method requires macroinvertebrate identification to family level. However, King and Schael (2001), Chessman (1995) and Lenat and Resh (2001) stated species-level identification to be compulsory for detailed assessments of ecological integrity of aquatic systems, and essential in gaining a deep understanding of lotic ecosystem functioning (de Moor 2002). Because of this, in deriving spatial and temporal reference conditions at the three near-to-pristine sites (PR, K-3a and OR), specimens were identified to species where possible. Species data were not used to ascertain ecological categories for sites, but species- and family-level data were compared in multivariate analyses to ascertain whether family-level taxonomy adequately describes and accounts for spatial and temporal differences.

Plate 1 and 2: Site PR (Palmiet River), a "reference condition" site, situated in the upper reaches of the Palmiet catchment, Nuweberg. Picture 1 taken looking upstream from the site. Picture 2 taken illustrating the aquatic and marginal vegetation. Site code: PR.

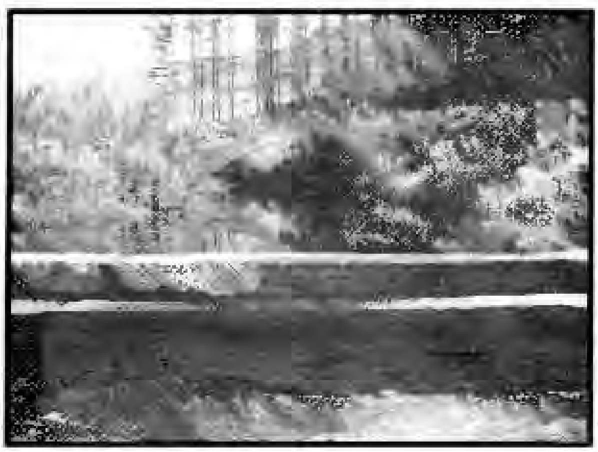


Plate 3 and 4: Site 2 (Palmiet River - upstream of weir), situated in the upper reaches of the Palmiet catchment, Nuweberg. Picture 3 taken illustrating the pooling/damming effect the weir has on upstream conditions. Picture 4 taken from left bank of river, illustrating the limited/impacted riverine flow conditions. Site code: PRAW.

Plate 5 and 6: Site 3 (Palmiet River - downstream of weir), situated in the upper reaches of the Palmiet catchment, Nuweberg. Picture 5 taken from mid-stream of the river, centre of weir, looking downstream. Picture 6 taken from right bank of river. Site code: PRBW.

Plate 7 and 8: Site 4 (Keeroms River), situated 50m upstream of the Eikenhof Dam. Located within the Grabouw State Forest. Picture 7 illustrates riverine substrate resulting from deposition. Picture 8 taken from the right river bank, looking downstream. Eikenhof Dam seen in distance. Site code: KR.

Plate 9 and 10: Site 5 (Wesselsgat River), situated 50m upstream of the Eikenhof Dam. Located within the Grabouw State Forest. Picture 9 illustrates the elevated roadway through the course of the river, and highlight the riverine substrate resulting from deposition. Picture 10 taken from the right river bank, looking at sampled biotopes and further upstream. Site code: WR.

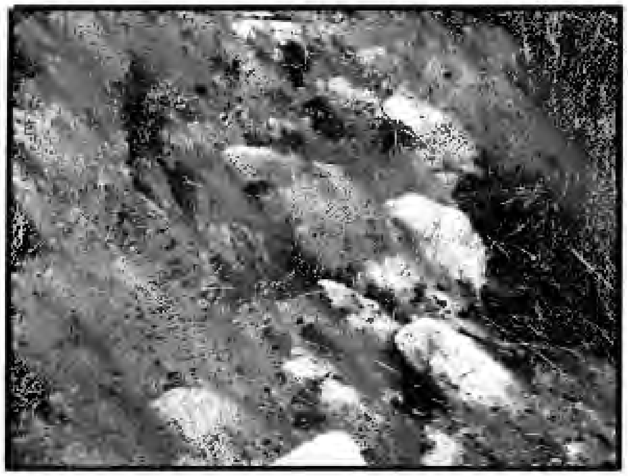
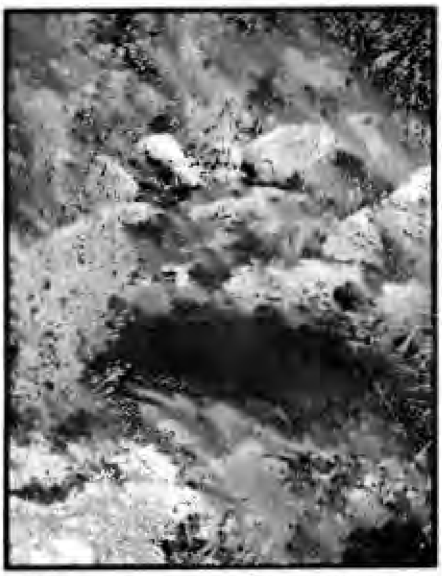


Plate 11 and 12: Site 6 (Stokoe River) a tributary of the Palmiet River, situated within the Kogelberg Nature Reserve. Picture 11 taken mid-stream looking upstream. The presence of a semi- dirt/cement road forces the stream flow through a 50cm cement culvert (seen at the top-middle within this photo), located beneath the road. Photo 12 taken from dirt/cement road, looking downstream. Site code: SR.



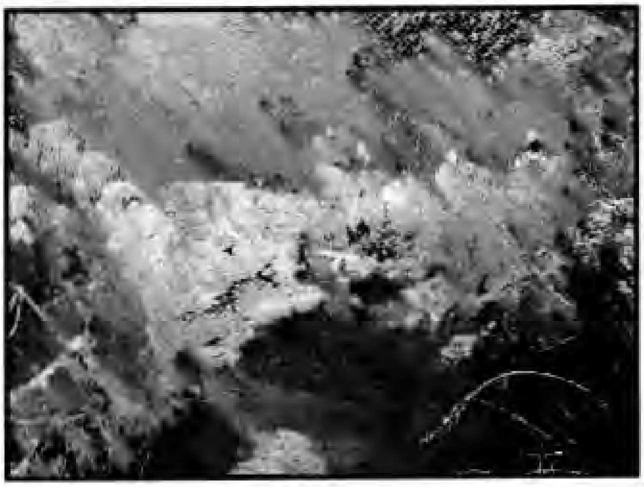


Plate 13 and 14: Site 7 (K-3a river channel), a "reference condition" site, situated within the heart of the Kogelberg Nature Reserve. Photo 13 taken looking upstream. Photo 14 taken looking upstream, from the base/bottom of the sampling site. Site code: K-3A.

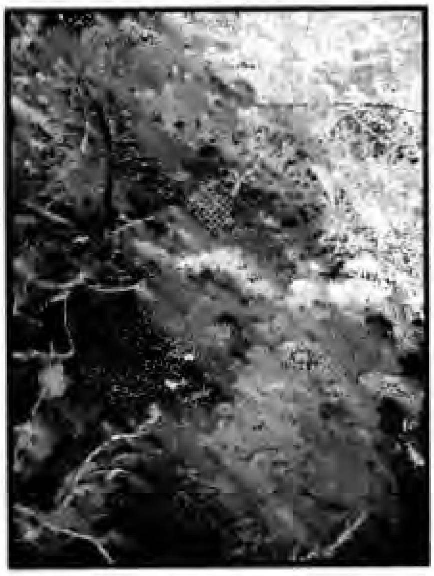


Plate 15 and 16: Site 8 (Oudebos River), a "reference condition" site, situated within southern section of the Kogelberg Nature Reserve. Photo 15 taken looking downstream. Photo 16 taken from the right bank of the river, looking at in-stream vegetation and stones. Site code: OR.

CHAPTER 3

ESTABLISHMENT OF REFERENCE CONDITIONS WITHIN THE PALMIET RIVER CATCHMENT

3.1 INTRODUCTION

River ecosystems are longitudinal systems that integrate the characteristics of the catchment they drain. They exhibit a high degree of spatial and temporal variability, particularly in semi-arid environments as is the case across large areas of South Africa (Eekhout *et al.* 1997). Spatial differences in environmental variables and biological assemblages are widely studied characteristics of lotic environments (Hawkes 1975, Statzner and Higler 1986, Hawkins *et al.* 1997) and, in context with bioassessment, spatial heterogeneity is often taken into account by partitioning areas into relatively homogenous regions. The primary goal of many assessment systems is to provide a spatial framework within which aquatic resource management, including bioassessment, is conducted.

Since aquatic organisms are known to have specific physico-chemical and hydraulic requirements, seasonal variation in factors such as stream discharge (McElravy *et al.* 1989) and temperature (Hawkins *et al.* 1997) may lead to differences in the distribution and abundance of benthic macroinvertebrates. The temporal variability in taxon richness, as a result of seasonal changes in discharge and temperature, may affect biotic indices that are based on macroinvertebrate assemblages. Thus when macroinvertebrate assemblages are used for bioassessment, temporal differences of individual taxa may influence judgement as to whether a site is impacted.

The ultimate objective of bioassessment is to facilitate and evaluate the effect of disturbance on biological resources, and it is necessary to be able to distinguish those differences in ecosystem characteristics that stem from natural heterogeneity and variability in the system from those caused by anthropogenic activities. This requires an understanding of the natural conditions with respect to the component of the ecosystem being examined. Reference conditions define what is expected, or what is found naturally at a site, or in a particular area or region, and provide a means of comparing observed conditions with expected conditions. The degree of impairment or deviation from natural conditions can thereby be ascertained, serving as a foundation for developing criteria for the protection of aquatic ecosystems.

This chapter specifically explores the identification of reference conditions with regard to both abiotic and biotic components within the Palmiet River catchment. Historical lack of attention to conservation of pristine areas has resulted in the scarcity of non-impacted streams in most regions; thus only three sites were identified as near-to-pristine (minimally-disturbed) and used to characterise the best attainable reference conditions. A near-to-pristine site was selected from each of three rivers within the Palmiet catchment: Oudebos River (OR), K-3a River (K-3A) and Palmiet River (PR). All three sites were located within the Mountain Stream Zone (after Rowntree and Wadeson 2000).

The physico-chemical and biological components were explored statistically, both spatially and temporally, to define characteristics and ranges of values which describe near-to-pristine conditions found within the Palmiet catchment. The habitat component was used for descriptive purposes only. Changes in physico-chemical components were related to biological responses of aquatic macroinvertebrates to ascertain which of the physico-chemical variables were strongly driving the biological variability.

3.2 AIMS

The overall aim of this study was to define reference physico-chemical conditions and macroinvertebrate assemblages within the Palmiet River catchment. This was achieved by:

1. characterising spatial and temporal differences in physico-chemical data, including objectively dividing the year up into seasons based on time-series of instrument-based water temperature data.
2. ascertaining whether patterns in biological data are linked to physico-chemical data collected spatially and temporally.
3. examining the impacts of spatial and temporal variability on macroinvertebrate SASS5 scores.

The spatial and temporal variability in physico-chemical variables and macroinvertebrate assemblages, and in particular the ability to identify reference catchment conditions for the Palmiet River, will be discussed in relation to aquatic bioassessment and the establishment of reference sites within identified homogenous regions.

3.3 METHODS

3.3.1 SAMPLING PROTOCOL

To identify reference conditions, and investigate naturally occurring spatial and temporal variability at these sites, physico-chemical, biological and habitat data were collected. Physico-chemical and habitat data were acquired through in-field sampling. Continuous temperature and discharge time-series data were captured from installed piezometer and water-level monitors, respectively, at each site. Biological data were collected using the SASS5 protocol.

3.3.1.1 Physico-chemical conditions

At each primary site, on every sampling trip, temperature, conductivity (Crison CM 35 conductivity meter, accuracy of ≤0.5%), pH (Crison pH 25 pH meter, accuracy of ≤0.01), dissolved oxygen concentration (Aqualytic OX 22 oxygen meter, accuracy of ±2.5%) and turbidity (Turbidity Meter TN100, accuracy of ±3%) were measured. Discharge was calculated by measuring depth, width and velocity (using a GLOBAL FP101 Digital Velocity meter, accuracy of ±2%). Velocity and turbidity were not measured at secondary sites.

3.3.1.2 GPS

The geographical coordinates of each primary sampling site were recorded during the first round of sampling (Spring 2009) and confirmed during the second round of sampling (Summer 2010), using a Garmin III hand-held Geographic Positioning System (GPS). All coordinates (see Table 1) were geo-referenced using the WGS 84 (World Geodetic Survey 1984) datum with an accuracy of ±12m. GPS coordinates for secondary sites were obtained from Ollis (2005).

3.3.1.3 Biological data

All macroinvertebrates collected within this study were captured using SASS sampling protocols, to derive spatial and temporal reference conditions at three near-to-pristine sites (PR, K-3a and OR).

SASS 5 (South African Scoring System, Version 5)

Aquatic macroinvertebrates were collected according to the SASS sampling protocol (Dickens and Graham 2002). The SASS 5 procedure is conducted on site and each macroinvertebrate family present is recorded. Qualitative kick and sweep samples were taken, using nets of 1mm mesh size, in a 30cm by 30cm frame. Stones-in-current (SIC) were sampled for 2 minutes, stones-out-of-current (SOOC) for 1 minute, and gravel, sand and mud (GSM) for a total of 1 minute, while 2 metres of marginal vegetation and 1m^2 of aquatic vegetation were sampled. However, not all biotopes were always present at a site. Sampling of each biotope was conducted within a 20m sampling area. Hand-picking and visual observation of aquatic macroinvertebrates on stones was undertaken for 1 to 2 minutes to capture specimens missed by the sampling procedure.

To examine spatial and seasonal variability at reference sites, five separate areas were sampled at randomly selected localities within the stones-in-current riffle biotope at each site, because Dallas (1995) ascertained that 2-5 replicates will normally allow collection of ≥ 75% of taxa present. Samples were placed into separate sampling trays for sorting and identification. White plastic trays, approximately 30cm by 45cm in size with a depth of 10cm, were used. After adding river water from the site to each tray, and removing debris, the macroinvertebrates collected from each biotope group were identified in the field to the pre-defined taxonomic levels required for SASS (family level for most taxa), using a photographically illustrated identification guide (Gerber and Gabriel 2002) and a field guide (Gerber and Gabriel 2002) for aquatic invertebrates of South African rivers. Organisms were immediately fixed in 70% alcohol and transferred to the laboratory for more detailed identification at a later period.

A standard SASS5 score-sheet (see Appendix A) was completed per site. Abundance estimates were recorded according to a log-scale as follows: 1 = single individual present, A = 2-10 individuals; B = 11-100 individuals; C = 101-1000 individuals; and D = >1000 individuals. The SASS 5 Score, Number of Taxa and Average Score Per Taxa (ASPT), none of which take abundance estimates into account, were calculated for each sample. These scores are summed to give a Total Score per site. The number of taxa is calculated and divided into the Total Score to provide an Average Score Per Taxon (ASPT) value. Interpretation of the Total Score and the ASPT values provide means of establishing the quality of water at each site.

SASS5 *calculations of Ecological Categories (Biological Banding)*

A modified method of Dallas and Day (2007), derived from Dallas (2007), was used to generate Ecological Categories from SASS5 Score and ASPT values. This method utilizes natural variation in SASS5 Scores and ASPT at reference sites within a spatial group (i.e. specific to an ecoregion) to determine the percentiles and band (category) widths, which are used to define ecological categories. In the case of this study, the Palmiet River catchment is located within the greater Southern Folded Mountains, and reference percentiles and band widths calculated by Dallas (2007) were used (Table 3.1). Data were plotted with ASPT as a function of SASS5 Score.

Dallas (2007) derived percentiles (90th, 67.5th, 45th, 36th and 22.5th) for SASS5 Score and ASPT used, with category A representing the top 10% of SASS5 Score and ASPT. She calculated the remaining categories, B to E/F, using an equal category width represented by 22.5% of the data per category. Categories E and F were combined (E/F), and thus the number of categories is five.

Table 3.1: Percentage quartiles calculated by Dallas (2007) for SASS5 Score and ASPT within the Southern Folded Mountains Ecoregion

	90th	67.5th	45th	22.5th
SASS5 Score	171	133	103	76
ASPT	8.3	7.3	6.4	5.3

Percentiles provide a means of dividing the data into ranges based on the distribution of data. The 90th percentile is a value such that 90% of the values of the variable fall below that value (or alternatively 10% fall above that value). The 22.5th percentile is a value such that 22.5% of the values of the variable fall below that value. Interpretation is based on the premise that if either SASS5 Score or ASPT is above the category value it will fall in the category. For example, a site would fall in ecological category A (defined as SASS5 Score > 150 or ASPT > 8.0) if the site had a SASS5 Score of 160 and an ASPT of 7.2; or a SASS5 Score of 130 and an ASPT of 8.5.

3.3.2 DATA ANALYSES

In order to investigate spatial and temporal variation in macroinvertebrate assemblages and environmental data captured at each site, univariate and multivariate analysis methods were used.

3.3.2.1 Univariate Analysis

Establishing a stage-discharge relationship

To calculate flow discharge, a linear regression was produced, which compared the measured water level recorded by a WL gauge, to that of the discharge calculated (according to Gore 1996) matching up to the relevant time and days of sampling. The linear regression in turn provided a straight line relationship equation for each reference site, to which all WL measurements could be converted into discharge values.

Calculating mean daily water temperature and discharge

Water temperature and calculated discharge were measured every 30 minutes within a 24 hour period for the duration of this study and were averaged to gain mean daily water temperature and discharge values.

Defining seasonal periods

The Statistical T-test Algorithm for analysing Regime Shifts (STARS) (Rodionov 2004) was applied to calculated mean daily temperatures recorded at each of the three reference sites, to detect possible seasonal changes - thus determining the onset and conclusion of each of the three seasons (spring, summer and autumn) under investigation in an objective manner.

Multivariate statistical methods have been used to detect long-term ecosystem changes, such as regime shifts, mainly within marine systems (Howard *et al.* 1997). This approach has been applied once within aquatic riverine ecosystems (*J. Ewart-Smith, Freshwater Consulting Group, pers. comm., 2012*), and is briefly outlined (see Rodionov 2004 for greater detail).

Rodionov (2004) developed a method known as the STARS for time-series data, where a regime shift is seen as an abrupt change from one relatively stable state to another. The algorithm comprises seven steps, following which the test continues in a loop until all the data for a certain variable are processed (Rodionov 2004). Each new observation is tested to see if it differs significantly from the mean of the current regime under the statistical criteria of a Student's t-test (i.e. a regime shift occurs when a statistically significant difference exists between the mean value of the variable before and after a certain point based on the t-test). If the current value is found to be greater or less than the critical level of the current regime mean, then the current time (e.g. monthly, seasonally or annually) is marked as a possible change point, and subsequent observations are tested in the same manner to confirm this change point as a new regime or merely an outlier.

Time-series data often show serial correlation, but the STARS method assumes that there is no auto-correlation within the time-series. Therefore, prior to running analyses with the

STARS method, auto-correlation was removed using a 'prewhitening' method called IP4 (inverse proportionality with four corrections), available within the STARS program. This process involves subsampling and bias correction of the least-squares estimate of the serial correlation. Shifts that are detected in the time-series under the prewhitening procedure are smaller in magnitude than those detected without prewhitening under 'straight' analyses (Rodionov 2006).

STARS can be tuned to detect the regimes of certain time scales and magnitudes, by altering the "cut-off length", the level of probability and the "Huber parameter". The time scale to be detected is controlled primarily by the cut-off length (e.g. as the cut-off length is reduced, the time scale of regimes detected becomes shorter). Increasing the probability level, increases the potential for a regime shift to be detected (e.g. as p=0.01 increases to p=0.05, the probability of detecting a regime shift in that period, increases.)
In the case of this study:

Cut-off length	=	10
Probability level	=	0.01
Huber parameter	=	1

Test for normality and homogeneity of variances

All univariate analyses were performed using the STATISTICA v10 statistical analysis software. To determine whether or not there were seasonal differences in either discharge or temperature, or both, at each reference site (PR, K-3a and OR), temperature was square-root and discharge was *log*(X) transformed.

Homogeneity in variances was tested for using Levene's Test for Equality of Variances, and the Kolmogorov-Smirnov (K-S) Test was used to test whether or not the data were normally distributed spatially and seasonally, at each site (PR, K-3a and OR).

One-way ANOVA's (parametric ANOVA)

Where data were found to confirm parametric assumptions, one-way ANOVA's were used to explore differences in spatial and seasonal temperatures and flow discharges at each of the three reference sites (PR, K-3a and OR.) separately. Where significant spatial or temporal differences were detected, a Post-hoc Tukey Test was performed, to identify where these differences occurred, specific to each site (PR, K-3a and OR). Results of all analyses were considered significant at $p < 0.05$.

Kruskal-Wallis Test (non-parametric ANOVA)

Where data were found to be not normally distributed, the Kruskal-Wallis test was used to explore spatial and seasonal differences in temperature and flow discharges at each of the three reference sites (PR, K-3a and OR). Where spatial and seasonal significance was found, a Multiple Comparisons Test was performed, to identify where these differences occurred, specific to each site (PR, K-3a and OR). Results of all analyses were considered significant at $p < 0.05$.

3.3.2.2 Multivariate Analysis (explore spatial and temporal biotic changes)

Multivariate analyses of macroinvertebrate assemblage data were performed using the PRIMER (Plymouth Routines in Multivariate Ecological Research, Version 6) (Clarke and Gorley 2006) computer software package. Spatial and temporal community structure at each sampling site, was compared by means of the Bray-Curtis Coefficient of Similarity (Bray and Curtis 1957), which is regarded as one of the most robust similarity coefficients for biological community applications (Clarke and Warwick 2001). Patterns in community structure were presented in two-dimensional space by means of cluster analysis (classification) and ordination, both based on the triangular matrix of similarity/dissimilarity coefficients computed between pairs of samples for each data set analysed. Data obtained from sampled sites were not standardised, and were fourth-root transformed to reduce the influence of very abundant taxa.

Classification involved hierarchical, agglomerative clustering with group-average linking, as recommended by Field *et al.* (1982) and Clarke and Warwick (1994), and results are displayed as a dendogram. Group-average sorting essentially joins groups of samples together at the average level of similarity between all members of one group and all members of the other (Field *et al.* 1982). As cluster analysis may force data into artificially distinct classes when, in reality, continua exist, a complementary method of analysis is advisable to confirm groupings (Field *et al.* 1982). Ordination by means of non-metric multi-dimensional scaling (MDS), which was used in this investigation, was one such method. An ordination is a map of the samples, usually in two or three dimensions, in which the placement of samples reflects the similarity of their biological communities (Clarke and Warwick 1994). The distance between samples attempts to match dissimilarities in community structure: nearby points have similar communities and distant points have dissimilar ones. Advantages of MDS include its flexibility and its basis on very few underlying assumptions (Field *et al.* 1982). All MDS ordinations were generated using 25 restarts. Distortions of the underlying data in two-dimensional MDS ordinations (and the subsequent reliability of the ordinations) were determined by the respective 2-D stress (Table 3.2).

Table 3.2: Guidelines for interpretation of 2-D stress values for MDS diagrams (Clarke and Warwick 1994). The greater the stress value, the greater the risk of drawing false inferences from an ordination.

