Spatial and temporal heterogeneity in lotic systems: implications for defining reference conditions for macroinvertebrates

H.F. Dallas

Southern Waters Ecological Research and Consulting

and

Freshwater Research Unit, Zoology Department, University of Cape Town

Report to the Water Research Commission on

"Ecological reference conditions for riverine invertebrates"

February 2002

WRC Project No. KV 138/02

TABLE OF CONTENTS

EXECUTIVE SUMMARY

E1. BACKGROUND AND MOTIVATION

This report is based on data collected in the Western Cape during two previous Water Research Commission (WRC)-funded projects. The first focused on the "effects of water quality variables on riverine ecosystems" (Dallas & Day 1993, Dallas *et al.* 1995) and the second on "the development of tools for assessing regional water quality guidelines" (Dallas *et al.* 1998). During these projects, research into and development of the key bioassessment tool used in South Africa, namely SASS4, was undertaken (Dallas 1995, Dallas 1997). A subsequent project, commissioned by the Department of Water Affairs and Forestry (DWAF), focused on the "derivation of ecological reference conditions for riverine macroinvertebrates" (Dallas 2000a, 2000b, Dallas & Fowler 2000, Fowler *et al.* 2000).

This report, therefore, represents an amalgamation and analysis of data from the WRC and DWAF projects, addressing specific objectives related to aquatic bioassessment and defining ecological reference conditions for riverine macroinvertebrates. The current project duration was from January 2001 until 31 December 2001. The greater part of the report formed the basis of a thesis submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy, Department of Zoology, University of Cape Town (December 2001).

E2. INTRODUCTION

The protection of water resources in order to ensure their long term sustainability and the utilisation of these resources in the most efficient and effective manner, within the constraints set by the requirements for their protection, are the key interdependent components of the South African National Water Act (Act No 36 of 1998). This integrated approach to resource protection requires that measurable and verifiable resource quality objectives (RQOs), that clearly define acceptable levels of protection for water resources, be established. The RQOs have four components: requirements for water quantity (water level and flow), requirements

for water quality (chemical, physical and biological characteristics of the water), requirements for habitat integrity (of instream and riparian habitats), and requirements for biotic integrity (health, assemblage structure and distribution). These components highlight the complexity and interactive nature of aquatic ecosystems. An alteration in any one of the independent components, namely water quantity, water quality or habitat integrity will invariably lead to a change in biotic integrity. Biological assessment, or bioassessment, is a tool that integrates the effects of these components. Its utility in assessing environmental condition, especially water quality and general river condition, and in defining reference conditions for macroinvertebrates in river ecosystems, forms the basis of this report.

In particular, the extent of spatial and temporal variability in macroinvertebrate assemblages in South Africa and the implications of this variability for bioassessment and defining reference conditions, are examined in this report. Briefly, a reference condition is the condition that is representative of a group of minimally-disturbed sites, i.e. reference site, organised by selected physical, chemical and biological characteristics (Reynoldson *et al*. 1997) and that enables the degree of degradation or deviation from natural conditions to be ascertained. Two key questions addressed in this report are:

1) to what extent is spatial heterogeneity a feature of lotic ecosystems in South Africa, and is it possible to partition intrinsic spatial variability in such a way that defining reference conditions based on several similar reference sites is feasible?

2) to what extent is temporal heterogeneity a feature of lotic ecosystems in South Africa, and is it possible to account for intrinsic temporal variation in macroinvertebrate assemblages such that an anthropogenic disturbance at a monitoring site may be detected when comparing it to a derived reference condition?

E3. AIMS OF THE PROJECT

General aim

Central to this report is the question of whether ecological reference conditions are realistic and attainable entities, or whether intrinsic spatial and temporal heterogeneity of and variability in lotic systems are such that establishing reference conditions is not possible. The key questions posed, therefore, relate to the extent to which macroinvertebrate

assemblages vary spatially and temporally, and the implications of this variability to bioassessment and defining reference conditions. The question has been addressed by examining regional variability of macroinvertebrate assemblages within the context of assessing the utility of the spatial framework for regional classification of reference sites; by examining variability at the level of habitat; by examining temporal variability; and by identifying the environmental variables contributing to the variability in macroinvertebrate assemblages. To answer these questions patterns of spatial and temporal heterogeneity were examined in two distinct geographic regions, and at the level of individual taxa, macroinvertebrate assemblages and the derived biotic index, i.e. SASS scores.

Specific aims are:

- To test the protocol developed during an Institute for Water Quality Studies (IWQS, DWAF) project (Dallas 2000b) by applying it to another region, i.e. the Western Cape.
- To examine the spatial variability of macroinvertebrate assemblages between and within different ecoregions/bioregions, subregions and river-types, i.e. testing the validity of ecoregions/bioregions, subregions and river-types as units for defining homogenous regions and to discuss this variability in relation to establishing reference conditions.
- To examine the utilisation of SASS biotopes/habitats by macroinvertebrates and discuss implications with respect to the influence of biotope availability on Reference Condition SASS Scores and expected reference communities.
- To examine temporal variability of macroinvertebrate assemblages and discuss implications for establishing reference conditions.
- To derive ecological reference conditions for macroinvertebrates for specific river types of the Western Cape.
- To examine methodological aspects of SASS4 with a view to incorporating abundances into SASS sore calculations and to discuss implications for establishing reference conditions.