2-D stress value	Interpretation
< 0.05	Excellent representation with no prospect of misinterpretation
0.05 – 0.1	Good ordination with no real prospect of misleading interpretation
0.1 – 0.2	Potentially useful 2-D ordination, but too much reliance should not be placed on the plot for values at upper end of range
0.2 – 0.3	2-D ordination should be treated with great deal of scepticism and discarded in upper half of range, especially with <50 data points
> 0.3	Points close to being arbitrarily placed in a 2-D ordination

One-way Analysis of Similarities (ANOSIM), a non-parametric statistical analysis, was used on the same Bray-Curtis matrix, to test the null hypothesis that there were no differences in macroinvertebrate assemblages between seasons (Clarke 1993). For ANOSIM, 999 permutations were used to calculate the rank similarity matrix. The ANOSIM test statistic, R, lies between the range (0-1). R=1 only if all replicates within sites are more similar to each other than any replicates from the same site at different seasons; and R=0 if the null hypothesis is true, so that similarities between and within seasons will be the same on average, at the same site. The taxa responsible for 90% within-group similarity was determined using SIMPER (similarity percentages), which examines the contribution of individual taxa to the Bray-Curtis similarity measure.

3.4 RESULTS

3.4.1 Physico-chemical component

3.4.1.1 Defining seasonal periods

The STARS (Statistical Regime Shift) Test (Rodionov 2004) was applied to calculated mean daily water temperature data collected at each of the three reference condition sites (Figure 3.3) over the course of the entire study. The STARS Test provides specific dates on which temperature increased or decreased significantly (by performing T-tests), indicating the start and end of each season within the localised catchment of the study (Table 3.3).

Table 3.3: STARS Test regime shift analysis of all three reference sites, calculated from Figures 1-3, indicating the onset and conclusion of each season sampled.

	Start	End
Spring	01/10/2009	18/11/2009
Summer	19/11/2009	23/03/2010
Autumn	24/03/2010	05/06/2010

3.4.1.2 Instantaneous physico-chemical variables

To explore spatial differences in physico-chemical variables, the univariate, non-parametric Kruskal-Wallis test was used. No significant spatial differences ($p>0.05$) were found between near-to-pristine sites PR, K-3A and OR in conductivity, water temperature, pH, turbidity, velocity or percentage dissolved oxygen saturation. Temporally significant seasonal differences were found in conductivity ($H=6.924$, $df=2$, $n=18$ $p=0.031$), water temperature ($H=12.551$, $df=2$, $n=18$, $p=0.002$), pH ($H=6.676$, $df=2$, $n=18$, $p=0.036$), velocity ($H=10.020$, df=2, n=18, p=0.007), and percentage dissolved oxygen saturation (H=13.053, df=2, n=18, p=0.002), however. No significant temporal difference was found in turbidity (p>0.05).

The univariate, non-parametric post-hoc Multiple Comparisons Test was used to explore when these specific significant seasonal differences occurred. Conductivity was found to be significantly higher in autumn than in either spring (H=6.924, df=2, n=18, p=0.032) or summer (H=6.924, df=2, n=18, p=0.048). Water temperature was significantly higher in summer than in autumn (H=12.551, df=2, n=18, p=0.001). pH was significantly higher in autumn than in spring (H=6.676, df=2, n=18, p=0.044). Velocity was significantly higher in spring than in summer (H=10.020, df=2, n=18, p=0.006). Percentage dissolved oxygen saturation was significantly higher in summer than in autumn (H=13.053, df=2, n=18, p=0.001).

The lack of significant spatial differences in the magnitude of instantaneous physico-chemical variables between sites OR, K-3A and PR, allowed for the collective averaging (combining) and creation of spatial variables to determine near-to-pristine instream riverine conditions (seen in Table 3.4). However, the high degree of significant temporal difference found in five of six instantaneous physico-chemical variables measured, suggested that the variability was too great to combine the data. Thus, seasons were kept separate when defining reference conditions (Table 3.4).

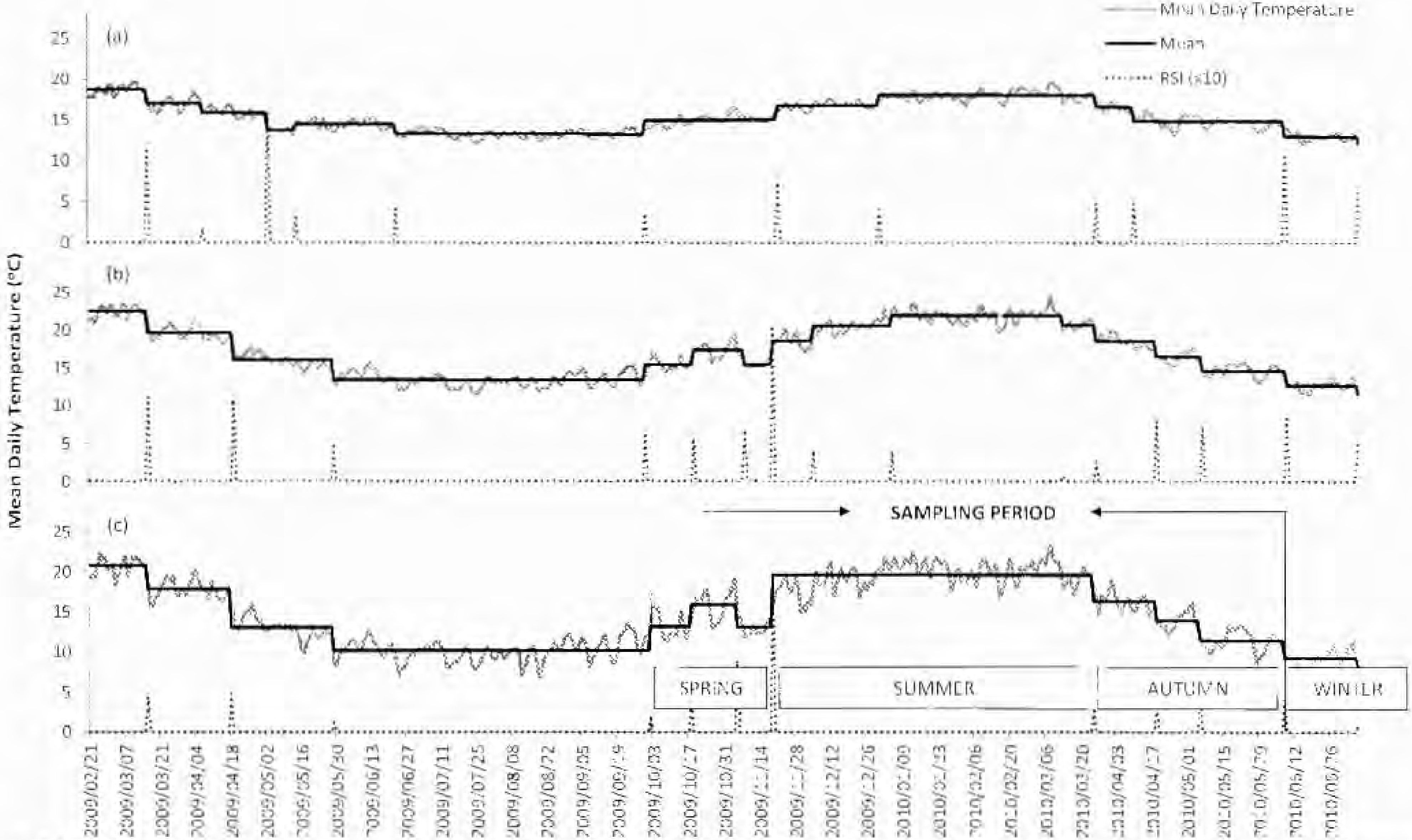


Figure 3.1: STAR analysis of mean daily water temperature within reference sites, (a) OR, (b) K-3A and (c) PR. "RSI" = Regime Shift Index. The RSI is a STAR Test calculated value that indicates where a regime shift had taken place, and the degree of regime shift, be it a positive or negative direction. RSI values have been multiplied tenfold to more clearly indicate where these regime shifts have taken place.

Table 3.4: Summary statistics for physico-chemical conditions spatially averaged from three reference sites sampled in the Palmiet River catchment, separated by season. Abbreviations: "Min" = minimum value, "Max" = maximum value, & "SD" = standard deviation, (n=6).

Physico-chemical Variables	Basic Statistics	Spring	Summer	Autumn
Conductivity (µS/cm)	Avg.	36.88	49.13	100.62
	SD	4.21	4.24	49.97
	Median	38.25	50.05	121.9
	Min.	31.5	43.9	37.2
	Max.	40.9	53.4	144.1
Water Temperature (°C)	Avg.	19.42	21.98	13.08
	SD	1.37	2.13	2.0
	Median	20.25	23.15	14.3
	Min.	17.6	19.2	10.4
	Max.	20.35	23.6	14.5
pH	Avg.	4.17	4.62	4.75
	SD	0.28	0.33	0.4
	Median	4	4.66	4.55
	Min.	3.97	4.22	4.44
	Max.	4.54	4.98	5.27
Turbidity (NTU)	Avg.	0.55	0.56	0.37
	SD	0.11	0.13	0.16
	Median	0.59	0.53	0.35
	Min.	0.41	0.43	0.2
	Max.	0.67	0.73	0.57
Velocity (m/s)	Avg.	0.3	0.17	0.28
	SD	0.04	0.06	0.04
	Median	0.31	0.16	0.3
	Min.	0.25	0.1	0.22
	Max.	0.33	0.24	0.32
% Oxygen Sat.	Avg.	97.56	100.65	93.12
	SD	1.77	0.62	3.54
	Median	98.65	100.6	95.15
	Min.	94.97	100	88.5
	Max.	98.76	101.4	95.7

3.4.1.3 Water temperature time-series data

Mean daily water temperatures were measured within the temperature-derived regime shift seasons. To explore spatial differences in mean daily water temperatures, Kruskal-Wallis Test was used, revealing highly significant spatial differences in temperature between the three sites in spring (H=23.913, df=2, n=105, p=0.001), summer (H=203.408, df=2, n=105, p=0.001) and autumn (H=41.280, df=2, n=105, p=0.001). Multiple Comparisons Tests found that site K-3A had significantly higher spring temperatures than OR (H=23.913, df=2, n=105, p=0.001) and PR sites (H=23.913, df=2, n=105, p=0.001) (Figure 3.2). Site K-3A had significantly higher temperatures than sites OR (H=203.408, df=2, n=105, p=0.001) and PR (H=203.408, df=2, n=105, p=0.001) in summer, and furthermore, PR had significantly higher temperatures in summer than OR (H=203.408, df=2, n=105, p=0.001) (Figure 3.2). Site K-3A had significantly higher temperatures than sites OR (H=41.280, df=2, n=105, p=0.001) and PR (H=41.280, df=2, n=105, p=0.001) in autumn, and PR had significantly higher temperatures in autumn than OR H=41.280, df=2, n=105, p=0.026) (Figure 3.2).

Temporally, Kruskal-Wallis Tests indicated significant seasonal differences in mean daily temperature (H=360.877, df=2, n=672, p=0.001) (Figure 3.2). Multiple Comparisons Tests revealed that mean daily temperatures in summer were significantly higher than in spring (H=360.877, df=2, n=672, p=0.001) or autumn (H=360.877, df=2, n=672, p=0.001) (Figure 3.2). No significant temporal difference was found between spring and autumn (p>0.05).

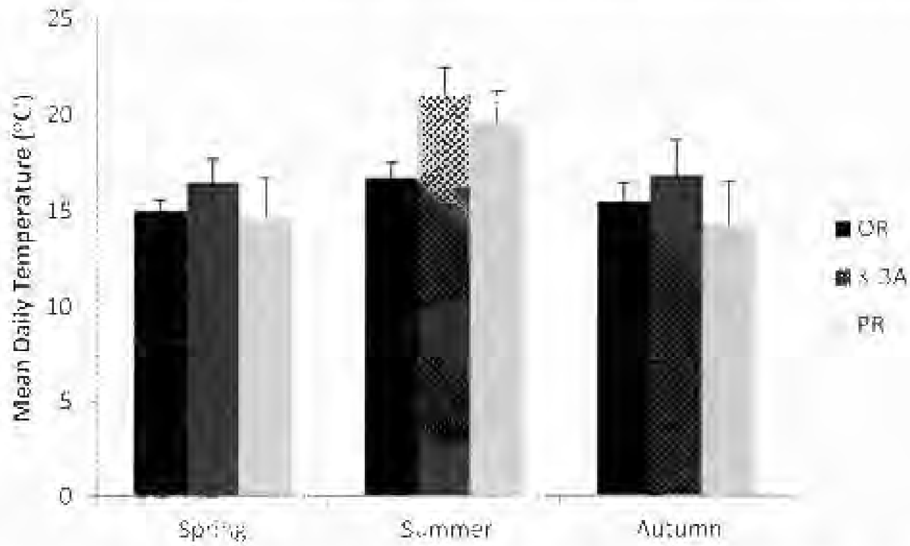


Figure 3.2: Mean daily temperature compared spatially (OR, K-3A and PR) and temporally (Spring, Summer and Autumn) within near-to-pristine condition river sites. Error bars represent standard deviation in temperature measurements (n=105).

The high degree of spatial and temporal differences in mean daily temperature time-series data prevented the formation of spatial and temporal conditions in defining near to pristine conditions. Instead, the examination of mean daily temperature time-series data suggests that conditions should be defined on a site- and season-specific basis.

3.4.1.4 Discharge data

Water-level data were obtained from water-level gauges in each of the steams being studied. To calculate discharge from water-level data, a water-level – discharge relationship was derived for each of the three near-to-pristine sites (Table 3.5), based on three data points, one for each site.

Table 3.5: The water-level – discharge linear relationship. Derived linear equation was the formula used to convert measured water-level values to useful discharge values specific to each site. R^2 values indicate the strength of the water-level – discharge relationship.

Site (n=3)	Linear Equation	R^2 value
OR	y=145.72x - 40.43	0.9604
K-3A	y=32.192x - 12.972	0.9855
PR	y=120.61x - 55.257	0.9997

Despite the small sample size (n=3), water-level – discharge relationship was relatively strong at all three sites, with R^2 values > 0.96 (Table 3.5). The strong relationship enabled the use of the appropriate linear equation to convert water-level values into estimated discharge values. Mean daily discharge values were calculated for every day within the seasonal periods derived from temperature-defined regime shift.

Kruskal-Wallis tests revealed highly significant spatial differences in mean daily discharge between OR, K-3A and PR sites, in spring (H=92.453, df=2, n=105, p=0.001), summer (H=329.78, df=2, n=105, p=0.001) and autumn (H=172.449, df=2, n=105, p=0.001). Multiple Comparisons Tests found that site PR had significantly higher spring discharge than sites OR (H=92.453, df=2, n=105, p=0.001) and K-3A (H=92.453, df=2, n=105, p=0.001) sites (Figure 3.3). Site K-3A had significantly higher discharge than site OR (H=92.453, df=2, n=105, p=0.001). Site PR had significantly higher discharge than sites OR (H=329.78, df=2, n=105, p=0.001) and K-3A (H=329.78, df=2, n=105, p=0.001) in summer, and K-3A had significantly higher discharge in summer than OR (H=329.78, df=2, n=105, p=0.001) (Figure 3.3). Site PR had significantly higher discharge than sites OR (H=172.449, df=2, n=105, p=0.001) and K-3A

(H=172.449, df=2, n=105, p=0.001) in autumn, and K-3A had significantly higher discharge in autumn than OR (H=172.449, df=2, n=105, p=0.001) (Figure 3.3).

Kruskal-Wallis Tests indicated that there were significant seasonal differences in mean daily discharge (H=19.946, df=2, n=672, p=0.001) between sites (Figure 3.3). Multiple Comparisons Tests found that mean daily discharge in spring were significantly higher than in summer (H=19.946, df=2, n=672, p=0.001) or autumn (H=360.877, df=2, n=672, p=0.019) (Figure 3.3). However, no significant difference was found between mean summer and autumn discharge values ($p>0.05$).

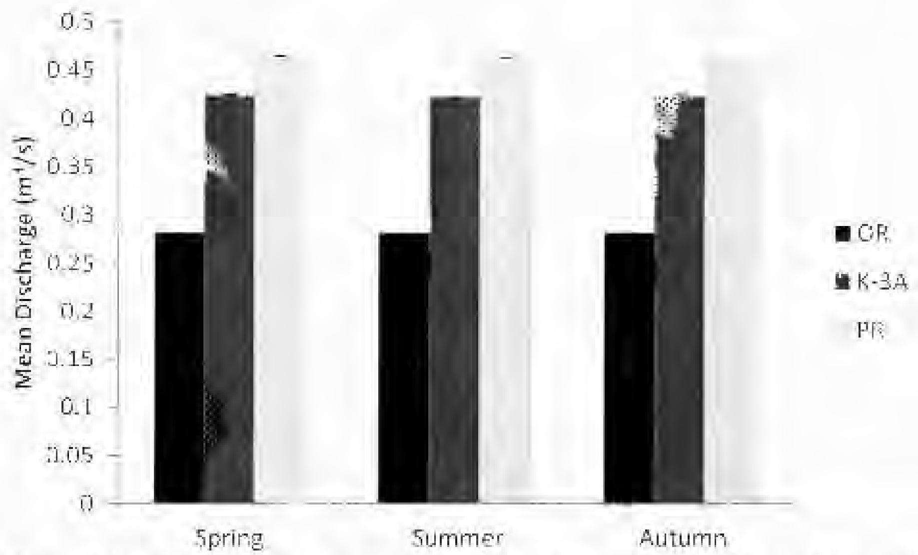


Figure 3.3: Mean daily discharge compared spatially (OR, K-3A and PR) and temporally (Spring, Summer and Autumn) within near-to-pristine condition river sites. Error bars represent standard deviation of temperature (n=105).

As in the temperature time-series data, the high degree of spatial and temporal differences in mean daily discharge time-sites data suggested that near-to pristine conditions have to be defined on a site- and season specific basis.

3.4.2 Macroinvertebrate community assemblage

The SASS5 protocol requires the separate collection of instream invertebrates from three different biotopes. However, for the purposes of this study, all macroinvertebrate specimens that were captured from the different biotopes were grouped together, enabling a community assemblage analysis, for sites as a whole.

3.4.2.1 Univariate analyses

A total of 11 286 macroinvertebrate individuals were captured, comprising 46 macroinvertebrate families identified throughout the course of sampling. The amphipod family Paramelitidae, the trichopteran family Leptoceridae and the dipteran family Simulidae accounted for 18.06 %, 16.21% and 12.37% of cumulative community abundance, respectively. The coleopteran family Scirtidae, the plecopteran family Notonemouridae and the ephemeropteran family Leptophlebiidae accounted for a further 10.74%, 8.01% and 4.9% of cumulative community abundance, respectively. These six families accounted for 70.28% of total cumulative community abundance.

Table 3.6: Descriptive statistics for macroinvertebrate abundance and the number of taxa according to site and season. Macroinvertebrates were captured using SASS5 protocol. "SD" = standard deviation, (n=5).

Site	Basic Stats	Spring	Summer	Autumn
OR	Mean Abundance	229.8	285.6	344.2
	SD Abundance	48.02	69.21	45.32
	Mean No. Taxa	18.6	21.6	20.2
	SD No. Taxa	2.07	2.07	0.84
K-3A	Mean Abundance	190.2	170.4	327
	SD Abundance	64.13	58.24	55.80
	Mean No. Taxa	17.8	17.8	20.4
	SD No. Taxa	1.92	2.77	1.82
PR	Mean Abundance	352.6	121.4	236
	SD Abundance	68.94	16.47	53.73
	Mean No. Taxa	20	21.8	17.6
	SD No. Taxa	1.58	5.17	2.79

Kruskal-Wallis tests revealed highly significant spatial differences in mean abundance of macroinvertebrates between the sites in spring (H=9.780, df=2, n=15, p=0.008), in summer (H=9.380, df=2, n=15, p=0.009) and in autumn (H=7.994, df=2, n=15, p=0.018). Multiple Comparisons Tests revealed that in spring, site PR had significantly higher mean abundance of macroinvertebrates than site K-3A (H=9.780, df=2, n=15, p=0.009) (Table 3.6). In summer

(H=9.380, df=2, n=15, p=0.007) and autumn (H=7.994, df=2, n=15, p=0.017), site OR had significantly higher mean abundance of macroinvertebrates than at site PR (Table 3.6).

Kruskal-Wallis tests revealed that mean number of taxa was significantly different between sites in autumn (H=7.994, df=2, n=15, p=0.018), but not in spring or summer (p>0.05) (Table 3.6). Multiple Comparisons test revealed that mean number of taxa in autumn at site OR was significantly higher than at site PR (H=7.994, df=2, n=15, p=0.017) (Table 3.6).

Temporally, Kruskal-Wallis tests revealed highly significant seasonal differences in mean abundances of macroinvertebrates (H=14.555, df=2, n=45, p=0.001), and even though a higher mean number of taxa was collected in summer (Figure 3.4), no significant differences were found when examining mean number of taxa (p>0.05). Multiple Comparisons tests revealed that mean number of macroinvertebrates was significantly higher in spring (H=14.555, df=2, n=45, p=0.48 and in autumn (H=14.555, df=2, n=45, p=0.001) than in summer.

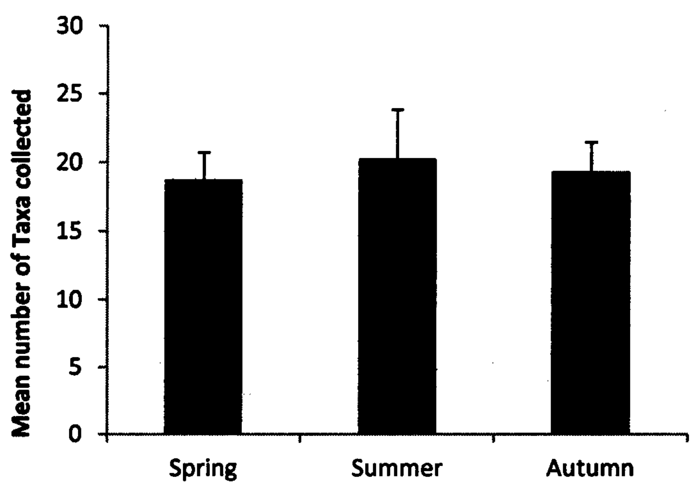


Figure 3.4: Mean number of taxa collected temporally at all reference condition sites.

3.4.2.2 Multivariate analyses of biological data

Multi-dimensional Scaling (MDS) was performed on a Bray-Curtis similarity matrix based on fourth-root transformed macroinvertebrate abundance data. The effects of varying levels of taxonomic resolution were also explored, in particular the use of family- versus species-level identifications (Figure 3.5). The results of the family-level MDS ordination (Figure 3.5) revealed spatially and temporally distinct groupings with minimal overlap. A similar trend was observed in the species-level ordination (Figure 3.5). In both ordinations, however, the stress value was higher than 0.2, which has been used as a guideline limiting the strength in confidence in the MDS, indicating that the ordinations should be treated with caution (see Table 3.2).

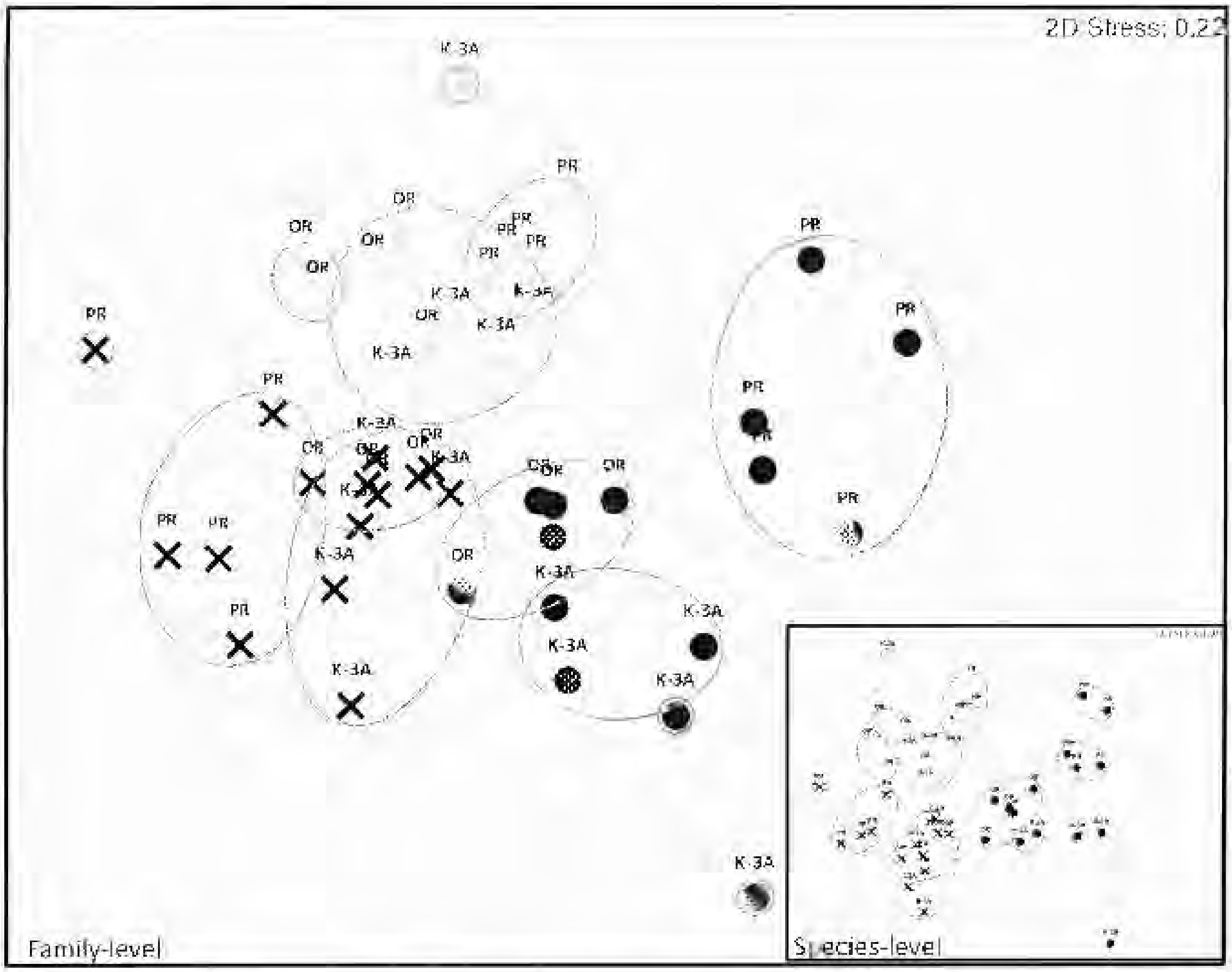


Figure 3.5: Non-metric Multi-Dimensional Scaling (MDS) ordination of macroinvertebrate family-level (left) and species level (lower right) abundance data, separated by site (OR, K-3A and PR) and season (Spring - triangle, Summer - circle and Autumn - cross). Curtis-Bray similarity resemblance matrix was used. Data were fourth-root transformed. Groupings shown at a similarity level of 70% according to the cluster analysis of the data.

The ANOSIM (Analysis of Similarities) test (Two-Way Crossed Analysis) was used to assess whether the apparent differences seen in Figure 3.5, were statistically significant. ANOSIM added more strength to observed spatial and temporal differences in macroinvertebrate assemblages as opposed to the high-stress value generated from MDS ordination, by confirming visual spatial and temporal trends. Spatially, replicates within each of the three near-to-pristine sites were more similar to each other than replicates from other sites, and were significantly different in macroinvertebrate assemblages (Global R = 0.809, p=0.1%). Temporally, replicates within each of the seasons are more similar to each other than replicates from other seasons, and were highly significantly different in macroinvertebrate assemblages (Global R = 0.884, p=0.1%). The presence and degree of significant differences, both spatially and temporally may be explained further by examining dissimilarities in macroinvertebrate assemblages.

The taxa responsible for the Bray-Curtis dissimilarity between sites and seasons was computed using SIMPER. Spatially, Notonemouridae, Chironomidae and Potamonautidae accounted for 14.7% of total cumulative dissimilarity between sites OR and K-3A (Table 5). Paramelitidae, Simuliidae and Chironomidae accounted for 19.5% of total cumulative dissimilarity between sites OR and PR, and Paramelitidae, Simuliidae and Leptoceridae accounted for 19.2% of total cumulative dissimilarity between sites K-3a and PR (Table 3.7).

Table 3.7: SIMPER analysis of spatial macroinvertebrate assemblage data. Overall Dissimilarity % is the total percentage dissimilarity between sites in comparison. Only top three taxa accounting for the highest percentage of dissimilarity between sites were included, with respective contributing dissimilarity percentages.

Spatial Comparison	Overall Dissimilarity %	Family-taxon	Dissimilarity %
OR & K-3A	**34.8**		
		Notonemouridae	5.5
		Chironomidae	4.7
		Potamonautidae	4.4
OR & PR	**39.4**		
		Paramelitidae	10.7
		Simuliidae	4.5
		Chironomidae	4.3
K-3A & PR	**40.5**		
		Paramelitidae	9.2
		Simuliidae	5.5
		Leptoceridae	4.5

Temporally, Leptoceridae, Chironomidae and Notonemouridae accounted for 15.4% of total cumulative dissimilarity between spring and autumn (Table 3.8). Notonemouridae, Paramelitidae and Leptoceridae accounted for 18.6% of total cumulative dissimilarity between spring and autumn, and Chironomidae, Paramelitidae and Simuliidae accounted for 18.7% of total cumulative dissimilarity between summer and autumn (Table 3.8).

Table 3.8: SIMPER analysis of temporal macroinvertebrate assemblage data. Overall Dissimilarity % is the total percentage dissimilarity between seasons in comparison. Only the top three taxa accounting for the highest percentage of dissimilarity between sites were included. Cumulative Dissimilarity % is the combined total dissimilarity of the three taxa.

Temporal Comparison	Average Dissimilarity %	Family-taxon	Dissimilarity %
Spring & Summer	**40.8**		
		Leptoceridae	5.6
		Chironomidae	4.9
		Simuliidae	4.9
Spring & Autumn	**35.9**		
		Notonemouridae	6.9
		Paramelitidae	6.8
		Leptoceridae	4.9
Summer & Autumn	**40.3**		
		Chironomidae	7.0
		Paramelitidae	6.1
		Simuliidae	5.6

3.4.3 Spatial and temporal variability in SASS5 indices

Though the near-to-pristine sites are not known to be subjected to any anthropogenic impacts, it was important to ascertain whether the near-to-pristine sites conform as "reference condition sites" according to the protocol created by Dallas and Day (2007) for upper river sites. Invertebrate replicates (SASS and ASPT values) (n=15) for each site were projected against the reference condition quartiles derived from Dallas and Day (2007) (Figure 3.6).

On average OR, K-3A and PR sites, according to regional biological bands developed by Dallas and Day (2007), fell into ecological category (EC) "A", indicating that they are of reference condition.

Earlier in this study significant spatial and temporal differences were found in physico-chemical components and in response, similar trends were observed in macroinvertebrate assemblages. As per SASS5 protocol, SASS5 scores (SASS5 and ASPT) were derived from biological data, and it would be expected that SASS5 scores would follow patterns observed in abundance and number of taxa data, and if so, may too differ spatially and temporally.

SASS5 scores were calculated for each of the five replicates taken for each season. Kruskal-Wallis Tests revealed no significant difference in SASS5 Scores between the reference sites (df=2, n=15, p>0.05). Kruskal-Wallis tests performed on spatial and temporal ASPT scores revealed no significant site specific differences (df=2, n=15, p>0.05), but did indicate highly significant seasonal differences in ASPT scores (H=29.560, df=2, n=45, p=0.001). The Multiple Comparisons test showed that ASPT scores in Spring (H=29.560, df=2, n=45, p=0.001) & Autumn (H=29.560, df=2, n=45, p=0.002) were significantly higher than ASPT scores calculated for Summer (Figure 3.7).

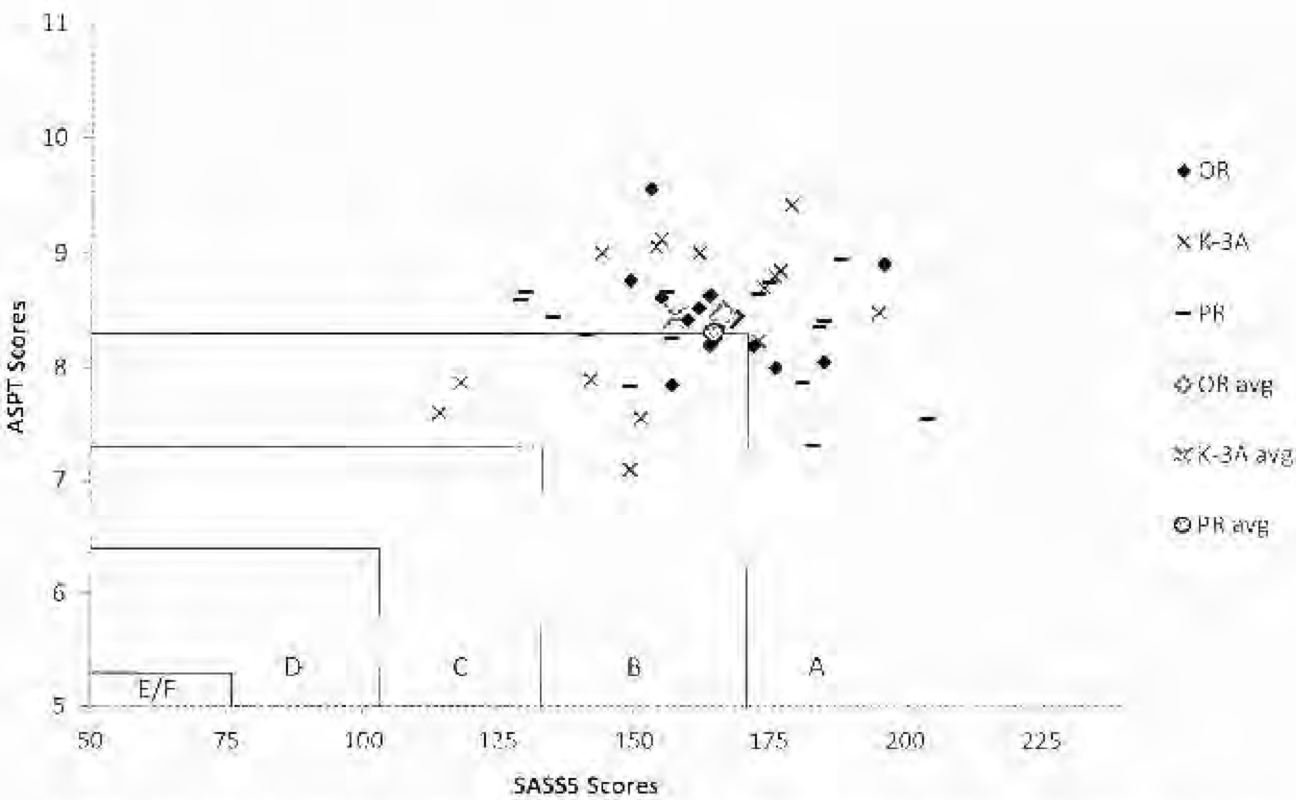


Figure 3.6: SASS5 figure for determining Ecological Categories for spatial replicates by using Biological Banding derived from Dallas and Day (2007), for the upper region sites. Abbreviations: "OR" – Oudebos River, "K-3A" – K-3a River, and "PR" – Palmiet River sites, and "avg" – averages calculated for SASS5 and ASPT scores for the three near-to-pristine sites (n=45). Reference condition indicated by invertebrate assemblages falling within the "A" Band.

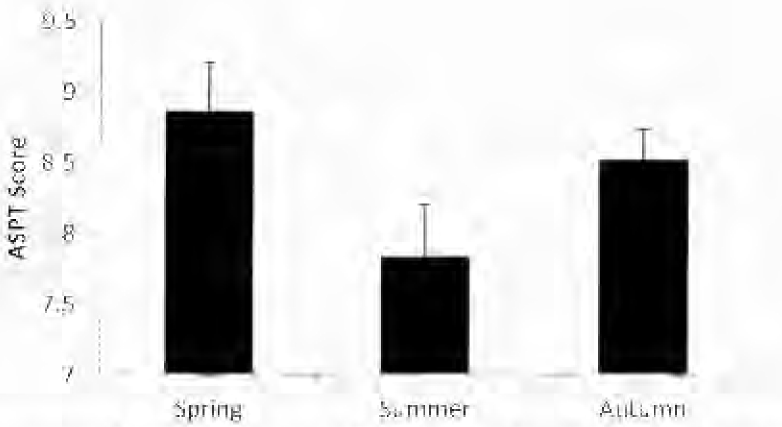


Figure 3.7: Mean temporal ASPT scores at reference condition sites. Abbreviations: "ASPT" – average score per taxa.

The lack of significant spatial differences in the biologically derived SASS5 and ASPT scores suggests that it is valid to collectively group and classify a range of SASS5 and ASPT scores for reference conditions, ascertained from the three sites sampled (Table 3.9). However, due to the high degree of temporal differences observed in ASPT scores, the SASS5 and ASPT scores could not be collectively grouped to form a common range of reference values, instead were forced to remain seasonally separate (Table 3.9).

Table 3.9: Summary statistics for SASS5 and ASPT scores calculated from spatially combined data collected at three reference sites sampled in the Palmiet River catchment, separated by season. Abbreviations: "Min" = minimum value, "Max" = maximum value, & "SD" = standard deviation.

Biological Indices	Basic Statistics (n=15)	Spring	Summer	Autumn
SASS 5 Score	Avg.	165.73	158.27	164.53
	SD	16.05	25.91	17.69
	Median	157	157	168
	Min.	144	114	130
	Max.	196	204	195
ASPT Score	Avg.	8.86	7.83	8.52
	SD	0.35	0.37	0.22
	Median	8.8	7.87	8.45
	Min.	8.26	7.1	8.24
	Max.	9.56	8.6	9

Interest was directed towards examining whether observed temporal differences in ASPT scores altered calculated EC symbols for seasonal replicates (Figure 3.8). Temporally, spring and autumn replicates were located within the "A" EC biological band (Figure 3.8). The summer replicates averaged within the "B" or "minimally-impacted" biological band. The

"B" EC symbol in this case indicates some form of impact on water quality within the stream in the summer period, which may have resulted in the reduction of highly sensitive macroinvertebrates within the summer period, lowering SASS5 scores and ASPT values (Table 3.9 and Figure 3.8). However this is untrue. Neither site nor any season across the study was exposed to impacts that may lead to reduced water quality. Perhaps taxa that are better adapted to survival and colonise within the summer period are taxa that are also found within impacted sites. If that were the case, that may explain the lower SASS5 scores and ASPT values ascertained in the summer period. What's more, to avoid the seasonal difference in EC symbols, biological bands were defined temporally for spring, summer and autumn (Table 3.10). Once the new temporal biological bands were developed, seasonal replicates (n=15) were averaged and plotted, and all three reference sites were found to aggregate within the upper 90th percent quartile with an EC equal to an "A", as was expected due to the pristine nature of each of the streams.

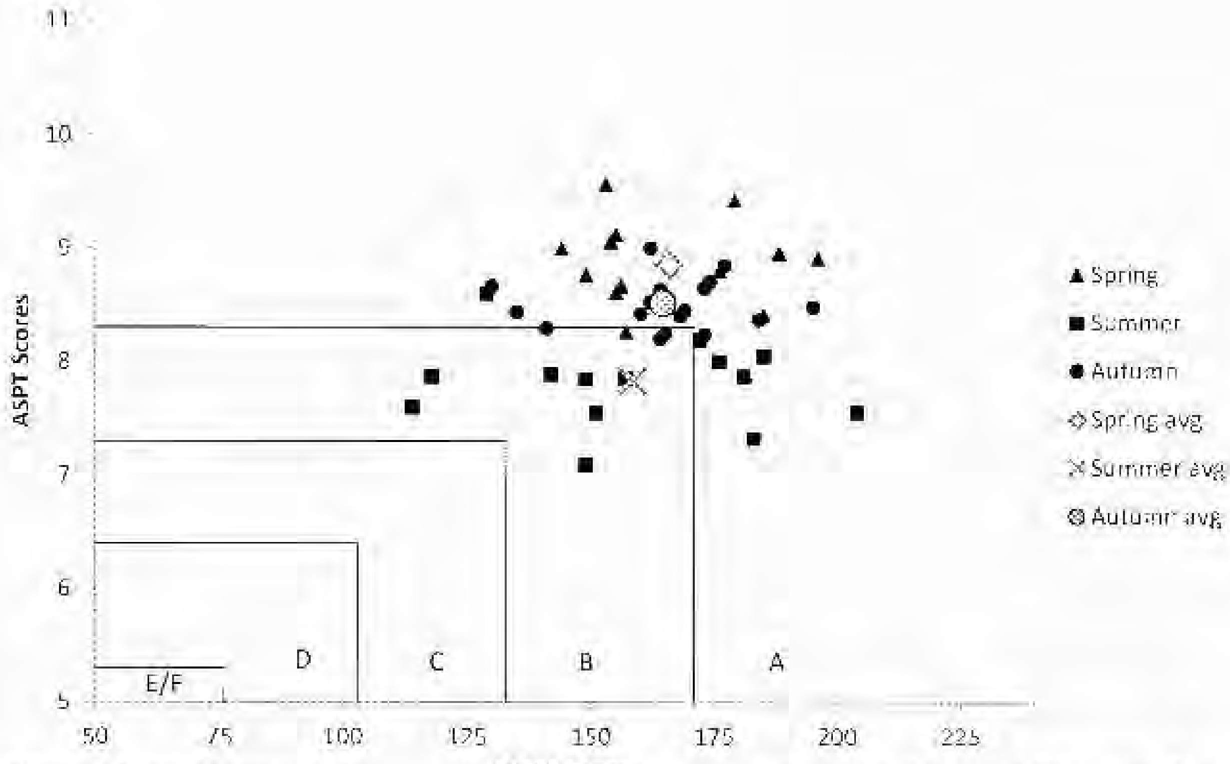


Figure 3.8: SASS5 figure for ascertaining Ecological Categories for seasonal replicates by using Biological Banding derived from Dallas and Day (2007), for upper region sites. Abbreviations: "OR" – Oudebos River, "K-3A" – K 3a River, and "PR" – Palmiet River sites, and "avg" – averages calculated for SASS5 and ASPT scores for the three near-to-pristine sites (n=45). Reference condition indicated by invertebrate assemblages falling within the "A" Band.

Table 3.10: Calculated quartiles for "Palmiet River catchment Seasonal Biological Bands". Palmiet River catchment reference bands (90, 67.5, 45, 22.5 % quartiles calculated from the overall combined SASS & ASPT scores from the three reference sites). Average SASS5 and ASPT scores for OR, K-3a and Palmiet sites were calculated. Spatially combined SASS5 and ASPT scores were averaged (n=15) for spring, autumn and summer.

	SASS5 Score				**ASPT Score**			
% Quartiles	**90th**	**67.5th**	**45th**	**22.5th**	**90th**	**67.5th**	**45th**	**22.5th**
Spring	149.2	111.9	74.6	37.3	8.0	6.0	4.0	2.0
Summer	142.4	106.8	71.2	35.6	7.1	5.3	3.5	1.8
Autumn	148.1	111.1	74.1	37.0	7.7	5.8	3.8	1.9

3.5 DISCUSSION

The objective of this chapter was to define abiotic and biotic reference components within the Palmiet River catchment. In establishing naturally occurring physico-chemical conditions and macroinvertebrate assemblages, spatial and temporal variability within three reference condition sites were investigated statistically, including the effects such variability may have on the SASS5 bioassessment protocol. The limited nature of spatial (three sites only) temporal three seasons only) replication heeds caution to interpreting the strength of findings.

Spatial patterns of physico-chemical conditions and macroinvertebrate assemblages

Reference sites were *a priori* selected, on the knowledge that sites shared similar underlying geology (Table Mountain Group), surrounding vegetation (Kogelberg Sandstone Fynbos), similar land-use practices (all three sites located within provincial nature reserves). They were all sampled from within the mountain stream zone and riffle biotope to minimise potential spatial impacts. Examination of environmental variables revealed no significant differences with respect to physico-chemical variables between the three reference sites. In terms of deriving reference conditions for the Palmiet River catchment, the lack of spatial difference in physical-chemical variables implied that sites were similar enough to use these values to derive a set of reference conditions (Table 3.4).

However, highly significant differences were observed between sites when investigating temperature and discharge (Figure 3.2 and 3.3). Identifying possible causes of such spatial variability fell outside of the scope of this study, and reasons could only be postulated from previous scientific literature.

The significantly higher temperatures at site K-3A may be a result of the pools found in between sampled sections of the river at the site. McRae and Edwards (1994, cited by Allan and Castillo 2007) found that increased number of ponds or deeper sections within a stream tend to increase stream temperature, because they increase the residence time of water and the surface area exposed to solar radiation. Pooled sections were avoided in sampling, with sampling restricted to riffles only, but, water flowing from these warmer pools into a riffle section sampled, would result in elevated temperatures when compared to sites such as OR and PR that lack water pooling.

Discharge was calculated by combining measurements of width, depth, and velocity, and was found to be higher at site PR than at the other two sites. A site with higher values in any of the three components would result in a higher discharge. With this in mind, the lack of spatial differences in velocity, in addition to the expected similarity in rainfall due to all three sites being located within the same catchment, the greater stream dimensions of site PR may have solely resulted in the significantly higher discharges recorded.

Similarly, macroinvertebrate taxon richness, abundance and community assemblages revealed spatially distinct patterns (Table 3.6, Figures 3.4 and 3.5). Individual inspection of invertebrate families found that paramelitidae, notonemouridae, simuliidae, leptoceridae and chironomidiae contributed for the greatest amount of spatial variability between three the sites. Previous literature suggests that these spatial differences in invertebrates may be attributed to discharge and its effects on food availability (Schael 2005).

Paramelitidae were more abundant at sites OR and K-3A than at PR, this in turn contributing to an overall dissimilarity in macroinvertebrate assemblage of 10.7% and 9.2% between the sites, respectively. Paramelitidae are shredders (Schael 2006) and feed by fragmenting leaves and other large pieces of plant material, and were found to be more abundant at sites OR and K-3A which had visually more canopy cover than site PR. With the increased food availability from the riparian trees, and the significantly lower discharge rates, food availability would be expected to be higher because the lower discharge would allow food to linger for longer in stream. The greater food availability may in turn result in the higher shredder abundances observed in sites OR and K-3A than at site PR. In the same sense, Notonemouridae, also shredders, were more abundant at site OR than K-3A, and comparatively site OR had visually higher canopy coverage and lower discharge than K-3A, and may explain higher numbers of Notonemourids captured.

The high spatial dissimilarity in Simuliidae may be related to the significantly higher discharge rates recorded at site PR. Simuliids, which were found to be more abundant at site PR than sites OR and K-3A, are known to be filter-feeders collecting small particles of food suspended in the water-column. They feed on a range of micro-organisms (bacteria to zooplankton), and rely heavily on stream flow to bring food to them (Schael 2006). Increased discharge may result in a greater opportunity of food passing through and thus being filtered, and may explain the greater abundances of Simuliidae at site PR than at sites OR and K-3A.

Leptoceridae contributed to 4.5% dissimilarity in macroinvertebrate assemblages, between sites PR and K-3A. Leptocerids feed on algae and detritus (Schael 2006). The PR site had less canopy cover than K-3A. The lesser canopy cover may result in higher solar radiance instream, and in turn may result in increase algal production. The increase in algal mats could lead to an increase in abundances of organisms that feed of algae, such as leptocerids sampled within this study.

Chironomidae too showed marked spatial differences in abundance. Interestingly, the chironomids identified within this study could be split into two functional feeding groups: deposit feeders and predators (Schael 2006). The deposit-feeding chironomids were more abundant at site OR and K-3A, and the predatory chironomids were more abundant at site PR than OR. The greater abundances of deposit-feeding chironomids may be attributed to a modification in diet from algae (common food source in less-canopied streams) to predominately detritus (in more canopied sections of streams). It was not tested in this study, but it can be postulated that an increase in deposit feeders may be the result of an increase in shredders at OR, with the deposit-feeding chironomids feeding on the remains of what is left after shredders feeding on plant material. The greater abundance of predatory chironomids at site PR compared to site OR was an unexpected situation. Many studies have established that predator abundances increase with the increase in prey (Metz 1974, Allan 1978), supported by increased abundance of Potamonautidae in this study, at site OR. However, predatory chironomids were most abundant at site PR where prey abundances were comparatively lower. Allan and Castillo (2007) stated that predator foraging mode affects prey vulnerability, interacting with aspects of prey movement to influence localised encounter rates and departures. Mobile prey are likely to flee if able to detect the approach of large, actively searching predators, and so predator impact may be greatest with least mobile prey. The observed increases in Simuliidae at site PR, a sessile prey to predator-feeding chironomids, may in turn lead to the increased abundances of chironomidae found at site PR when compared to site OR.

Relating spatial results to bioassessment, SASS5 metrics did not reflect observed significant spatial patterns and differences in macroinvertebrate assemblages between the three sites. The SASS protocol was designed to use invertebrate data as a proxy for water quality, and because of this, spatial patterns in SASS metrics would be expected to shadow observed patterns in community assemblages. However, this is not so. With that said, the lack of similarity in observed patterns between macroinvertebrate assemblages and SASS5 metrics does not undermine SASS as a biotic index, and may be due to the fact that univariate and multivariate analyses performed in this study, analysed macroinvertebrate community abundances, whereas SASS was designed to assess presence/absence of taxa. The Kruskal-Wallis test, MDS ordination and ANOSIM analyses were performed on abundance data, and significance was attributed to taxa where abundances varied greatly, not on the presence or absence of a specific taxa.

The lack of spatial differences in SASS5 and ASPT scores, for the sake of this study, allowed for the collective spatial grouping of each metric within the Palmiet River catchment, i.e. the three reference sites can be used to create a spatially combined SASS and ASPT score range suitable for reference conditions in the upper reaches of the Palmiet River catchment.

Temporal heterogeneity

Physico-chemical variables revealed that conductivity, instantaneous water temperature, pH, velocity and percentage dissolved oxygen saturation differed significantly between seasons at the three sites, as did long-term water temperature and discharge data. Allan and Castillo (2007) mentioned that as a result of the influence of seasonal change in discharge regime, precipitation inputs and biological activity, river chemistry can vary over time. Flow variation has especially strong effects on ionic concentrations (Allan and Castillo 2007). Conductivity, a measure of total dissolved ions, was expected to be higher with the first increase in discharge of the rainy season, i.e. within spring of this study due to runoff of accumulated salts and ions from surrounding catchment. However, conductivity was found to be significantly higher in autumn than spring and summer. Winter rainfall may have removed soluble surface salts prior to spring rainfall. Lewis and Saunders (1990) cited by Allan and Castillo (2007) discovered the lack or minimal rainfall received in dryer periods allowed for the build-up of soluble surface salts and ions, and the first rains, in this case autumn, would wash away accumulated soluble surface salts and ions into the stream, and may explain the significant increase in dissolved ions in autumn compared to spring and summer.

Similarly, pH was significantly higher in autumn than in spring or summer. pH of water is the measure of hydrogen ions concentration, and is influenced by geographical and atmospheric characteristics. Most fresh waters are relatively well buffered and more or less neutral, with pH ranges from 6 to 8 (Dallas and Day 1993). South-western Cape streams are naturally far more acidic than others and their biotas are adapted to these conditions. The range of pH noted in this study was between 3.97 and 5.27 which was similar to that measured at the same PR site in 1987 by Gale (1989), and in particular differences were apparent between autumn and spring. These differences in pH cannot be solely attributed to geographical or lithostratigraphic characteristics of the catchment; pH is also influenced by the indigenous vegetation, which is characteristic of the upper-catchment areas of the Western Cape. Fynbos plants are rich in polyphenols and when the plants decay, the polyphenols are released into the soil, where they undergo transformation into a complex of chemicals known as "humic substances" (Davies and Day 1998). These humic substances are organic acids and, when dissolved in water, pH is reduced. The significantly higher recorded discharge in spring, would flush out humic substances from the soils, into the stream, increasing the stream acidity, thus lowering the pH. Conversely the comparatively lower discharges recorded in summer and autumn limited organic acidic input and thus higher pH levels were found in respective seasons.

Water velocity was significantly higher in spring than in summer. This may be simply due to the significantly higher discharge recorded in spring. An increase in discharge, i.e. volume of water down a stream, would result in a lower degree of contact between surface waters and substrate, thus limiting amount of friction and in turn resulting in the higher velocities recorded in periods of higher discharge.

Oxygen, is influenced by partial pressure, the decomposition of organics and presence of photosynthesis, was found to be significantly higher in the summer season than in spring or autumn. Importantly, oxygen levels in riverine water are known to be influenced on top of the already mentioned factors, by temperature, input of groundwater and stream velocities (Allan and Castillo 2007). The solubility of oxygen in water is reduced as the stream temperature increases. Perennial streams such as those under study here, rely heavily on groundwater input during periods of no or minimal rainfall. The influx of groundwater in comparison to rainfall received within the stream lowers dissolved oxygen within the stream due to microbial processing of organic matter as water passes through the soil (Allan and Castillo 2007). With this in mind, dissolved oxygen saturation values would be expected to be at a minimum within the summer (dry) period. And oxygen concentrations are affected by instream velocities, as a result of increased turbulence. An increase in turbulence results in an increase in surface area exposed to air, allowing for increased diffusion of oxygen from air into the water. The greater the turbulence, the greater the expected instream oxygen concentrations.

With all of this in mind, the significantly higher temperatures, significantly lower velocities and the potential (but not investigated) influx of groundwater in summer, it would be expected that oxygen concentrations instream would be considerably lower than in spring or autumn. Surprisingly however, this was not so. Dissolved oxygen saturation differences were very slight and found to be significantly higher in the summer season.

As Allan and Castillo (2007) noted, photosynthesis is an important process that may alter the concentration of oxygen. A study by Minshall *et al.* (1983) exploring the instream primary production over a range of stream sizes in four distinct biomes of the United States, found that Gross Primary Production (GPP) was highest in summer, periods of increased temperature and irradiance. Similarly, Morin *et al.* (1999) developed empirical models to predict primary production from chlorophyll *a* and water temperature, and found that production was strongly related to water temperature in stream periphyton. In my study, the elevated temperatures and increased irradiance expected to reach the stream in summer, may increase photosynthetic activity and in turn increase GPP instream and as a by-product of photosynthesis, oxygen concentrations instream would be elevated, even to supersaturated levels (88.5 to 101.4%) as in the case of this study.

Macroinvertebrate patterns

The greatest number of taxa were recorded in summer, which also exhibited the most within-season variability, and the lowest abundance of macroinvertebrate individuals (Table 3.6). Autumn on the other hand, revealed the greatest macroinvertebrate abundances (Table 3.6). These marked differences in abundances were reflected in the ordination analysis illustrating distinctly separate seasonal groupings. Britton (1991), in a study of the Swartboskloof stream, Western Cape, noted that most taxa were least abundant in summer, which is a stressful period due to pressures imposed from temperature and discharge. The lotic environment in the Western Cape consists of periods of naturally high baseflow and low temperatures, and periods of lowest baseflow coupled with high temperatures, and these events create extremely stressful environments, which may in turn result in seasonally distinct patterns of macroinvertebrate assemblages at upper-catchment sites of this region.

Dallas (2004a) stated that invertebrate assemblages have developed varying life cycles to overcome such harsh environments, and therefore any temporal differences in taxonomic makeup of macroinvertebrate assemblages within streams may be due to the differences among insect life cycles. In mountain streams of the Western Cape, many insects are univoltine, (i.e. have a single generation per year), and at any given time a single species may be represented by eggs, larvae, pupae or nymphs and adults (Davies and Day 1998). A study examining the life histories of two leptocerids discovered that the univoltine detritus-feeding *Ceraclea transversa* had five larval instar stages and overwinter as inactive pupae before emergence in late spring (Resh 1976a). A similar pattern in emergence in spring may be observed in leptocerids captured within this study, because leptocerid abundances were significantly higher in spring, dropping off considerably, after emergence, in summer and autumn.

Chironomidae, which are known to be multivoltine, were found to be significantly more abundant in summer than in spring or autumn. Boothroyd (1999) found that chironomid growth and emergence was highly correlated with increases in air temperature, and subsequent increases in instream temperatures. In spring when water temperatures were significantly lower than summer, early instars may be too small for the sampling method, SASS, in collecting invertebrates with comparatively large mesh-size (1mm), thus smaller instars of aquatic insects, in this case chironomids, may not be collected and observed seasonal differences may solely be due to the apparatus used for sampling. The significant increase in temperature from spring to summer may cue rapid growth in chironomids, to a size that is capable of being captured in the SASS nets, and this may explain the significant numbers of chironomidae found in summer as opposed to spring. And in turn, after late summer emergence, individuals of the next generation were too small to be collected, thus explaining the dramatic decline in chironomid abundance in autumn.

Notonemouridae and Paramelitidae were both significantly more abundant in autumn than in either spring or summer. Some aquatic macroinvertebrates, particularly stonefly and caddisfly larvae, as in the case of this study, feed on leaves (Winterbourn 1982). Furthermore, some microdistribution surveys have demonstrated close instream associations between the biomass of coarse leaf matter and members of herbivorous fauna (Linklater 1995). The notonemourid and paramelitid life cycles and thus abundances are cued with leaf litter fall, which is abundantly available in autumn when deciduous trees shed their leaves in the Western Cape.

Several authors have also suggested that filter-feeders such as Simuliids can benefit and may depend for food on coarse organic matter produced by the feeding activities of shredders (Linklater 1995). Britton (1991) postulated that microbial processing of leaf-litter during autumn, aided by physical abrasion of leaf material at comparatively higher discharges recorded than in summer, may result in high-quality course particular organic matter (CPOM) becoming available to filter-feeders in autumn. It is postulated that Simuliidae are more abundant in autumn than any other season, because they have life cycles synchronized to exploit this food resource once it becomes available.

SASS5 indices

As required by SASS5 protocol, SASS5 indices scores and values were derived from biological data, and due to the sole reliance on biological data, it would be expected that scores and values would shadow those temporal patterns observed in biotic analyses. This was true for mean seasonal ASPT values, which revealed marked temporal differences, but no seasonal differences were found in SASS5 scores.

The lack of temporal differences in SASS5 scores may be explained by the number of taxa found within a season and the assigned SASS5 scores to each taxon. For example, the summer season consisted of a greater number of taxa than either spring or autumn which were more tolerant of harsh conditions occurring in the summer period and thus have been assigned lower SASS5 scores individually, and may have in turn attributed to a greater SASS5 score for that season than expected when compared to the calculated ASPT values.

Generally, more sensitive and high-scoring taxa, such as Glossosomatidae, Philopotamatidae, Teloganodidae, Petrothrincidae and Dryopidae were more common in spring compared to summer, whilst Notonemouridae, Barbarochthonidae, Leptophlebiidae, Paramelitidae and Scirtidae were more common in autumn. These observations were reflected in macroinvertebrate clustering into predominately spring- versus summer- versus autumn-groups, and explains the high degree of differences found in temporal ASPT scores.

Relatively high ASPT scores in spring and autumn, due to the dominance of more sensitive taxa, led to SASS5 and ASPT replicates being localized within the "A" ecological category or unimpacted biological band. The summer season samples, which comprised more taxa but of lower sensitivity scores were mostly found to aggregate within the "B" ecological category or minimally-impacted biological band. The decrease in EC symbols from spring to summer, and increase from summer to autumn, indicates according to Dallas and Day (2007) some form of impact of water quality on the stream in the summer period. However, these sites fall within provincially protected nature reserves and were chosen for their pristine nature. Thus, observed differences in macroinvertebrate communities and biotic indices throughout the course of this study can be solely attributed to the seasonal variation. These seasonal changes in reference-site data revealed the necessity to adopt a seasonal approach and modify the bands produced by Dallas and Day (2007), i.e. bands specifically created for each season, such that unimpacted reference condition summer sites were assigned to an EC equivalent to an "A" symbol. Following this adjustment, all three reference sites, in all three seasons sampled, fell within the unimpacted-pristine biological band.

Conclusion

River ecosystems are longitudinal systems that integrate the characteristics of the catchments they drain. It has often been assumed that regions with similar abiotic characteristics will have similar biotic characteristics. Thus if sites are in the same region, i.e. in this case catchment, and river type (i.e. similar in terms of hydrological type, size and substratum), it is assumed that their macroinvertebrate assemblages would also be transmitted into spatial homogeneity with respect to abiotic factors It was shown, however that is not always the case, even though water-chemistry variables were found to be spatially similar. Factors such as discharge and temperature influenced macroinvertebrate assemblages spatially.

Similarly, since many aquatic organisms have specific physico-chemical and hydraulic requirements, temporal variation in factors such as stream discharge and temperature have lead to seasonally distinct water-chemistry, and may have lead to the observed temporal variation in the distribution and abundance of macroinvertebrate assemblages.

Caution was exercised in placing too great an emphasis on calculated reference physico-chemical conditions due to the limited sample size (n=6). However, in a study examining the effect of stream regulation on the physico-chemical properties of the Palmiet River, Gale

(1989) used site PR as a guide for an undisturbed site, and median-measurements of pH, turbidity, velocity and percentage dissolved oxygen saturation over a course of 18 months fell within the range of reference-deemed conditions defined within this study, placing confidence in the physico-chemical conditions calculated within this study for the same catchment. Conductivity and water temperature median-measurements fell below the set reference range, and this may be due to the study by Gale (1989) incorporating winter, which is a season known to have low temperatures and conductivity levels within the Western Cape, thereby lowering the median calculated within her study.

The seasonal patterns in macroinvertebrate assemblages reflected life history characteristics of individual taxa exploiting conditions of high food availability and ideal conditions for emergence. The lack of spatial variability and the presence of temporal heterogeneity in taxon abundance and richness were reflected in biotic indices that were based on the macroinvertebrate assemblages. Whilst more taxa were recorded in summer, a higher proportion of sensitive and high-scoring taxa were recorded in spring and autumn. This in turn skewed ASPT scores and placed either season in biological bands described as reference conditions, but subjected the summer season replicates to a lower ecological category represented as minimally-impacted. Thus in this study when macroinvertebrate assemblages were used for bioassessment, temporal variation of individual taxa influenced judgment as to whether or not a site was disturbed.

The significant temporal differences in macroinvertebrate assemblage, ASPT and the lower ecological category given for pristine condition sites within the summer season, highlighted that these streams are changing significantly between seasons, influences established biotic indices. To establish reference conditions appropriate for these systems, a generalized regional non-seasonal biological banding system cannot provide an accurate ecological category or "health indicator" within the Palmiet River catchment. Instead, a seasonal approach at catchment scale is required where biological bands need to be calculated temporally for reference conditions, for the seasons of spring, summer and autumn. For example, if a monitoring site is assessed in autumn only, it should preferably be compared to the reference condition for autumn, if you were to sample say in summer, and compare it to the overall, seasonally combined SASS biological bands generated by Dallas and Day (2007), the absence or diminished importance of "summer" taxa due to seasons being combined, may reflect water quality impairment or reduced river health, and ignore the presence of temporal variability. Sampling season and producing SASS biological bands for reference conditions within those seasons is thus particularly crucial.

CHAPTER 4

COMPARISON OF TWO INVERTEBRATE-BASED BIOMONITORING TECHNIQUES

4.1 INTRODUCTION

Lotic freshwater ecosystems hold one of the most important natural resources on Earth (Dudgeon *et al.* 2005), and yet are also among the most threatened of ecosystems on the planet (Malmqvist and Rundle 2002), especially in South Africa (e.g. Nel *et al.* 2011). The semi-arid climate combined with increasing human populations (Ferreirra 2008) and encroachment of activities along river courses (Moya *et al.* 2007) places tremendous pressure on freshwater systems in the country. Preservation of intact river systems, together with efficient management practices on already impacted rivers remains a priority to ensure restoration of their integrity. For this reason, the development and application of various monitoring programmes and tools are important steps in trying to balance the protection of our water resources with those created by social and economic development (Ferreira 2008).

The current monitoring programme used in South Africa to assess ecosystem integrity of river systems is known as the RHP. River Health Programme is a national program designed specifically for the South African environment to assess and monitor the ecological state of rivers (Malherbe *et al.* 2010), using both chemical water quality and biotic monitoring. It is based on the concept that aquatic communities (including fish, invertebrates and riparian vegetation) reflect the effect of anthropogenic disturbances in rivers over extended periods of time (Ballance *et al.* 2001). The rationale is that the ecological condition of the aquatic biota provides a direct, holistic and integrated approach to measure the ecological condition of a river as a whole (Roux *et al.* 1999). Aquatic macroinvertebrates have been the most widely used biotic group for biomonitoring (Milner and Oswood 2000, Sandin *et al.* 2001, Dallas 2002). They include aquatic insects (of various life stages of insects), worms, molluscs and crustaceans, that occur in or on the riverbed, within the water column and surrounding vegetation (Allan 1995). They are adapted to live in certain environmental conditions, and changes within this environment may adversely affect community composition and abundance. Resident aquatic macroinvertebrates are good, short-term indicators of ecological integrity because they integrate the effects of physical and chemical changes.

The index currently being used to assess the status of riverine macroinvertebrates by the RHP, is the South African Scoring System (SASS). SASS was developed by Chutter (1994) as a univariate index that indicates water quality of a river based on the macroinvertebrate community living there. The index is based on the presence of aquatic macroinvertebrate families and the perceived sensitivity of these families to water quality changes. Different families show different sensitivities to pollution. These sensitivities range from highly tolerant families (e.g. Culicidae) to highly sensitive families (e.g. Notonemeuridae). The index has undergone several upgrades and version 5 is currently in use. SASS is an accredited (through the DWA) protocol that has been tested and widely used n South Africa as a biological index of water quality. SASS results are expressed both as an index score (SASS score) and the average score per recorded taxa (ASPT value). From these data, it is possible to establish the integrity of a river, by comparing results to established Biological Bands derived by Dallas and Day (2007).

Through the establishment and course of the RHP, biomonitoring of rivers gained impetus, leading to the development and refinement of various RHP objectives and a range of biomonitoring indices to assess diverse components of the aquatic ecosystem (Malherbe *et al.*2010). A new procedure was developed to further support management and protection of rivers. This procedure is known as EcoClassification, which refers to the determination and categorisation of the present ecological state (PES) of rivers relative to their natural state. An ecological evaluation is undertaken in terms of expected reference conditions to conditions currently present, and is assigned a category representative of the ecological status (EcoStatus). A new index, the MIRAI (Macroinvertebrate Response Assessment Index) has been developed for use in the EcoClassification and EcoStatus procedures. The MIRAI is used to determine the ecological condition of macroinvertebrate communities. It integrates the environmental requirements of the invertebrate taxa in an assemblage and their responses to modified habitat conditions (Thirion 2007). Although MIRAI uses the SASS sampling protocol and can be calculated using information collected during a standard SASS survey (Dickens and Graham 2002), it can also be calculated using more detailed information. The calculation of the MIRAI Ecological Condition (EC) is calculated by comparing the current assemblage structure with a reference condition, in aspects of flow and habitat modification, water quality, connectivity and seasonality. Each of these metric components undergoes a ranking, weighting and rating systems adopted by MIRAI as part of the EcoClassification and EcoStatus procedures (Kleynhans and Louw 2008). If no appropriate reference conditions exist, historical data or data from rivers in the same zone and within the same ecoregion can be used.

During the last decade within South Africa, biomonitoring protocols such as SASS have been successfully tested, scientifically scrutinised and upgraded to the current version 5 (e.g. Chutter 1994, 1998, Dallas 1995, 1997, 2000a, b, 2001, 2004a, b, 2005, 2007a, b, 2010). Less work has gone into objectively examining the MIRAI and its diagnostic capabilities, and no effort has been devoted to comparing SASS and MIRAI protocols. The frequently tested SASS5 protocol, and the newly developed approach used in the MIRAI, presents water managers with the problem of choosing the more appropriate tool for their purposes.

4.2 AIMS

The aim of the study was to compare the ability of the two biomonitoring protocols to assess ecosystem condition of rivers within the Palmiet River catchment. SASS5 (Graham and Dickens 2002) and the MIRAI (Thirion 2007) are both multihabitat, field-based methods that require identification of macroinvertebrates to family level. For each, sensitivity weightings, which have been pre-assigned to individual taxa based on their tolerance to water-quality impairment, are used to calculate biotic indices scores. SASS5 and the MIRAI protocols use similar sampling equipment, but differ in that the MIRAI integrates a multi-metric procedure including assessment of flow, habitat and water-quality modifications; both final outputs reflect a single ecological category, however. This study was designed to examine the applicability and differences in derived ECs using both SASS5 and the MIRAI techniques. It sought to:

1. Compare physico-chemical conditions at impacted sites that were sampled to the reference conditions established for the Palmiet River catchment (see Chapter 2).

2. Using multimetric techniques, investigate differences between reference sites (PR, K-3A and OR) and sites selected *a priori* as being impacted (PRAW, PRBW, WR, KR and SR) and additional impacted sites for which secondary data were obtained (GRABO, ARIES, STOKO and KOGFR).

3. Explore which environmental variables are the main drivers behind the patterns observed in macroinvertebrate communities, using a multivariate linear modelling approach.

4. Ascertain SASS5 ecological categories for all impacted sites.

5. Ascertain the MIRAI ecological categories for all impacted sites, and investigate the overall MIRAI scoring sensitivity by altering rating scores (stringent, moderate and lenient).

6. Compare the Ecological Categories derived for all impacted sites using SASS results with regional reference conditions, SASS results with seasonally-specific local (catchment-scale) reference conditions, and MIRAI.

7. Highlight the implications of the findings from this study for macroinvertebrate-based bioassessment in South Africa, and identify specific aspects requiring further research.

4.3 METHODS

Established reference conditions of the Upper Reaches of the Palmiet River catchment (Chapter 3) were used to compare physico-chemical conditions and biological assemblages at five *a priori* selected impacted sites (PRAW, PRBW, WR, KR and SR) and four secondary sites (GRABO, ARIES, STOKO and KOGFR) originating from a study conducted by Ollis (2005). In the rest of this thesis these are referred to as "the Ollis sites".

4.3.1 Physico-chemical conditions

Physico-chemical variables (conductivity, temperature, pH, turbidity, velocity, discharge and percentage dissolved oxygen saturation) (see Section 3.2.11 for instrument equipment) were measured on one occasion only, and are compared to those established as Upper Reaches reference conditions (Chapter 3) within the Palmiet River catchment for summer in 2011. However, at the Ollis sites, stream velocity and turbidity readings were not recorded.

4.3.2 GPS

The geographical coordinates of each primary sampling site were recorded during the summer of 2010, using a Garmin III hand-held Geographic Positioning System (GPS). All coordinates (see Table 2.1) were geo-referenced using the WGS 84 (World Geodetic Survey 1984) datum with an accuracy of ±12m. GPS coordinates for secondary sites were obtained from Ollis (2005).

4.3.3 Biological assemblages

The SASS sampling protocol was followed to collect macroinvertebrates, as per both SASS5 (Dickens and Graham 2002) and the MIRAI (Kleynhans 2005) procedures (refer to sections 3.3.1.3 for SASS5 sampling protocol).

MIRAI (Macroinvertebrate Response Assessment Index) as stipulated in MIRAI manual (Thirion 2007) and EcoStatus determination (Kleynhans and Louw 2008)

The MIRAI, one of six modelled indices developed for EcoClassification and EcoStatus determination, aims to provide an Ecological Category of instream conditions by assessing the deviation of aquatic macroinvertebrate assemblage from the reference condition. The MIRAI process consists of two integral parts, namely determining reference conditions, and deriving how the PES deviates from established reference conditions.

In this study, sites PR, K-3A and OR were used as reference or near-to-pristine sites, and macroinvertebrates captured within, constituted the construction of a reference assemblage of presence/absence and abundance of taxa. This reference assemblage was then used to compare to the PES at impacted sites. Any deviation observed in macroinvertebrate assemblage would be seen as a result of anthropogenic influence. MIRAI protocol requires the use of the SASS procedure to collect invertebrate samples. No replicates were taken at each site, as per the norm with MIRAI investigations and identification of invertebrates was to family level.

The PES of a river, as defined by MIRAI in Kleynhas and Louw (2008), is expressed in terms of various components. That is, drivers (physico-chemical, geomorphological and hydrology) and biological responses (fish, riparian vegetation and specifically for MIRAI, aquatic invertebrates). The principle followed by MIRAI, is that the biological responses (i.e. of macroinvertebrates in this study) integrate the effect of the modification of the drivers and that this results in an ecological endpoint, which is quantifiable and in the form of an ecological category.

The MIRAI comprises four different metric-groups (flow modification, habitat modification, water quality modification, and system connectivity and seasonality). Each metric-group measures the deviation of the invertebrate assemblages from the reference (expected) assemblage to current (observed) invertebrate assemblage found at each site. The term "metric-group" is derived from each specific metric group collectively containing numerous individual metrics. The group-metric "system connectivity and seasonality" was not explored within this study due to sampling for this investigation being limited to within one season, and received a weighting of 0%.

The first step in ascertaining the PES of the invertebrates is to complete the data sheet (see Appendix B). This includes the abundance and frequency of occurrence of the different invertebrate taxa under natural (reference) conditions, as well as the abundance and frequency of occurrence of the invertebrate taxa actually present at the site. For this index a deviation from reference in abundance and/or frequency of occurrence is seen as an impact or difference compared to natural. The six-point rating system works as follows:

0 = No change from reference

1 = Small change from reference

2 = Moderate change from reference

3 = Large change from reference

4 = Serious change from reference

5 = Extreme change from reference

For example, if an invertebrate assemblage present at an impacted site were to differ greatly from reference conditions, a rating value of 4 or 5, depending on the severity of deviation, would be assigned. These qualitative ratings are expert-knowledge based and are assessed by the relevant expert in a particular specialty. It is preferable that the relative difference between ratings for example, 0-1 be the same as between 3-4 (Joubert 2004, cited by Kleynhas and Louw 2008). However, this is difficult to control and is currently exclusively based on expert knowledge.

- Ranking and Weighting

In addition to the rating of the different metrics, each metric (and metric-group) is also ranked and weighted according to its importance in determining the Ecological Category (EC) of the invertebrate assemblage. The principle of following a ranking-weighting approach is that not all driver or biological response metrics have the same relative ecological significance in all types of rivers. That is, the aspect(s) on which a particular metric may be seriously modified, but it may be of relatively little significance in terms of the functioning and integrity of the river. In another river (or a different section of the same river) in a different ecoregional context, this metric may, however, be of great ecological importance. Thus, the ranking-weighting process is done separately from the rating and should not be influenced by it (Kleynhans *et al.* 2004).

- Ranking is done as follows: (exclusively based on expert knowledge)

The metric of a metric-group that is considered to be most important in influencing the EC of the metric-group, if it changes, is ranked as 1. This can be formulated as: considering the range from 0 to 5, if a particular metric-group is considered, which metric would contribute most to improving (or decreasing) the Present Ecological State? The next most important metric is ranked as 2, then 3, and so on. It is important to note that the ranking procedure is only used to guide the weighting and is not used in any calculation.

- Weighting is done as follows: (exclusively based on expert knowledge)

The metric ranked 1 (most important) is awarded a weight of 100%. The weight of the metric with a rank of 2 is considered relative to its importance when compared to the metric with a rank = 1, and this can be any percentage lower than the awarded 100% (i.e. other metrics are then ranked as a percentage relative to the most important metric). It is important to remember that all metrics with the same rank must have the same weight, and that a lower ranked metric = 3, say – must have a lower percentage weight than a higher ranked metric =2, for instance.

1. *FLOW MODIFICATION* metric-group

In order to facilitate the evaluation of the impact of different flows on the invertebrate community, four different velocity categories have been defined:

Very fast flowing water	>0.6 m/s
Moderately fast flowing water	0.3-0.6 m/s
Slow flowing water	0.1-0.3 m/s
Very slow flowing/standing water	<0.1 m/s

Each invertebrate taxon has been assigned a velocity preference score (0-5), based on Thirion (2007) and already mentioned Reserve determination projects DWAF (2001) and DWAF (2004a). These velocity preference scores were assigned to specific taxa which prefer one of the four different velocity categories (i.e. are found more abundant in this type of velocity) (Appendix C), and are indicated on the Data sheet of the MIRAI set of spreadsheets (see Appendix B). The velocity preference scores are allocated according to the following system:

0 = No preference

1 = Very small preference

2 = Small preference

3 = Moderate preference

4 = High preference

5 = Very high preference

In the flow modification metric-group, the presence/absence, as well as the abundance and/or frequency of occurrence of taxa in all velocity categories, are evaluated. The MIRAI makes provision for assessing the presence/absence of taxa as well as their abundance and frequency of occurrence. Although the frequency of occurrence will generally be more useful than abundance, the paucity (i.e. low species richness) of data necessitates the use of abundance information. However, if sufficient information is available it is preferable to use the frequency of occurrence, rather than the abundance information only. It is important to assign a taxon to only one of the velocity categories. If, for example, a taxon has a high preference for very fast flowing water, but only a moderate preference for moderately fast flowing water, it will assessed in the very fast flowing water.

2. *HABITAT MODIFICATION* metric-group

In order to facilitate the evaluation of the impact of habitat changes on the invertebrate community, five different habitat types are defined:

Bedrock/boulders	Bedrock and boulders include all hard surfaces larger than 256mm. It includes bedrock/boulders both in- and out-of-current.
Cobbles	The cobble biotope includes all hard surfaces within the 16-256mm size range, both in- and out-of-current cobbles.
Vegetation	The vegetation biotope includes all vegetation that can provide habitat for invertebrates. As such it includes both marginal and aquatic vegetation, in- and out-of-current.
Gravel, Sand and Mud	Gravel, sand and mud includes grain types <16mm in diameter in- and out-of-current.
Water column	This biotope includes the water surface and the water column.

Habitat preference scores were allocated in the same way as the velocity preference scores (Appendix D). Each invertebrate taxon has been assigned a habitat preference score (0-5), based on Thirion (2007), DWAF (2001) and DWAF (2004a). These habitat preference scores are indicated on the Data sheet of the MIRAI set of spreadsheets (see Appendix B). The habitat preference scores are allocated according to the following system:

0 = No preference

1 = Very small preference

2 = Small preference

3 = Moderate preference

4 = High preference

5 = Very high preference

3. *WATER QUALITY MODIFICATION* metric group

To facilitate the evaluation of deviation from reference conditions in water quality on the invertebrate community, invertebrates are divided into four sensitivity categories, based on tolerance of impaired water quality (see Appendix E):

- Intolerant of modified physico-chemical conditions – SASS5 sensitivity 12-15
- Moderate tolerance of modified physico-chemical conditions – SASS sensitivity 7-11
- High tolerance of modified physico-chemical conditions – SASS5 sensitivity 4-6
- Very tolerant of modified physico-chemical conditions – SASS5 sensitivity 1-3.

These groups are based on SASS5 scoring-weights (see Appendix A). At this stage, because SASS score-weightings are restricted to family-level, the water quality evaluation can therefore only be performed at family level.

In addition to the normal set of metrics regarding presence/absence and the abundance and/or frequency of occurrence of taxa, two additional metrics – the SASS5 score and ASPT value are included.

Guidelines for rating SASS and ASPT deviations from reference conditions as follows:

- SASS scores as a percentage of the calculated reference SASS score

>90%	=	0
80-90%	=	1
60-80%	=	2
40-60%	=	3

20-40%	=	4
<20%	=	5

- ASPT scores as a percentage of calculated reference ASPT value

>95%	=	0
90-95%	=	1
85-90%	=	2
80-85%	=	3
75-80%	=	4
<75%	=	5

- The Ecological Category (EC)

The calculation of the Ecological Categories of drivers and biological responses is done by totalling the weighted scores and expressing this as a percentage of the maximum. This value indicates the percentage deviation away from the expected reference condition and must be subtracted from the 100 to arrive at the percentage value that represents the EC. This value is used to place the EC of the metric-group in a particular category that ranges from A to F (Table 4.1). The model automatically calculates the EC based on the percentage deviation of reference conditions.

Table 4.1: Generic ecological categories for EcoStatus components (modified from Kleynhans (1996).

ECOLOGICAL CATEGORY	DESCRIPTION	SCORE (% OF TOTAL)
A	Unmodified, natural.	90-100
B	Largely natural with few modifications. Small change in natural habitats and biota may have taken place, but the ecosystem functions are essentially unchanged.	80-89
C	Moderately modified. Loss and change of natural habitat and biota have occurred, but the basic ecosystem functions are still predominantly unchanged.	60-79
D	Largely modified. A large loss of habitat, biota and basic ecosystem functions has occurred.	40-59
E	Seriously modified. The loss of natural habitat, biota and basic ecosystem functions is extensive.	20-39
F	Critically/Extremely modified. Modifications have reached a critical level and the system has been modified completely with an almost complete loss of habitat and biota. In the worst instances the basic ecosystem functions have been destroyed and the changes are irreversible.	0-19

Presence-absence transformed biological data were analysed using multivariate analyses (ordination, clustering, ANOSIM and SIMPER) to ascertain differences between assemblages at established reference sites and those considered to be impacted (refer to section 3.3.2.2 for multivariate analyses).

Distance-based Linear Modelling (DISTLM) and distance-based Redundancy Analysis (dbRDA) (Anderson *et al.* 2008) analyses were used to determine which physico-chemical variables appear to be driving responses in macroinvertebrate assemblages at all nine impacted sites.

DISTLM and dbRDA (relate temporal abiotic changes to seasonal biotic responses)

The relationship between the biological Bray-Curtis dissimilarity matrices, and environmental variables was explored by using distance-based linear models (DistLM) and distance-based redundancy analysis (dbRDA) (McArdle and Anderson 2001). DistLM is used for partitioning data (analogous to linear multiple regression) and dbRDA for visualizing the results as principal component ordinations (PCO), constrained to linear combinations of the predictor variables (Anderson *et al.* 2008).

- Formation of "predictor" metrics

All temperature and most of the flow discharge metrics were created according to Rivers-Moore (2004), and from unpublished work by Ewart-Smith (2012). The deviations from the above literature involved the inclusion of all physico-chemical variables, and formation of flow discharge "Event Classes".

Due to the nature of the time-series data collected, Drift Defined Flood Classes (classes of flow) could not be created with strong confidence. To calculate such classes, the time-series data must consist of multi-year records. Thus, a study focussing on only three consecutive seasons prevents the application of this approach. The solution to the shortage in time-series data was over-come by creating individual "Event Classes" defined according to calculated quartiles in flow discharge, per site.

Baseflow discharge for each site was considered as the minimum discharge, without the actual stream drying up. "Event flooding classes" were calculated by subtracting the baseflow (i.e. minimum discharge) from that of the largest discharge measured within the study, at each site. The difference in the maximum and baseflow discharges were then split up into quartiles by using Excel 2007, and the three upper most quartiles (25-49% = Class Event 1, 50-74% = Class Event 2, and 75-99% = Class Event 3) were used to develop flow discharge metrics (i.e. Days since "specific" Class Events, and Duration (in days) of "specific" Class Events, within seven, 30, 60 days to sampling, and finally over the whole season prior to sampling.

- Over-coming "multi-collinearity" within predictor metrics

Once all predictor metrics were formulated, the PRIMER package was used to analyse for multi-collinearity in data. This was done by creating Principal Component Analyses (PCA's), and running Draftmans plot, on each of the three sets of predictor metrics:

  - o Temperature metrics, Flow Discharge metrics and Physico-chemical metrics

PCA's visually illustrate relationships between environmental variables within a MDS-type ordination. The closer the different variables are within the ordination, the stronger the relationships are and the potential for high multi-collinearity, between those variables. Draftmans plots further support the graphic illustration create by PCA's, by creating a correlation matrix of all environmental variables within the designed set. Values within this matrix range from (0:1), where the higher the values (i.e. closer to 1), the higher the degree of correlation between those variables. A cut-off for multi-collinearity within these Draftmans plots was assigned to values exceeding a value of 0.90 (any value above 0.9, indicating severely high multi-collinearity). Those metrics that fell below the 0.90 multi-collinearity cut-off were used as final predictor metrics within the DISTLM and dbRDA process.

4.3.4 SASS5 (South African Scoring System, version 5)

SASS score and ASPT values were calculated for all sites, and plotted on biological bands derived by Dallas and Day (2007) to ascertain an ecological category (EC) for each impacted site. The SASS score and ASPT values of sites were too, plotted on seasonally-derived biological bands ascertained in Chapter 3 (see section 3.3.13).

4.3.5 MIRAI (Macroinvertebrate Response Assessment Index)

Ecological categories were calculated using the MIRAI for all nine impacted sites. The MIRAI's sensitivity to adjusting the rating scale was tested by altering rating scores (at varying degrees: lenient, moderate and stringent) for flow, habitat and water quality modification. The rating scales worked on a three-tier system, with stringent scores being one value lower, and lenient scores being one value higher than that of the originally calculated moderate rating scales.

4.3.6 SASS5 versus MIRAI

SASS5-derived ECs using both the biological bands created by Dallas and Day (2007) and seasonal-derived bandings in Chapter 3, specific to the Palmiet River catchment, were compared to MIRAI-derived default (i.e. moderate sensitivity) ECs.

4.4 RESULTS

4.4.1 Physico-chemical conditions

To investigate differences in physico-chemical conditions between reference and impacted sites, seven variables were measured (Table 4.2). These values were in turn compared to established reference conditions (Chapter 2) for the relevant (summer) sampling season. Surprisingly, conditions at summer 2011 reference site PR, impacted sites PRAW, PRBW, WR, KR and all Ollis sites (Ollis 2005) was lower than values established as reference conditions.

Water temperatures were comparatively higher at sites STOKO and KOGFR than at all the other sites.

pH at all sites, except K-3A and the impacted Ollis sites, fell within the established reference values.

Turbidity, which was not measured by Ollis (2005), was marginally higher at summer 2011 reference sites than the established reference conditions, and even higher at sites WR and KR. The range in turbidity measurements between reference and impacted sites were was minimal (generally less than 2 NTU), however, and thus any differences may be ecologically meaningless.

Velocity was not reported in Ollis (2005). Impacted sites PRAW, PRBW, WR and KR had flow rates lower than the established reference conditions.

Despite spatial differences in discharge discovered in Chapter 3, comparisons could be made between data generated for reference and impacted sites in the last sampling period. Generally, discharge was lower at impacted sites than at reference sites, with the exception of sites SR and KOGFR.

Dissolved oxygen saturation was relatively high at all sites, except for those samples by Ollis (2005).

Table 4.2: Physico-chemical variables measured at "Summer 2011" sites, compared with established reference conditions within the Upper reaches of the Palmiet River catchment, including data sampled by Ollis (2005). The high degree of spatial variability in discharge values prevented the derivation of reference conditions, and thus the "n/a". The dashed line "-" indicates were physico-chemical variables were not measured by Ollis (2005).

	Palmiet River catchment Reference Conditions			Summer 2011 Reference Sites			Summer 2011 Impacted Sites								
							Primary Impacted Sites					Ollis' Sites			
	Median	Min	Max	PR	K-3A	OR	PRAW	PRBW	WR	KR	SR	GRABO	ARIES	STOKO	KOGFR
Conductivity (µS/cm)	50.05	43.9	53.4	18.77	62.3	40.75	18.83	15.89	26.8	20.7	43.1	11.04	8.63	14.95	10.24
Temperature (°C)	23.15	19.2	23.6	16.9	22.1	17.65	18.2	19.4	22.1	22.7	20.4	19.1	18.8	24.2	24.3
pH	4.66	4.22	4.98	4.63	3.87	4.28	4.9	4.63	4.38	4.33	4.8	5.7	6.2	6.2	5
Turbidity (NTU)	0.53	0.43	0.73	0.81	0.98	0.82	0.22	0.53	2.3	2.08	0.9	-	-	-	-
Velocity (m/s)	0.16	0.1	0.24	0.15	0.5	0.2	0.04	0.07	0.03	0.07	0.61	-	-	-	-
Discharge (m^3/s)	n/a	n/a	n/a	0.21	0.17	0.15	0.08	0.05	0.04	0.02	0.2	0.07	0.05	0.12	0.26
% Oxygen Saturation	100.6	100	101.4	101	105.9	95.28	97.1	99.5	88.8	81.9	105.6	26.65	85.33	67.7	75.96

4.4.2 Biological community assemblage

Macroinvertebrate Community Structure

A total of 36 macroinvertebrate taxa, accounting for 1894 individuals, were identified in the summer season of sampling. Of these, ten taxa were found only at impacted sites, and two only at the reference sites.

Multivariate cluster and MDS analyses of macroinvertebrate assemblages, revealed that Summer 2011 reference sites share a 50% similarity with reference sites used to establish reference conditions (Chapter 3), and were thus closely aggregated in the MDS ordination (Figure 4.1). Interestingly, the "impacted" sites PRAW, PRBW and SR grouped within the 50% similarity band that included Summer 2011 reference sites. Presence-absence data of invertebrates from sites WR and KR were 50% similar, and were seen as both composing of different macroinvertebrate composition in comparison to reference sites. A similar pattern was observed with sites ARIES and KOGFR. Invertebrates from sites STOKO and GRABO were <50% similar to those from all other sites.

A SIMPER analysis was performed to ascertain the degree of difference between invertebrates at the reference and impacted sites, and to determine which invertebrate families were most responsible for the dissimilarity. SIMPER analyses supported the patterns observed in the MDS ordination. Reference sites PR, K-3A and OR sites revealed an average dissimilarity of 11.63, 16.67 and 12.82% respectively from the established reference invertebrate community assemblage. The presence of Culicidae and Tipulidae at site PR explained a combined total 40% dissimilarity from reference. The absence of Barbarochthonidae and Notonemouridae, and Athericidae and Sericostomatidae at sites K-3A and OR respectively, explained a combined 33% and 40% of total cumulative dissimilarity from reference at each site.

Impacted sites PRAW, PRBW and SR, which were located within the 50% average similarity grouping in Figure 4.1 (MDS), revealed an average dissimilarity of 31.71, 19.05 and 35.14% respectively from established reference macroinvertebrate community assemblage.

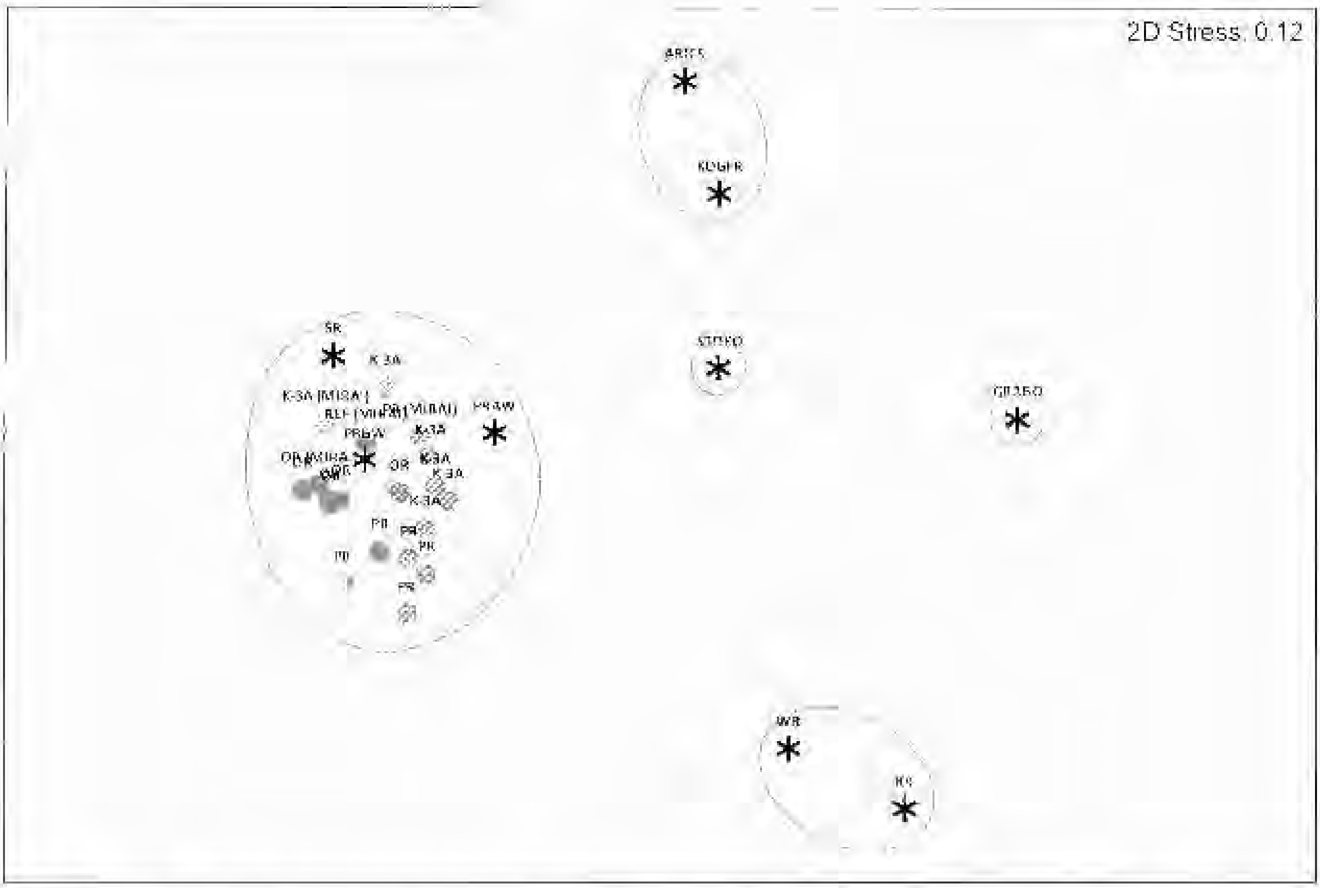


Figure 4.1: Multi-Dimensional Scaling (MDS) presence-absence ordination based on single sample macroinvertebrate assemblages of all sampled summer sites (Reference indicated by circles, and impacted sites - indicated by stars). Ovals represent 50% similarity

The presence of Aeshnidae, Lestidae, Dytiscidae and Hydrophilidae, and the absence of Barbarochthonidae, Paramelitidae, Crambidae and Glossosomatidae at site PRAW, accounted for a combined 61.52% dissimilarity from reference. The presence of Athericidae, Platycnemidae and Tipulidae, and absence of Crambidae, Glossosomatidae and Paramelitidae, at site PRBW accounted for a combined 75% of cumulative dissimilarity from reference. The presence of Athericidae, Platycnemidae and Culicidae, and the absence of Corydalidae, Petrothrincidae and Notonemouridae to name a few, at site SR, accounted for a combined 46.14% of cumulative dissimilarity from reference.

The remaining impacted sites, STOKO, KOGFR, ARIES, WR, GRABO and KR revealed an average dissimilarity of 56.76, 59.18, 61.9, 71.43, 76.47 and 86.21% respectively from the reference invertebrate assemblage,. The presence particularly of Hydracarina, Aeshnidae and Culicidae, and the absence of Teloganodidae, Sciritidae and Philomatidae at site STOKO explained a combined 28.56% dissimilarity from reference. The presence of Chlorolestidae, Heptageniidae and Culicidae, and absence of Barbarochthonidae, Glossomatidae and Notonemouridae, at site KOGFR, explained a combined 20.7% cumulative dissimilarity from reference.

The presence of Aeshnidae, Baetidae, Muscidae and Oligochaeta, and the absence of Teloganodidae, Sciritidae and Crambidae, at site ARIES, explained a combined 26.95% dissimilarity from reference. The presence of Caenidae, Gomphidae and Culicidae, and the absence of Barbarochthonidae, Crambidae and Paramelitidae, at site WR, explained a combined 24% dissimilarity from reference. The presence of Gerridae, Dytiscidae, Oligochaeta and Culicidae, and the absence of Leptophlebiidae, Notonemouridae and Corydalidae, at site GRABO, explained a combined 26.95% dissimilarity from reference. And lastly, the presence of Gomphidae and Culicidae, and the absence of Glossomatidae, Sciritidae and Sericostomatidae, at site KR, explained a combined 20% dissimilarity from reference.

Ascertaining influential indicators and relative biological responses

Two of the five physico-chemical variables were significantly related to macroinvertebrate assemblage composition (Table 4.3). Percentage dissolved oxygen saturation was found to display a highly significant (p<0.01) relationship with presence-abundance macroinvertebrate assemblage data, explaining the highest proportion of variation (Table 4.3). pH too, displayed a significant relationship (p<0.05) with presence-absence data, but to a lesser state, explaining just more than half of the variation explained by percentage dissolved oxygen saturation. The remaining physico-chemical variables (discharge, temperature and conductivity) were not significantly related to presence-abundance assemblage data, explaining a further 9.6, 10.8 and 6.1% of variation in transformed biological data. The percentage variation in macroinvertebrate assemblage composition explained by the overall selected physico-chemical variables was high, explaining 69.9%.

Table 4.3: dbRDA Ordination of normalised physico-chemical predictive metrics, and presence-absence macroinvertebrate family-based response matrix, for all Summer 2011 sampled sites. Step-wise procedure and Adjusted R^2 criterion were used. Significant *p* values are presented in italics ($p<0.05$). "Individ. Prop" is abbreviated, representing the individual proportion of variation in macroinvertebrate assemblages explained by the individual predictor variable. "Cumul. Prop." represents the cumulative proportion of variation explained by predictor variables.

Variable	Pseudo-F	*p*	Individ. Prop.	Cumul. Prop.
% Oxygen Saturation	4.067	*0.005*	0.289	0.289
pH	2.310	*0.048*	0.145	0.434
Temperature (°C)	1.628	0.179	0.096	0.530
Discharge (m^3/s)	2.092	0.103	0.108	0.638
Conductivity (mS/m)	1.208	0.335	0.061	0.699

The remaining 30.1% of unexplained variation can be attributed either to factors which were not measured uniformly throughout all sites, or not measured at all in this study, or to stochastic factors (nondeterministic fluctuations) that cannot be measured (Borcard *et al.* 1992). Due to the scale of the study, it was not logistically feasible to record detailed environmental information at each site. Therefore it is not surprising that a considerable degree of the macroinvertebrate variation could not be explained by the set of variables measured in this study. This amount of unexplained variation is comparable to other ecological studies that have incorporated variation partitioning procedures (e.g. Borcard *et al.* 1992).

4.4.3 SASS5 (South African Scoring System, version 5)

Table 4.4: SASS5 metrics calculated for each site sampled, compared to established reference SASS5 median, minimum and maximum values for the Palmiet River catchment. Refer to chapter 2 methods for full site names instead of abbreviations.

	Palmiet River catchment Reference Conditions			PR	K-3A	OR	PRAW	PRBW	WR	KR	SR	GRABO	ARIES	STOKO	KOGFR
	Median	Min	Max												
SASS5 Score	157	114	204	178	128	164	143	179	76	35	125	46	122	85	183
ASPT	7.87	7.10	8.60	8.09	8.53	9.11	7.15	8.14	5.43	4.38	7.81	3.5	5.8	5.0	6.5

All three summer 2011 PR, K-3A and OR sites fell within the calculated reference SASS5 metric range, although the ASPT score for site OR exceeded that of the reference score (Table 4.4). Unexpectedly, supposedly impacted sites PRAW, PRBW, KOGFR and SR fell within the pre-determined reference conditions. Sites WR and KR, and the Ollis sites fell outside (below) the set reference scores for the Palmiet River catchment, indicating an impairment in water quality at each of the these sites.

SASS5 Biological Banding

Calculated SASS5 and ASPT scores were plotted against scores derived by Dallas and Day (2007) (Figure 4.2), and then according the seasonal bands (Figure 4.3) developed in the previous chapter (Chapter 3), to ascertain ecological categories indicating site condition according to SASS5 protocol.

As illustrated in Figure 4.3, summer 2011 reference sites PR, K-3A and OR were located within the "A" pristine biological band, surprisingly including summer 2011 sites PRBW and KOGFR, which had been selected as impacted. The remaining impacted sites varied in degree of impairment in water quality, with sites SR and PRAW located within the "B" or minimally-impacted band, site ARIES within the "C" band, sites WR and STOKO in the heavily impacted "D" band, and sites KR and GRABO were within the "E/F" band indicating severe water quality impairment.

When SASS5 scores and ASPT values for each site were plotted against seasonally-derived summer biological bands (ascertained in Chapter 3), summer 2011 reference sites PR, K-3A and OR, including "impacted" sites PRBW and KOGFR, all retained the ECs class attributed to them by Dallas and Day (2007) biological banding (Figure 4.4). The summer season-derived biological bands, however, elevated the remaining impacted sites (excluding ARIES site which retained its original EC) by at least one EC class (Figure 4.3). Site PRAW changed from a "B" to an "A" category, similarly sites STOKO (D to C) and GRABO (E/F to D) .Sites WR and KR increased by two ECs from a D and E/F symbol, to B and C.

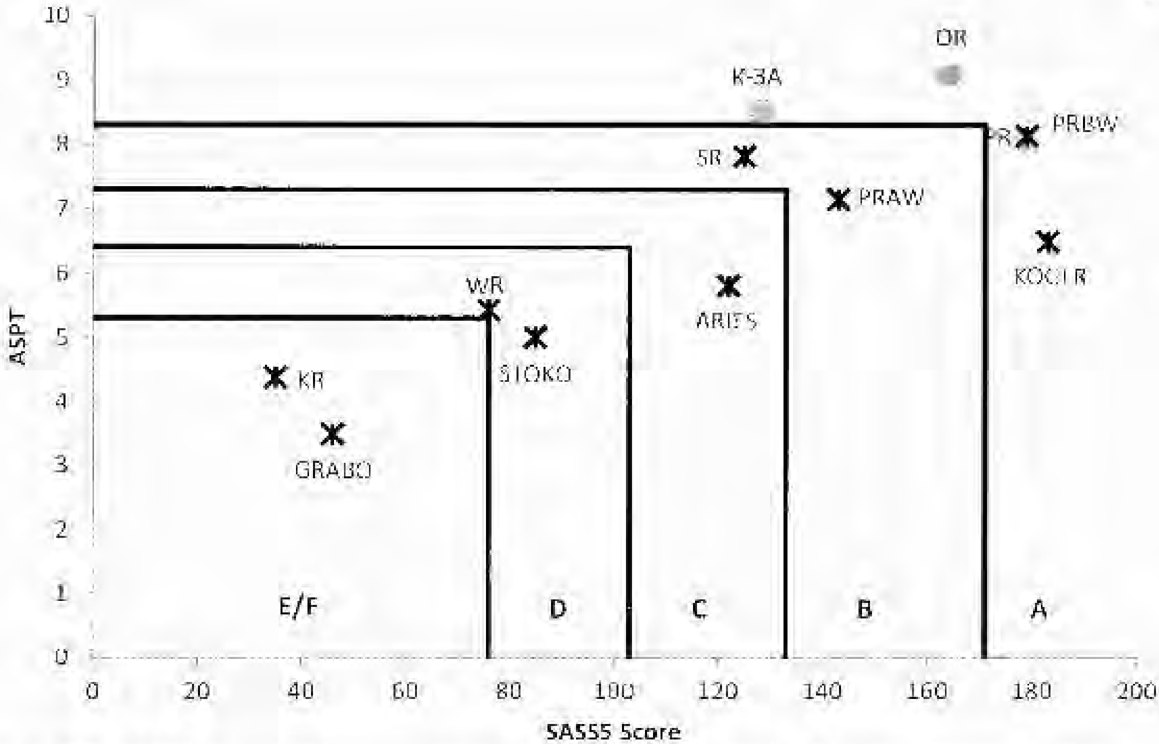


Figure 4.2: SASS5 protocol for ascertaining ecological categories by using Biological Banding derived from Dallas and Day (2007) for the Southern Folded Mountains (Upper). Reference sites indicated by circles, and impacted sites by stars.

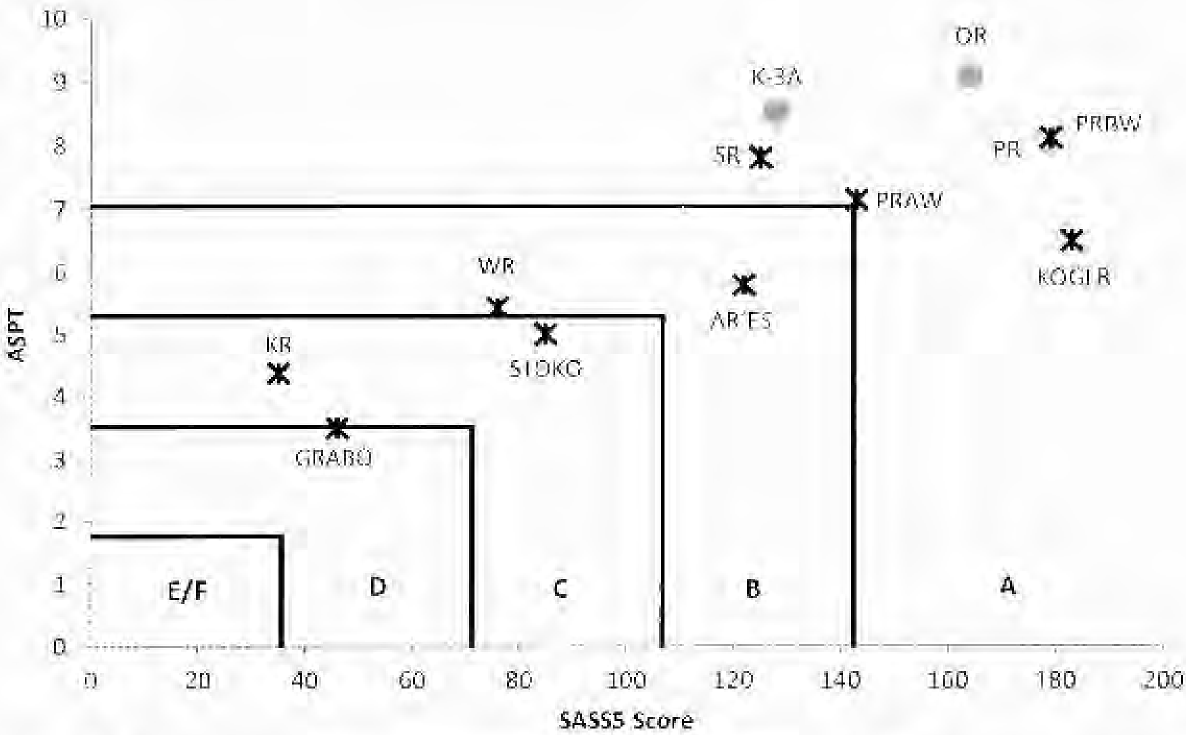


Figure 4.3: SASS5 protocol for ascertaining ecological categories by using seasonally derived Summer Biological Banding derived for the Palmiet River catchment. Reference sites indicated by circles, and impacted sites by stars.

4.4.4 MIRAI (Macroinvertebrate Response Assessment Index)

Sensitivity Test of MIRAI Scores and ascertained Ecological Categories

MIRAI protocol involved the rating, ranking and weighting of individual metrics within three metric groups. All three scoring processes (rating, ranking and weighting) were scored according to expert-based knowledge of the catchment and of the specific sites (Kleynhans 2005). The knowledge, on which the rating scores are based, may vary between personas, and thus different scientists may assign different ratings scores for a particular site. The sensitivity to altered degrees of rating scores was explored at three levels, lenient, moderate and stringent, comparing the final derived ecological category assigned by the MIRAI process (as in Table 4.5).

Table 4.5: MIRAI Sensitivity Scores (lenient, moderate and stringent) for the three modification metric groups from reference conditions, and derived Ecological Categories (EC). Each score ranges from 0-100, with 0 representing no change from reference, whereas 100 represent severe/extreme change from reference conditions. Ecological categories range from "A" pristine to "F" severely-impacted.

	Sensitivity	PRAW	PRBW	WR	KR	SR	GRABO	ARIES	STOKO	KOGFR
FLOW MODIFICATION	Lenient	27	9	40	52	33	64	55	44	44
	Moderate	47	29	60	72	52	84	75	64	64
	Stringent	67	49	80	84	72	94	95	84	84
HABITAT MODIFICATION	Lenient	14	0	30	39	18	42	32	22	35
	Moderate	33	18	50	59	37	62	52	42	55
	Stringent	53	38	70	79	57	82	72	62	75
WATER QUALITY MODIFICATION	Lenient	20	0	60	82	26	91	60	74	55
	Moderate	34	12	73	96	40	105	73	88	59
	Stringent	48	26	87	107	53	112	87	95	72
EC	Lenient	B - 79	A - 97	D - 57	D - 42	C -74	E - 34	D - 51	D - 53	D - 59
	Moderate	C - 62	B - 80	E - 39	E - 25	D 57	F - 16	E - 33	E - 35	D - 41
	Stringent	D - 44	C - 62	E - 21	F - 10	E -39	F - 4	F - 15	E - 20	E - 23

On average, examination of metric-group sensitivities revealed that the flow modification metric-group changed, be it an increase/decrease, by a score of 19 when sensitivity ratings were altered. Habitat modification group-metric on average changed by a score of 20, and water-quality modification group-metric changed by a score of 12 when rating sensitivity was altered. An overall average increase/decrease in score-rating leniency resulted in a score modification adjustment of roughly 17. The large average increase /decrease of 17 more often (sites PRAW, PRBW, KR, SR, and ARIES) than not (WR, GRABO, STOKO and KOGFR) led to a site being placed in a higher or lower EC than would have occurred with a more moderate approach.

4.4.5 SASS5 versus MIRAI assigned Ecological Categories

Derived ecological categories ascertained through the use of three separate protocols (SASS5 Dallas and Day 2007) Biological Bands, SASS5 Palmiet River catchment Summer Biological Bands, and MIRAI, were compared to ascertain whether ECs assigned were similar, at all sites (Table 4.6). As expected, both SASS5 (Dallas and Day 2007) and SASS5 (Seasonal) bands assigned re-sampled reference sites an EC equal to an "A" pertaining to each site being of reference condition. Comparatively, neither protocol could be compared to MIRAI, due to the nature of MIRAI by incorporating reference conditions from all three sites to create a reference list of macroinvertebrate taxa present in pristine conditions. Comparison of EC's derived using SASS5 (Dallas and Day 2007) bands and that of MIRAI revealed varying differences in categorised symbols. Only two of the nine sampled impacted sites shared similar EC's (KR and GRABO). Sites PRAW, PRBW, WR and STOKO revealed a SASS5 EC higher than MIRAI-derived EC's for respective sites. More notably, the remaining three impacted sites (SR, ARIES and KOGFR), marked with an asteriks in Table 4.6, revealed MIRAI EC's of two of more categories lower than the SASS5-derived counterpart.

Table 4.6: A comparison of ecological categories from SASS5 Dallas and Day (2007), the biological bands developed in chapter 3, and Macroinvertebrate Response Assessment Index (default rating setting - i.e. moderate). An asterisk (*) indicates where SASS5 (Dallas and Day 2007) EC differ by more than one symbol when compared to MIRAI-derived EC. Refer to Table 2.9 for descriptions of EC symbols.

Site	SASS5 ECs Dallas and Day (2007) Ecoregion Bands	SASS5 ECs Seasonal-catchment specific Bands	MIRAI ECs
PR	A	A	n/a
K-3A	A	A	n/a
OR	A	A	n/a
PRAW	B	A	C
PRBW	A	A	B
WR	D	B	E
KR	E/F	C	E
SR	B	A	D*
GRABO	E/F	D	F
ARIES	C	B	E*
STOKO	D	C	E
KOGFR	A	A	D*

4.5 DISCUSSION

The freshwater resources of our country will not be able to support the needs of humans and the living organisms alike indefinitely, unless managed in a different way to the present. Environmental degradation and public pressure have led to a need for accurate assessment, both of the damage caused by human activities and of the improvement due to wise management practises (Ferreira 2008). Because research has shown that our rivers are under constant threat and the fact that our government assumes responsibility for our freshwater resources, new procedures are always being developed to assess the health of our rivers. Both SASS5 and the MIRAI procedures, currently in use in South Africa, make use of the most direct and widely used measure of riverine health i.e. monitoring instream macroinvertebrates. The objective of this study was to sample and compare *a priori* selected impacted sites to already established reference conditions (Chapter 3) found within the

Palmiet River catchment, with the use of SASS5 and the MIRAI biomonitoring protocols. Analysis of results were structured according to the aspect examined (e.g. physico-chemical, biological, SASS and MIRAI), although results will be discussed site-specifically moving downstream from headwaters to lower reaches in the Kogelberg Reserve, incorporating the four-above mentioned aspects, followed by a comparison of both procedures and a conclusion to the study.

Summer 2011 Reference Sites

PR, K-3A and OR

Reference sites PR, K-3A and OR were sampled in summer and compared to the established physico-chemical and macroinvertebrate assemblages presented in reference conditions in Chapter 3. Physico-chemically, all three sites had values varying inside and out of the reference range. Sites PR and OR had values of conductivity and temperature that were lower, site K-3A had pH values lower, all three sites had greater turbidity readings, site K-3A had greater velocity measurements and site OR had lower percentage dissolved oxygen saturation values, than pre-established reference conditions for the catchment. Those values falling outside of established physico-chemical reference conditions, according to Chapter 3, stipulate that these sites are in fact not of reference condition. However, caution must be taken when assigning an ecological condition to a stream according to established physico-chemical variables, especially, based on once-off sampling, as in this study.

Multivariate analysis of the invertebrate data revealed that the assemblages at all three summer 2011 reference sites (PR, K-3A and OR) were of great similarity to reference presence-absence macroinvertebrate assemblage, supported by the cluster ordination grouping sites within a minimum of 50% average similarity, and by the low (<20%) calculated dissimilarity percentages by SIMPER analysis.

When SASS5 (Dallas and Day 2007) was applied to biological data for summer 2011 reference sites, site PR had the highest SASS5 score and K-3A the lowest score, site OR had the highest ASPT value, and PR the lowest value. With that said, as expected, all three sites' scores and values fell within the established reference condition ranges and when plotted against Dallas and Day (2007), were assigned an ecological category symbol of "A", indicating each site to be of reference condition with respect to SASS5.

Summer 2011 Impacted Sites

PRAW, PRBW

PRAW and PRBW were selected as impacted sites due to their locations, up- and downstream of a concrete weir and causeway, respectively. With the knowledge that only one (PRAW) and three (PRBW) physico-chemical variables satisfied reference conditions, it was surprising to find that macroinvertebrate assemblages at the two sites shared a high percentage similarity (>50%), and relatively low dissimilarity (<32%), from reference biological data. Importantly though, specific examination of site PRAW revealed a greater degree of difference (physico-chemically and biologically) from reference than PRBW, and may be attributed to the upstream damming effect caused by the presence of the weir and causeway. Temperature was found to be lower due to the increased depth of the water column, and stream velocity decreased due to the limiting of flow moving downstream. The reduction in flow in turn led to decreased percentage dissolved oxygen saturation, associated with the decrease in turbulence, and a decrease in suspended solids due to the lowering of the water carrying capacity. These alterations in turn have led to the presence of more tolerant taxa (SASS score <8) such as Dytiscidae and Hydrophilidae, and absence in sensitive taxa (SASS score >8) such as Barbarochthonidae and Crambidae, resulting in application of SASS5 Dallas and Day (2007) biological bands assigning the site to a "B" ecological category. Site PRBW which revealed a lesser degree of physico-chemical and biological difference from reference condition, was assigned an ecological category of an "A" based on SASS5, indicating that biologically, the site resembled that of reference condition, and furthermore that the presence of the weir and causeway did not alter macroinvertebrate assemblages downstream of the barrier.

The individual application of the MIRAI to site PRAW revealed that flow modification metric decreased the most from reference, due to the decrease in velocity and the damming effect created upstream by the presence of the weir and causeway. Habitat and water quality metrics also showed changes, however comparatively less than flow. The resulting derived-ecological category was a "C". Site PRBW situated directly downstream of the impact, was found to be most altered by flow modification, however to a lesser scale than PRAW, and was assigned a MIRAI ecological category of a "B".

WR and KR

Impacted sites WR and KR were selected due to their location, roughly 50m upstream of the Eikenhof Dam. Physico-chemically these sites shared very similar conditions, most of which (except temperature and pH) fell outside of the reference range. Analysis of presence-absence macroinvertebrate assemblages of the two sites revealed a similarity of roughly 50% to each other, both of which were aggregated some distance from reference in 2D-ordination, supported by the very high percentage of dissimilarities (>70%), indicated marked differences from taxa present in reference conditions.

The large-scale damming effect created by the presence of the Eikenhof Dam directly downstream of either site, has led to a comparative reduction in velocity and average discharge when compared to reference sites, and in turn reduced percentage dissolved oxygen saturation recordings. Most notably, the presence of *Pinus* plantation logging occurring upstream of either site has resulted in increased erosion and sedimentation which has in turn increased the turbidity of the river at each site, as found in (Dawson 2003). These physico-chemical alterations may have led to the marked deviations from reference found in biological data at both sites. The absence of sensitive taxa such as Crambidae and Paramelitidae and the presence of more hardy-tolerant taxa such as Caenidae and Gomphidae led to site WR being assigned a D based on SASS5 ecological categories. Similarly, the absence of sensitive taxa such as Scirtidae and Sericostomatidae, and the presence of Gomphidae and Culicidae, lowered SASS5 and ASPT values when compared to reference, and the site was assigned an E/F ecological category based on SASS5.

Application of MIRAI at sites WR and KR revealed that both sites had water-quality metrics that were severely modified from reference, followed by high flow and moderate habitat modifications. The result was "E" ecological categories for both sites.

GRABO

GRABO was selected as an impacted site due to its locality directly downstream of the town of Grabouw, encompassing urban and rural settlement, and a tarred road that crosses the river. Physico-chemical examination revealed that all variables fell outside of the reference condition ranges. Multivariate analysis of presence-absence macroinvertebrate assemblages at site GRABO, positioned the site furthest away in the ordination analysis, indicating the site to have the most different biological data from reference, supported by the large dissimilarity in macroinvertebrate community assemblage (>75%). These deviations in physico-chemical values and biological data from reference may be caused by urbanisation and subsequent development in floodplain areas, encroachment of residential areas into the river corridor, combined with rural use of the site for ablution facilities, and lastly, due to the vast amount of litter visually observed at the site. Of critical importance to biological assemblages were the recorded percentage dissolved oxygen saturation values. Oxygen levels were found to be far below the necessary 80% needed to protect all aquatic life, according to standards set by DWAF (1996).

The severe alterations in physico-chemical conditions has led to the distinctly different biological community, which lacks sensitive taxa such as Leptophlebiidae, Notonemouridae and Corydalidae, and includes more tolerant Gerridae, Dytiscidae, Oligochaeta and Culicidae, from reference. This in effect led to the drastic reduction in SASS5 score and ASPT values at the site, and as a result was assigned an "E/F" ecological category based on SASS5.

MIRAI results revealed that site GRABO was the most severely modified site, with the assigned "F" ecological category. Water-quality and flow modification were severely modified metrics, follow by a highly modified habitat metric. All three levels of modification may be a result of urban and rural development alongside the river, and associated impacts that accompany such activities (e.g. tarred bridge – may result in modified instream velocities, rural dumping of litter and human faecal matter instream).

ARIES

The ARIES site was selected as an impacted site due to the immediate downstream proximity of the site to the Arieskraal dam, and surrounding agricultural activities within the vicinity. The site had physico-chemical variables that all fell outside the range of reference conditions. Biologically, site ARIES shared a relatively high similarity percentage with site KOGFR, both of which had similar dissimilarities (within 2% of each other) when compared to reference taxa, and specifically, site ARIES having taxa that were slightly more dissimilar. Visually (2D-ordination) site ARIES differed in presence-absence macroinvertebrate assemblages from reference. The effect of the Arieskraal Dam, less than 5km upstream of the ARIES site, combined with water abstraction and traditional problems associated with surrounding agricultural activities (Dawson 2003) (e.g. sedimentation and eutrophication) has led to the addition of tolerant taxa Aeshnidae, Muscidae and Oligochaeta, and the loss of more sensitive taxa Teloganondidae and Scirtidae, which in turn resulted in the moderately-impact assigned ecological category of a "C" based on SASS5. The ecological category is unexpectedly high for a site which is surrounded by potentially harsh impacts. Dawson (2003) proposed a similar scenario at the same site, and stated that the potentially the Klein Palmiet tributary, which enters the Palmiet River between the Dam and Aries site, may in fact be mitigating the effect of upstream damming and abstraction, and in turn may explain the higher ecological category for the site than expected.

MIRAI of the ARIES site revealed that flow and water-quality metrics were equally-highly modified from reference, followed by moderate habitat modification, resulting in an EC of an "E".

STOKO

The STOKO site, one of the Ollis (2005) impacted sites, was selected due to its location roughly 9km and 4km downstream of the Arieskraal Dam and an agricultural region, respectively. All physico-chemical variables measured at this site fell outside of reference condition range, with conductivity and percentage dissolved oxygen below the reference minimum, and temperature and pH exceeding reference maxima. Multivariate analyses of biological taxa present at the site revealed a moderate alteration in presence-absence macroinvertebrate assemblages when compared to reference taxa, highlighted by the higher percentage dissimilarity (57%) from reference than impacted sites PRAW, PRBW and SR.

When SASS5 (Dallas and Day 2007) was applied to biological data from site STOKO, SASS5 score and ASPT values were relatively lower, and produced an ecological category symbol of "D" based on SASS5. The largely impacted nature of the "D" ecological category may be as result of the downstream nature of the site from two Palmiet River tributaries, the Huis and Krom Rivers, which are subject to high agricultural activities and flow modification within their respective catchments (Dawson 2003). Siltation, in the form of sandbanks, were visible up- and downstream of the site, which is uncharacteristic and oxygen levels, as in the case of GRABO, were below that necessary for ecosystem functioning (<80%).

Application of the MIRAI to site STOKO revealed that water-quality modification metric decreased the most from reference, followed by high flow- and lower habitat-modification metrics, resulting in an assigned EC of "E" for the site.

SR

The SR site was selected as an impacted site due to a gravel road crossing the mainstream of the river, and resulting channelization of flow underneath the road. Site SR had all but two variables (temperature and pH) fall outside the reference range. Conductivity, turbidity, velocity and percentage dissolved oxygen saturation all exceeded reference maxima. It is important to note that %DO and velocity was highest when compared to all sites. The elevated velocity and thus associated increase in oxygen levels may be as a result of channelization of flow underneath the gravel road which intersects the site. Despite this alteration, multivariate analyses of presence-absence macroinvertebrate assemblages surprisingly revealed a similarity of 50%, to reference as in the case of impacted sites PRAW and PRBW. The relatively low levels of dissimilarity (<36%) to reference, supported the 2-D ordination.

When SASS5 (Dallas and Day 2007) was applied to biological data from site SR, SASS5 score and ASPT values were found to be relatively high for a "deemed" impact site, categorising the biological community assemblage within a "B" ecological category based on SASS5, indicating the site to be minimally-impacted. Site SR was assigned an MIRAI EC of "D", due to combined moderate flow, water quality and habitat modification created by the presence of a gravel road bisecting the site and channelization of river flow underneath the road.

KOGFR

The KOGFR site was chosen to explore whether largely-upstream impacts on the Palmiet River reached further downstream to within the lower section of the Kogelberg Nature Reserve. The KOGFR had physico-chemical values that did not satisfy reference ranges, with conductivity and percentage dissolved oxygen saturation values far below the minimum set, and pH values that exceeded reference maxima. Interestingly, KOGFR recorded the highest temperature of all impacted sites. Biologically, the KOGFR site shared a relatively high similarity with impacted site ARIES, both of which had similar dissimilarities when compared to reference biological communities. Visually (2D-ordination) the KOGFR site differed in presence-absence macroinvertebrate assemblages from reference.

Interestingly, site KOGFR had a surprisingly high SASS5 score and a relatively low ASPT value, yet when plotted against Dallas and Day (2007) biological bands, was assigned to be of reference condition, with an SASS5 score of "A" .The pristine nature assigned to these sites indicates that all anthropogenic impacts upstream have had no effect and thus the river has recovered in health with distance from the impact. MIRAI application to the site assigned an ecological category of "D" with flow, habitat and water-quality metrics all of relative equal modification.

A comparison of SASS5 and MIRAI

The ability of SASS5 in ascertaining ecological categories was explored by comparing biological bands developed by Dallas and Day (2007) for the Southern Folded Mountains Ecoregion, to the Palmiet River summer-season catchment-specific Palmiet River biological bands developed in Chapter 3. Of all 12 sampled sites, only five shared similar assigned ecological categories using both bioassessment protocols. These five sites, the three reference and the two impacted sites PRBW and KOGFR, were classed as "A" and as a result were similar to reference conditions. Neither bioassessment procedure lacked the ability to access the condition of reference sites. The remaining impacted sites all revealed discrepancies in ecological categories between the two procedures.

Two patterns were evident in the seven sites differing in assigned ecological categories. Firstly, both procedures were able to identify water-quality impairment, except in the cases of site PRAW and SR, where the assigned ecological categories varied marginally, from minimally-disturbed to reference condition. Secondly, where SASS5-derived ecological categories from the Dallas and Day (2007) protocol indicated water-quality impairment, Palmiet River SASS5 seasonal-summer catchment-specific ecological symbols were on average at least one EC higher, indicating a lesser degree of water-quality impairment than found in the Dallas and Day (2007) protocol.

The difference in sensitivity of each protocol and thus the output of ecological categories to impacted sites may be attributed to the purpose and scope of each SASS5 procedure. SASS5 biological bands were originally developed by Dallas and Day (2007) to assist scientists with broad-scale biomonitoring. The idea was to create spatially distinct ecoregions, based on differences such as climate, geology and vegetation. Sites within an ecoregion, and thus sharing similar environmental traits, should be more similar in physico-chemical and biological make-up, than sites located within a spatially different ecoregion. This in turn allowed for the creation of ecological condition scores, such as those produced by SASS5, but specific to an ecoregion. The problem with such spatially-defined ecoregions, as in the case of the Southern Folded Mountains, is that the set of reference biological bands derived by Dallas and Day (2007) incorporate a vast degree of naturally occurring spatial variability of rivers within different catchments, all falling within the same ecoregion. The variability between rivers and catchments, within a specific ecoregion, may in turn increase or decrease the acceptable range of reference conditions and related SASS5 metrics to account for all reference site variation within the ecoregion. The dependence of the ecoregion approach on incorporating variability from numerous reference sites may mask the actual condition of streams within a specific catchment.

If reference bands were set too high or low, in order to account for all reference conditions within the spatial region, ecological categories might be lower, or higher, than assigned catchment ecological categories. Results of the two SASS5 procedures has revealed that ecological categories derived from the biological bands assigned by Dallas and Day (2007) ecological categories were higher than those assigned with the use of Palmiet River seasonal-summer catchment-specific bands.

It is suggested that to define ecological categories adequately for impacted sites, it is necessary to limit the degree of variability in reference defined ranges, and thus reference biological bands should be derived from within catchment conditions, other than using the more variable ecoregion approach developed by Dallas and Day (2007). It must be noted however, that a major criticism of the Palmiet River seasonal-summer catchment-specific bands was the lack of replication and limited reference sites, whereas Dallas and Day (2007) developed their bands re-sampling sites at least more than once, and incorporated more than 100 study sites (Dallas 2007).

Sensitivity of MIRAI:

One of the main principles, and part of the initial protocol on which the MIRAI procedure is based, is the ability to "rate" (score) the site, using four different metric-groups. For the sake of this study, the last metric-group, (seasonality and connectivity) was omitted because sampling occurred within one season only. Each of the four metric-groups involve a rating system (as mentioned in the methods of this chapter), and these qualitative ratings are expert-knowledge-based, and need to be assessed by an expert in a particular speciality (Kleyhans and Louw 2008). The main concern in relying on expert knowledge is that individuals may vary in their knowledge of the system and its requirements, therefore potentially assigning different rating scores to specific metrics. The issue thus arises whether a change in rating scores affected by varying expert knowledge, would alter the overall ascertained EC for a site.

Three levels of varying rating categories were applied to investigate MIRAI rating sensitivities: firstly moderately (or user default), secondly an increase in all rating scores by one value (referred to as stringent), and lastly a decrease in all rating scores by one value (referred to as lenient).

When rating scores were altered, habitat modification metric was found on average to change the most, closely followed by the flow modification metric, and lastly, water-quality modification metric. The combined average change in ecological category score was 17. It is important to note that the MIRAI ecological category score categories were designed to incorporate a specific range of scores: >89 for reference, 80-89 for minimally-impacted, 60-

79 moderately-impacted, 40-59 largely-impacted, 20-39 extremely-impacted and lastly <20 indicates severe impact. With the potentially average deviation in score of 17 between moderately rated and stringent or lenient ratings one would expect ecological categories to more likely than not, increase or decrease depending on the knowledge of the scientist or researcher. An elevation or reduction by one symbol, as found in this study, would not be seen as a big-leap in the wrong direction, because even though the ecological categories may differ slightly, the general pattern from sites sampled was that where an impact was observed in moderate rating scores, both stringent and lenient ratings did not prevent the identification of impact. More importantly for scientists and researchers was the comparison between stringent, lenient and extreme ratings.

MIRAI ecological category groupings were designed to incorporate a range score of roughly 19, however this range narrows with respect to minimally-impacted and reference sites. The combined potential in adjusted rating scores from stringent to lenient, or vice versa may potentially result in an altered ecological category score of 34. This range in difference far exceeds the created 19 score set range for ecological categories in MIRAI, and thus in this study, five of nine impacted sites revealed a change in ecological category by two ECs (Table 4). In riverine ecosystem management there is too great a difference in the health of a system, between being assigned for example a minimally-impacted "B" ecological category and a largely-impacted "D" ecological category. The possibility that more than half the ascertained ecological categories of those sites may differ by more than one symbol, creates too great a variability for scientists and researchers to assign with relative confidence an accurate ecological category, specific to a site. The difference between these two ecological categories has important ramifications with regard to conservation, management and restoration of a river. Caution thus has to be taken about personas undertaking bioassessment, especially using the MIRAI protocol. To limit variability, a robust procedure which limits variability is needed.

Which technique to use?

The SASS5 protocol (Dallas and Day 2007) was compared to the untested MIRAI procedure in its ability to assign ecological categories to nine *a priori* selected impacted sites, varying in type and degree of impacts. Both procedures required the same protocol in collection of biological data, and relied on the establishment of reference conditions to ascertain impairment, thus enabling techniques to be compared.

Examination of the ecological category outputs generated by the two protocols revealed that no sites shared matching results. Both procedures identified impairment where sites were in fact impacted; signifying agreement by for both approaches and provides mutual support that the Palmiet River system is indeed impacted. However, the degree of impairment varied depending which protocol was used. Generally, SASS5 produced ecological categories at least one symbol higher than those calculated by MIRAI. On three occasions MIRAI-derived ecological categories were two ECs lower than that assigned by SASS5. Because we do not know the actual condition of each site without considerably more collecting effort, it cannot be precisely stated which of the procedures was the more accurate in assigning ecological categories. Instead, the applicability and sensitivity of each protocol in defining ecological categories was explored independently.

SASS5 biological bands developed by Dallas and Day (2007) were created specifically to investigate instream water-quality, at an ecoregion-level, which encompassed over a 100 site's biological variability in defining categories of reference. The ecoregion-approach taken by SASS5 Dallas and Day (2007) was compared to a more specific-focussed SASS5 procedure generated in Chapter 3, where catchment biological bands, unique to the Palmiet River, were derived. Both SASS5 procedures revealed their ability to differentiate between reference and impacted sites. More importantly however, where a site was found to be impacted by the SASS5 ecoregion approach, the Palmiet River summer-season catchment-specific SASS5 approach revealed a lesser degree of impairment.

MIRAI, on the other hand, was heavily scrutinised for its expert-based rating approach which in a hypothetical situation with varying approaches taken to rating scores, led to more than half the assigned ecological categories differing by more than one category. Interestingly, when rating scores were altered, flow and habitat modification metric-groups were identified as the most influential, whereas the importance of water quality was lessened by the design of the MIRAI procedure. It is thus evident from the design and the make-up of MIRAI that a greater degree of emphasis has been placed on detecting flow and habitat modification, than on water-quality metrics, whereas, SASS5 was designed to specifically identify impairment of water-quality.

To ascertain which of the SASS5 or MIRAI procedures were better suited to assigning ecological categories for impacted sites within the Palmiet River catchment, water-quality variables such as water temperature, pH, conductivity, percentage dissolved oxygen saturation, and flow-related discharge values were regressed against macroinvertebrate assemblages found at all impacted sites. Results revealed that two of the four water-quality variables (percentage dissolved oxygen saturation and pH) were significantly related to the macroinvertebrate assemblages at impacted sites, and water-quality, instead of flow, was thus seen as driving these impacts at the sites. In cases where water-quality was observed to be the most altered group-metric (e.g. PRBW, WR, GRABO and STOKO), MIRAI-derived ecological categories closely mirrored those derived by SASS5.

From this finding, we can deduce that, due to the greater emphasis placed on flow and habitat modification by MIRAI, MIRAI derived EC's may mask water-quality impairment. This is especially important in a system where nearly half of the impacted sites indicated water-quality to be the main deviation from reference (see Table 4.5). Further application of MIRAI in ascertaining accurate ecological categories, requires that the water-quality group-metric be weighted equally to flow and habitat modification metrics.

Conclusions

"It is one thing to find fault with an existing system. It is another thing altogether, a more difficult task, to replace it with another approach that is better." – Nelson Mandela, 16 November 2000

(speaking of water resource management)

Direct comparisons of biomonitoring protocols, which are currently in use in this country, provide an important foundation for integrating and guiding bioassessment programmes, especially in vastly impacted catchments such as that of the Palmiet River. Comparisons such as this study can provide guidance for choosing between alternate procedures. Both SASS5 and MIRAI procedures were specifically designed to examine certain types of river modifications, each of which cannot encompass all characteristics associated by impact on a possible site. There is trade-off between the designed scope and accuracy of each protocol, with neither method being 100% adequate. Deciding on which protocol bests suits the system requires prior knowledge of the site and the potential impacts that an anthropogenic disturbance may have on the physico-chemical and flow conditions, and on the biological communities within the system. It is probably true to say that we will never have a full understanding of a specific site and the potential impacts thereon, and so judgement of expert scientists and water managers are key in the decision-making process.

Just as independent tests of results from clinical trials are important to ensuring public health safety, so may independent assessments provide confidence in judging whether stream biological integrity is intact. Repeated tests may provide greater certainty when results agree, especially when differences in procedures provide multiple lines of evidence that support the same conclusion (Herbst and Silldorf 2006).

CHAPTER 5

DISCUSSION

Riverine freshwater ecosystems house one of the most important natural resources, and are amoungst the most threatened ecosystems. With the semi-arid climate combined with increasing human populations, South Africa has recognised the scarcity of fresh water and thus the importance of protecting these riverine ecosystems. Environmental degradation and public pressure have led to a need for accurate assessment, development and application of various monitoring programmes to accurately assess the health of our rivers.

The current monitoring protocol used in this country is the South African Scoring System (SASS) developed by Chutter (1994), which uses a macroinvertebrate community index to assess the water quality of a river. SASS, combined with the biological banding system in placed by Dallas and Day (2007) is an accredited protocol that has been tested and widely used in South Africa as a biological index of water quality. SASS however has shortcomings, because the protocol was developed for application in broad synoptic assessments, and for this reason SASS does not have a strong cause-effect basis.

To overcome SASS short comings, a new index, the Macroinvertebrate Response Assessment Index (MIRAI) was developed, providing a habitat-based cause-and-effect foundation to determine the ecological condition of macroinvertebrate communities. It integrates the environmental requirements of the invertebrate taxa in an assemblage and their responses to modified habitat conditions. Comparatively, less work has gone into objectively examining the MIRAI, than its counterpart SASS.

In either approach, the ultimate objective is to facilitate and evaluate the effect of disturbance on instream riverine macroinvertebrate communities, and to accomplish this, it is necessary to be able to distinguish those differences in ecosystem characteristics that stem from natural variability, from those caused by anthropogenic activities. Either approach requires an understanding of natural conditions, or as used in this study, "reference conditions", providing a means of comparing observed with expect conditions. The degree deviation from natural conditions can thereby be ascertained, serving as a foundation for developing criteria for the protection of aquatic ecosystems.

Natural variability is of critical importance in deriving reference conditions, and may occur at multiple spatial and temporal scales, driving and affecting a multitude of abiotic and biotic factors, influencing and generating both patterns and processes in ecological systems. Understanding both the spatial and temporal scale of changes in abiotic variables, and the impact these may have on the biotic assemblages are important in the context of each protocol.

Establishment of reference conditions

Three *a priori* reference sites were selected within the Palmiet River catchment, on the knowledge that sites shared similar underlying geology, surrounding vegetation, similar land-use practices, all being sampled from within the mountain stream zone and riffle biotope, as to minimise potential spatial impacts.

Examination of environmental variables revealed no spatial differences with respect to instantaneous physico-chemical variables between the three reference sites. The lack of spatial difference in physical-chemical variables implied that sites were similar enough to use these values to derive a set of reference conditions. However, climatic conditions characteristic of the south-western Cape, with its Mediterranean climate, resulting in periods of high flow and low temperatures, and low flow and high temperatures, have lead to significant seasonal differences in long-term water temperature and discharge data, both of which are known to alter instream water chemistry, explaining the temporal differences found in conductivity, instantaneous water temperature, pH, velocity and percentage dissolved oxygen saturation. The presence of such seasonal variation prevented the collective grouping of variables; instead seasons were kept separate when trying to define reference conditions.

Biological analyses revealed that many of the sampled aquatic organisms had specific water chemistry requirements, and the seasonal differences in physico-chemical conditions, water temperature and discharge lead to distinct temporal variations of distribution, abundance and taxon richness of macroinvertebrate assemblages. In this study, macroinvertebrates overcame seasonal-change-induced "harsh environments", by developing life cycles queued to exploit more favourable conditions with respect to physico-chemical factors and food availability.

When biological data were applied to SASS, and combined spatially, all three sites resembled that of reference conditions, however temporally, the summer season was assigned a "B" category, whereas spring and autumn seasons were seen individually as resembling reference conditions. Macroinvertebrate sensitivity to changes instream, especially to the physico-chemical characteristics, resulted in changes in macroinvertebrate community assemblages, with generally more sensitive and high-scoring taxa being present in spring and autumn, than in summer. These results highlighted the necessity to adopt a more temporal approach, accounting for seasonal variation in expected macroinvertebrate assemblages, by creating what was termed "seasonally-distinct biological bands". Following the development of these seasonally-distinct bands, the known pristine nature of the three sites was echoed in the assigned reference condition category.

These results revealed that to establish reference conditions appropriate for these systems, a generalized regional non-seasonal biological banding system cannot provide an accurate ecological category or "health indicator". Instead, a seasonal approach at catchment scale is required where biological bands need to be calculated temporally for reference conditions, for the seasons of spring, summer and autumn. For example, if a monitoring site were to be assessed in autumn only, it should preferably be compared to the reference condition for the specific season. However, if you were to sample in summer and compare it to the overall, seasonally combined SASS biological bands generated by Dallas and Day (2007), the absence or diminished importance of "summer" taxa due to seasons being combined, may reflect water quality impairment, and ignore the presence of temporal variability. Sampling season and producing SASS biological bands for reference conditions within those seasons is thus particularly crucial.

Comparison of two invertebrate-based biomonitoring techniques

The use of SASS and MIRAI as biomonitoring protocols was explored by sampling three reference sites (Chapter 3), and nine *a priori* selected impacted sites, within the Palmiet

River catchment. Due to the inability to ascertain the actual condition of each site, physico-chemical variables and macroinvertebrate assemblages were compared to the established reference conditions in Chapter 3, to ascertain whether or not the *a priori* impacted sites, were in fact impacted.

Environmentally, all three summer 2011 reference sites had varying physico-chemical values inside and out of the defined reference range established in chapter 3. However, caution was taken when placing too great an emphasis when assigning an ecological condition to a stream based on once-off sampling. With that said, the nine impacted sites all revealed physico-chemical values that ranged from moderately to extremely impacted.

Multivariate analyses of macroinvertebrate presence-absence assemblage data revealed that the communities at the summer 2011 reference sites resembled that of the established reference conditions in chapter 3, sharing a similarity of more than 50% to reference. The remaining nine sites were found all vary in the degree of dissimilarity from reference, ranging from those falling immediately outside 50% similarity, to slightly exceeding 86% dissimilarity.

When the biological data were applied to SASS and the MIRAI procedures, examination of ecological category outputs revealed no similarities in assigned symbols. Both procedures identified impairment from reference, signifying some degree of agreement between either approach, both providing mutual support that the Palmiet River system is indeed impacted. However, the degree of impairment from reference varied depending on which protocol was used.

Generally, SASS produced ecological categories that were at least one symbol higher than those calculated by MIRAI. Due to the unknown actual condition of each site without considerably more collecting effort, it is impossible to precisely state which procedure was more accurate in assigning ecological categories. Instead, the applicability and sensitivity of each protocol in assigning ecological categories was explored independently.

The ability of SASS in ascertaining ecological categories was explored by comparing biological bands developed by Dallas and Day (2007) for the Southern Folded Mountains Ecoregion, to the seasonal –catchment specific Palmiet River biological bands developed in

Chapter 3. Both SASS procedures revealed their ability to differentiate between reference and impacted sites. More importantly however, where a site was found to be impacted in the SASS ecoregion approach, the Palmiet River seasonal-catchment specific SASS approach revealed a lesser degree of impairment. The difference in sensitivity, and final ecological output was attributed to the scope of each SASS procedure. Dallas and Day (2007) banding system was developed to assist scientists with broad-scale biomonitoring, at an ecoregion level. The problem with the ecoregions approach is that the set reference bands were generated to incorporate a vast degree of naturally occurring spatial variability of rivers within surrounding catchments. This variability may in turn mask the actual condition of streams within a specific catchment.

In a regional approach, variability clearly has to be reduced to adequately define ecological conditions. In this case, it is thus suggested that a catchment-approach be undertaken in formulating reference conditions and biological bands thereof. A major criticism of the Palmiet River seasonal catchment-specific bands was the lack of replication and limited number of reference sites, whereas Dallas and Day (2007) derived-bands were developed re-sampling each point at least once, incorporating more than 100 study sites.

Sensitivity examination of the MIRAIs ability in assigning ecological categories was flawed scientifically, and was scrutinised for its expert-based rating approach adopted. A hypothetical situation was created by altering rating scores with respect to three levels of leniency. On average, the adjustment in leniency rating scores lead to more than half the assigned ecological categories differing by more than one symbol. Furthermore, when rating scores were altered, flow and habitat modification metric-groups were identified as the most influential, whereas the importance of water quality was lessened by the design of the MIRAI procedure. It is thus evident from the design of MIRAI that a greater degree of importance has been placed on detecting flow and habitat modification, than on water-quality metrics; whereas SASS was designed to specifically identify impairment of water-quality.

To ascertain which of the SASS or MIRAI procedures were better suited to assigning ecological categories for sites within the Palmiet River catchment, water-quality variables and flow-related discharge values were regressed against macroinvertebrate assemblages found at all impacted sites. Results revealed that water-quality (specifically percentage dissolved oxygen saturation and pH) was driving the differences in macroinvertebrate assemblages. This finding was further supported in cases where water-quality was observed to be the most altered group-metric (e.g. PRBW, WR, GRABO and STOKO), MIRAI-derived ecological categories closely mirrored those derived by SASS.

From these results, we can deduce that, due to the greater emphasis placed on flow and habitat modification by MIRAI, MIRAI derived ECs may mask water-quality impairment. This is especially important in a system where nearly half of the impacted sites indicated water-quality to be the main deviation from reference. In this, SASS is thus seen as the more appropriate protocol in ascertaining ecological integrity. Further application of MIRAI requires the water-quality group-metric to be weighted equally to flow and habitat modification metrics.

The possibility that more than half the ascertained ecological categories of those sites may differ by more than one symbol, creates too great a variability for scientists and researchers to assign with relative confidence an accurate ecological category, specific to a site. The difference between these two ecological categories has important ramifications with regard to conservation, management and restoration of a river. Caution thus has to be taken about personas undertaking bioassessment, especially using the MIRAI protocol. To limit variability, a robust procedure which limits variability is needed.

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APPENDIX A

Appendix A: South African Scoring System Version 5 Score Sheet

5. SASS Version 5 Score Sheet - Note: do not complete details (shaded area) on SASS sheet if doing a full RHP assessment

Version date: Feb 2005

Date:	/ /		(dd.ddddd)	
RHP Site Code:		Grid reference (dd mm ss.s) Lat:	S	
Collector/Sampler:		Long:	E	
River:		Datum (WGS84/Cape):		
Level 1 Ecoregion:		Altitude (m):		m
Quaternary Catchment:		Zonation:		
Site Description:	Temp (°C):		Cond (mS/m)	
	pH:		Clarity (cm):	
	DO (mg/L):		Turbidity:	
	Flow:		Colour:	
	Riparian Disturbance:			
	Instream Disturbance:			

Biotopes Sampled	Rating (1 - 5)	Time (min)
Stones In Current (SIC)		
Stones Out Of Current (SOOC)		
Bedrock		
Aquatic Veg		
MargVeg In Current		
MargVeg Out Of Current		
Gravel		
Sand		
Mud		
Hand picking/Visual observation		

Taxon		S	Veg	GSM	TOT
PORIFERA (Sponges)	5				
COELENTERATA (Cnidaria)	1				
TURBELLARIA (Flatworms)	3				
ANNELIDA					
Oligochaeta (Earthworms)	1				
Hirudinea (Leeches)	3				
CRUSTACEA					
Amphipoda	13				
Potamonautidae* (Crabs)	3				
Atyidae (Shrimps)	8				
Palaemonidae (Prawns)	10				
HYDRACARINA (Water mites)	8				
PLECOPTERA (Stoneflies)					
Notonemouridae	14				
Perlidae	12				
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4				
Baetidae 2 sp	6				
Baetidae > 2 sp	12				
Caenidae (Squaregills/Cainflies)	6				
Ephemeridae	15				
Heptageniidae (Flatheaded mayflies)	13				
Leptophlebiidae (Prongills)	9				
Oligoneuridae (Brushlegged mayflies)	15				
Polymitarcyidae (Pale Burrowers)	10				
Prosopistomatidae (Water specs)	15				
Teloganodidae SWC	12				
Tricorythidae (Stout Crawlers)	9				
ODONATA (Dragonflies & Damselflies)					
Calopterygidae ST,T	10				
Chlorocyphidae	10				
Synlestidae (Chlorolestidae)(Sylphs)	8				
Coenagrionidae (Sprites and blues)	4				
Lestidae (Emerald Damselflies)	8				
Platycnemidae (Brook Damselflies)	10				
Protoneuridae	8				
Aeshnidae (Hawkers & Emperors)	8				
Corduliidae (Cruisers)	8				
Gomphidae (Clubtails)	6				
Libellulidae (Darters)	4				
LEPIDOPTERA (Aquatic Caterpillars/Moths)					
Crambidae (=Pyralidae)	12				

Taxon		S	Veg	GSM	TOT
HEMIPTERA (Bugs)					
Belostomatidae* (Giant water bugs)	3				
Corixidae* (Water boatmen)	3				
Gerridae* (Pond skaters/Water striders)	5				
Hydrometridae* (Water measurers)	6				
Naucoridae* (Creeping water bugs)	7				
Nepidae* (Water scorpions)	3				
Notonectidae* (Backswimmers)	3				
Pleidae* (Pygmy backswimmers)	4				
Veliidae/M...veliidae* (Ripple bugs)	5				
MEGALOPTERA (Fishflies, Dobsonflies & Alderflies)					
Corydalidae (Fishflies & Dobsonflies)	8				
Sialidae (Alderflies)	6				
TRICHOPTERA (Caddisflies)					
Dipseudopsidae	10				
Ecnomidae	8				
Hydropsychidae 1 sp	4				
Hydropsychidae 2 sp	6				
Hydropsychidae > 2 sp	12				
Philopotamidae	10				
Polycentropodidae	12				
Psychomyiidae/Xiphocentronidae	8				
Cased caddis:					
Barbarochthonidae SWC	13				
Calamoceratidae ST	11				
Glossosomatidae SWC	11				
Hydroptilidae	6				
Hydrosalpingidae SWC	15				
Lepidostomatidae	10				
Leptoceridae	6				
Petrothrincidae SWC	11				
Pisuliidae	10				
Sericostomatidae SWC	13				
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5				
Elmidae/Dryopidae* (Riffle beetles)	8				
Gyrinidae* (Whirligig beetles)	5				
Haliplidae* (Crawling water beetles)	5				
Helodidae (Marsh beetles)	12				
Hydraenidae* (Minute moss beetles)	8				
Hydrophilidae* (Water scavenger beetles)	5				
Limnichidae	10				
Psephenidae (Water Pennies)	10				

Taxon		S	Veg	GSM	TOT
DIPTERA (Flies)					
Athericidae	10				
Blephariceridae (Mountain midges)	15				
Ceratopogonidae (Biting midges)	5				
Chironomidae (Midges)	2				
Culicidae* (Mosquitoes)	1				
Dixidae* (Dixid midge)	10				
Empididae (Dance flies)	6				
Ephydridae (Shore flies)	3				
Muscidae (House flies, Stable flies)	1				
Psychodidae (Moth flies)	1				
Simuliidae (Blackflies)	5				
Syrphidae* (Rat tailed maggots)	1				
Tabanidae (Horse flies)	5				
Tipulidae (Crane flies)	5				
GASTROPODA (Snails)					
Ancylidae (Limpets)	6				
Bulininae*	3				
Hydrobiidae*	3				
Lymnaeidae* (Pond snails)	3				
Physidae* (Pouch snails)	3				
Planorbinae* (Orb snails)	3				
Thiaridae* (=Melanidae)	3				
Viviparidae* ST	5				
PELECYPODA (Bivalves)					
Corbiculidae	5				
Sphaeriidae (Pills clams)	3				
Unionidae (Perly mussels)	6				
SASS Score					
No. of Taxa					
ASPT					

Other biota:

Comments/Observations:

Procedure:

Kick SIC & bedrock for 2 mins, max. 5 mins. Kick SOOC & bedrock for 1 min. Sweep marginal vegetation (IC & OOC) for 2m total and aquatic veg $1m^2$. Stir & sweep gravel, sand, mud for 1 min total. * = airbreathers

Hand picking & visual observation for 1 min - record in biotope where found (by circling estimated abundance on score sheet). Score for 15 mins/biotope but stop if no new taxa seen after 5 mins.

Estimate abundances: 1 = 1, A = 2-10, B = 10-100, C = 100-1000, D = >1000 S = Stone, rock & solid objects; Veg = All vegetation; GSM = Gravel, sand, mud SWC = South Western Cape, T = Tropical, ST = Sub-tropical

Rate each biotope sampled: 1=very poor (i.e. limited diversity), 5=highly suitable (i.e. wide diversity)

APPENDIX B

Appendix B: Macroinvertebrate Response Assessment Index - Rating approach. A portion of the greater rating spreadsheet required for initial MIRAI procedure.

Taxon	Season	Ref abun	Ref freq	Pres Abun	Pres freq	<0.1	0.1-0.3	0.3-0.6	>0.6	BEDROCK	COBBLES	VEG	GSM	WATER	QUALITY
Porifera						2	2	2	2	3	2	1	0	0	LOW
Coelenterata						2	2	1	0	2	2	1	0	0	NONE
Turbellaria						1	2	3	4	1	4	0	0	0	NONE
Oligochaeta						2	2	2	1	0	1	0	4	0	NONE
Hirudinea						2	2	1	1	0	4	1	1	0	NONE
Amphipoda						1	2	3	2	0	2	2	3	0	HIGH
Potamonautidae						1	1	3	2	0	3	1	1	0	NONE
Atyidae						2	2	0	0	0	1	4	1	0	MODERATE
Paleomonidae						0	2	2	3	0	3	0	0	0	MODERATE
Hydracarina						0	2	2	0	1	1	2	3	1	MODERATE
Notonemouridae						1	1	2	4	1	4	1	0	0	HIGH
Perlidae						1	1	1	5	1	4	1	0	0	HIGH
Baetidae 1sp						2	2	2	2	2	2	2	2	1	LOW
Baetidae 2spp						2	2	2	2	2	2	2	2	1	LOW
Baetidae >2spp						2	2	2	2	2	2	2	2	1	HIGH
Caenidae						3	2	1	1	0	2	1	3	0	LOW
Ephemeridae						2	2	3	2	0	1	0	4	0	HIGH
Heptageniidae						1	1	3	2	1	4	1	0	0	HIGH
Leptophlebiidae						3	2	2	1	1	3	2	0	0	MODERATE
Machadorythidae						5	0	0	0	0	1	0	4	0	
Oligoneuridae						0	0	1	5	2	3	1	1	1	HIGH
Polymitarcyidae						2	2	2	3	0	1	0	3	0	MODERATE
Prosopistomatidae						1	1	2	3	1	4	1	0	0	HIGH
Teloganodidae						0	0	2	4	1	4	1	0	0	HIGH
Trichorythidae						0	1	1	4	1	4	1	0	0	MODERATE
Calopterygidae						1	3	1	0	0	1	3	1	0	MODERATE
Chlorocyphidae						2	3	1	0	1	4	1	0	0	MODERATE
Chlorolestidae						3	2	1	0	0	1	4	0	0	MODERATE
Coenagrionidae						1	2	3	1	0	1	4	1	0	LOW
Lestidae						4	1	0	0	0	1	4	1	0	MODERATE
Platycnemidae						2	3	1	1	0	2	3	0	0	MODERATE
Protoneuridae						2	3	1	1	0	1	4	1	0	MODERATE
Aeshnidae						1	2	2	2	0	3	2	0	0	MODERATE
Corduliidae						2	3	1	0	0	2	1	3	0	MODERATE
Gomphidae						0	2	3	0	0	1	0	5	0	LOW
Libellulidae						1	2	3	1	1	4	0	1	0	LOW
Pyralidae						1	1	3	2	0	2	3	0	0	HIGH
Belostomatidae						4	1	0	0	0	0	4	0	1	NONE
Corixidae						2	3	1	0	1	1	1	1	4	NONE
Gerridae						4	1	0	0	0	0	0	0	5	MODERATE
Hydrometridae						4	1	0	0	0	0	2	0	4	MODERATE
Naucoridae						2	2	3	0	1	1	1	1	4	LOW
Nepidae						4	1	0	0	0	0	5	0	0	NONE
Notonectidae						4	1	0	0	0	10	2	0	4	NONE
Pleidae						4	1	0	0	0	0	4	0	1	LOW
Veliidae						5	1	1	0	0	0	0	0	5	MODERATE
Corydalidae						0	0	3	2	0	3	0	1	0	MODERATE
Sialidae						4	3	1	0	0	1	0	4	0	LOW
Dipseudopsidae						4	1	0	0	0	1	0	4	0	MODERATE
Ecnomidae						1	5	0	0	2	3	2	0	0	MODERATE
Hydropsychidae 1sp						0	1	2	4	2	3	1	0	0	LOW
Hydropsychidae 2spp						0	1	2	4	2	3	1	0	0	LOW
Hydropsychidae >2spp						0	1	2	4	2	3	1	0	0	HIGH
Philopotamidae						0	1	2	3	1	4	1	1	0	MODERATE
Polycentropodidae						0	0	3	4	4	3	0	0	0	HIGH
Psychomyiidae						0	1	2	3	4	2	1	0	0	MODERATE
Xiphocentronidae						0	1	2	3	4	2	1	0	0	MODERATE
Barbarochthonidae						0	2	3	1	2	3	2	0	0	HIGH
Calamoceratidae						4	1	0	0	0	2	2	3	0	MODERATE
Glossosomatidae						0	2	3	4	1	4	0	1	0	MODERATE
Hydroptilidae						0	3	2	2	1	2	3	1	0	LOW
Hydrosalpingidae						0	1	3	4	2	3	2	0	0	HIGH
Lepidostomatidae						1	3	2	1	2	2	2	2	0	MODERATE
Leptoceridae						0	1	3	2	2	2	2	2	0	LOW
Petrothrincidae						0	0	1	4	4	1	0	0	0	MODERATE
Pisuliidae						1	3	2	1	2	3	2	2	0	MODERATE
Sericostomatidae						0	1	3	2	0	3	2	0	0	HIGH
Dytiscidae						4	2	1	0	1	2	3	1	2	LOW
Elmidae						0	0	4	2	1	4	1	0	0	MODERATE
Dryopidae						0	0	2	4	1	4	1	0	0	MODERATE
Gyrinidae						1	2	2	3	0	0	0	0	5	LOW
Haliplidae						3	4	1	1	1	1	4	1	1	LOW
Helodidae						2	2	2	1	0	2	3	0	0	HIGH

APPENDIX C

Appendix C: Macroinvertebrate Response Assessment Index – Velocity flow preference sheet.

Very Fast (>0.6 m/s)	Moderately Fast (0.3-0.6 m/s)	Slow (0.1-0.3 m/s)	Very Slow (<0.1 m/s)
Perlidae	Elmidae	Ecnomidae	Machadorythidae
Oligoneuridae	Naucoridae	Haliplidae	Veliidae
Glossosomatidae	Gomphidae	Tipuliudae	Lestidae
Hydropsalpingidae	Coenagrionidae	Hydroptilidae	Belostomatidae
Psephenidae	Libellulidae	Calopterygidae	Gerridae
Polycentropodidae	Barbarochthonidae	Lepidostomatidae	Hydrometridae
Blepharoceridae	Ephemeridae	Pisuliidae	Nepidae
Ceratopogonidae	Hydraenidae	Chironomidae	Notonectidae
Muscidae	Amphipoda	Chlorocyphidae	Pleidae
Simuliidae	Potamonautidae	Corduliidae	Dipseudopsidae
Notonemouridae	Heptageniidae	Corixidae	Calamoceratidae
Hydropsychidae	Pyralidae	Tabanidae	Ephydridae
Telagonodidae	Leptoceridae	Corbiculidae	Syrphidae
Dryopidae	Sericostomatidae	Sphaeridae	Dytiscidae
Elmidae	Corydalidae	Platycnemidae	Sialidae
Trichorythidae		Protoneuridae	Culicidae
Petrotrhincidae		Unionidae	Psychodidae
Paleomonidae		Limnichidae	Bulinae
Polymitarcyidae			Hydrobiidae
Gyrinidae			Lymnaeidae
Prosopistomatidae			Physidae
Philopotamidae			Planorbinae
Psychomyiidae			Thiaridae
Xiphocentronidae			Viviparidae
			Chlorolestidae
			Caenidae
			Dixidae
			Leptophlebiidae

APPENDIX D

Appendix D: Macroinvertebrate Response Assessment Index - Habitat preference sheet

Bedrock	Cobbles	Vegetation	Gravel, Sand, Mud	Water
Petrothirincidae	Hirudinea	Nepidae	Gomphidae	Veliidae
Psychomyiidae	Libellulidae	Belostomatidae	Syrphidae	Gerridae
Xiphocentronidae	Glossosomatidae	Peidae	Machadorythidae	Culicidae
Polycentropodidae	Chlorocyphidae	Lestidae	Dipseudopsidae	Dixidae
Porifera	Perlidae	Chlorolestidae	Sialidae	Gyrinidae
Ancylidae	Prosopistomatidae	Atyidae	Oligochaeta	Hydrometridae
	Notonemouridae	Protoneuridae	Ephemeridae	Notonectidae
	Heptageniidae	Coenagrionidae	Unionidae	Muscidae
	Telagonodidae	Haliplidae	Corbiculidae	Naucoridae
	Dryopidae	Hydrophilidae	Sphaeridae	Corixidae
	Empididae	Hydraenidae	Ephydridae	Psychodidae
	Elmidae	Calopterygidae	Polymitarcyidae	
	Trichorythidae	Helodidae	Tabanidae	
	Athericidae	Platycnemidae	Limnichidae	
	Philopotamidae	Pyralidae	Tipulidae	
	Psephenidae	Dytiscidae	Caenidae	
	Corydalidae	Hydrobiidae	Corduliidae	
	Paleomonidae	Physidae	Hydracarina	
	Potamonautidae	Thiaridae	Calamoceratidae	
	Aeshnidae	Viviparidae	Amphipoda	
	Sericostomatidae	Hydroptilidae		
	Leptophlebiidae	Bulinae		
	Blepharoceridae	Lymnaeidae		
	Oligoneuridae	Planorbinae		
	Hydropsychidae			
	Ceratopogonidae			
	Pisuliidae			
	Ecnomidae			
	Hydropsalpingidae			
	Simuliidae			
	Barbarochthonidae			

APPENDIX E

Appendix E: Macroinvertebrate Response Assessment Index – Water Quality preference sheet.

High	Moderate	Low	Very Low
Helodidae	Veliidae	Gyrinidae	Culicidae
Pyralidae	Gerridae	Pleidae	Notonectidae
Blepharoceridae	Dixidae	Porifera	Belostomatidae
Polycentropodidae	Hydrometridae	Ancylidae	Nepidae
Hydropsychidae >2spp	Petrothrincidae	Viviparidae	Coelenterata
Sericostomatidae	Chlorolestidae	Hydropsychidae 1sp	Hydrobiidae
Hydropsalpingidae	Psychomyiidae	Hydropsychidae 2spp	Physidae
Barbarochthonidae	Xiphocentronidae	Simuliidae	Thiaridae
Perlidae	Platycnemidae	Naucoridae	Bulinae
Prosopistomatidae	Paleomonidae	Haliplidae	Lymnaeidae
Notonemouridae	Aeshnidae	Coenagrionidae	Planorbinae
Heptageniidae	Leptophlebiidae	Dytiscidae	Turbellaria
Telagonodidae	Ecnomidae	Hydroptilidae	Muscidae
Oligoneuridae	Chlorocyphidae	Libellulidae	Corixidae
Baetidae >2spp	Dryopidae	Empididae	Potamonautidae
Amphipoda	Elmidae	Hydrophilidae	Hirudinea
Ephemeridae	Trichorythidae	Baetidae 1sp	Psychodidae
	Psephenidae	Baetidae 2spp	Chironomidae
	Hydraenidae	Leptoceridae	Syrphidae
	Calopterygidae	Ceratopogonidae	Ephydridae
	Lestidae	Tabanidae	Oligochaeta
	Atyidae	Tipulidae	Sphaeridae
	Protoneuridae	Caenidae	
	Corydalidae	Sialidae	
	Glossosomatidae	Unionidae	
	Athericidae	Corbiculidae	
	Philopotamidae	Gomphidae	
	Lepidostomatidae		
	Pisuliidae		
	Hydracarina		
	Polymitarcyidae		
	Limnichidae		
	Corduliidae		
	Calamoceratidae		
	Dipseudopsidae		