E4. SUMMARY OF MAJOR RESULTS

E4.1 The protocol for deriving reference conditions

The protocol developed in Dallas (2000b) formed a sound basis for data analyses when applied to another region, i.e. the Western Cape. Each of the steps described in the protocol are important when reference conditions are established. Of significance are the regional differences in the relative importance of biotopes, biotope preferences of individual taxa, and biotope and seasonal differences in macroinvertebrate assemblages. In the Western Cape, data limitations prevented the calculation of ratios. Instead absolute values were used and biological bands were derived based on the relationship of ASPT to SASS4 Score. This proved to be a useful means for data interpretation and subsequent detection of disturbance at a monitoring site.

E4.2 Spatial variability in macroinvertebrate assemblages at the regional level

In general, *a priori* regional classification of sites using the hierarchical spatial framework developed in South Africa provided a useful framework for preliminary classification of reference sites. Within-class variability (i.e. within a bioregion, ecoregion or bio-subregion etc.) was always lower than between-class variability (i.e. between bioregions, ecoregions, bio-subregions, etc.). Groups of sites based on *a posteriori* analysis of macroinvertebrate data, however, provided a more robust classification than any of the regional classifications.

Spatial classifications therefore offer geographic partitions within which to expect somewhat similar conditions and regional reference sites selected within the context of the hierarchical spatial framework are likely to be more representative of specific river types than those selected without using the spatial framework. Some variability within both regional classes and groups of sites with similar macroinvertebrate assemblages could not be accounted for at the regional or subregional levels, suggesting the presence of additional factors acting at a lower scale such as site or habitat.

The need for additional partitioning of variability at a lower scale is thus highlighted, as is the need for the classification of sites to be an iterative process that allows for subjective *a*

priori regional classifications to be modified on the basis of independent, objective *a posteriori* classification of biological assemblages. The lack of distinctiveness in macroinvertebrate assemblages from mountain streams and cobble-bed foothills, both of which are upland subregions, suggests that, from a practical perspective, and within the confines of bioassessment, mountain stream and foothill-cobble bed sites may be grouped together.

E4.3 Spatial variability in macroinvertebrate assemblages at the habitat level

Spatial variability at the level of habitat, specifically SASS-biotopes, revealed that several taxa exhibited a degree of biotope specificity, with some taxa recorded more frequently in one biotope rather than another. The relative importance of a biotope as a habitat for macroinvertebrates, as a reflection of both its availability and its utilisation by aquatic organisms, also varied regionally. The importance of hydraulic condition coupled with substrate type became apparent with differences in taxa observed within a biotope-group, e.g. stones-in-current versus stones-out-of-current. Seasonal differences in the distinctiveness of biotopes were observed in the Western Cape, with distinctiveness more pronounced in autumn, under low-flow conditions, in comparison with less pronounced biotope specificity in spring.

In terms of SASS Scores, stones-in-current/stones-out-of-current (SIC/SOOC) was shown to be the most important SASS biotope-group and taxa associated with it contributed the highest percentage to SASS Scores calculated at the site level. SIC/SOOC was also the most consistent in terms of its associated macroinvertebrate assemblage. There was a significant positive relationship between SASS4 Score and number of taxa with number of SASS-biotopes sampled and a negative correlation between ASPT and number of SASS-biotopes sampled.

The importance of sampling SASS-biotopes separately is clearly demonstrated. This enables SASS data to be interpreted on a "per SASS-biotope" basis in instances where one or other SASS-biotope is absent from a monitoring or reference site. By sampling SASS-biotopes separately, differences in the availability of SASS-biotopes between reference and monitoring sites may be taken into account, and subsequent results will thus reflect conditions other than those resulting from habitat differences. Flow conditions and season

are important additional factors that need to be taken into consideration when doing SASS, defining reference conditions and interpreting SASS data.

E4.4 Temporal variability in macroinvertebrate assemblages

Generally, seasonal differences were less pronounced than biotope-related differences and were more prevalent in the Western Cape compared to Mpumalanga. SASS Scores, specifically the number of taxa and ASPT, were significantly different among seasons in the Western Cape, with fewer taxa recorded in winter compared to summer and significantly higher ASPT values recorded in winter and spring in comparison to summer and autumn. Whilst more taxa were recorded in autumn than in spring, a higher proportion of sensitive and high-scoring taxa were recorded in spring. Temporal variability did not, however, curtail the detection of disturbance at monitoring sites.

In terms of defining reference conditions cognizance should be taken of the sampling season, particularly in regions that exhibit a relatively high degree of seasonal variability such as the Western Cape. When identifying expected or reference taxa for a seasonally variable region, details pertaining to the seasonal trends in individual taxa should be provided, since seasonal absences of certain taxa may affect the bioassessment results. Initial classification of reference sites based on seasonally-composite data provides a more robust classification of reference sites and is to be recommended.

E4.5 Environmental variables

Environmental variables at all scales were identified as potential predictor variables and were thus considered important in grouping sites with similar macroinvertebrate assemblages. In Mpumalanga, catchment-level variables included altitude and longitude, lending support to the observed distinction in macroinvertebrate assemblages between upland and lowland sites. Temperature, a correlate of altitude, was important, as was the depth of the shallow-water habitat (e.g. cobble riffle, bedrock rapid). Biotope-group predictor variables varied to some degree with aspects such as geological-type, canopy cover and the percentage of mud identified as important in the stony-habitat classification, in comparison to the depth of the deep-water habitat and the percentage of gravel/sand and mud in the vegetation classification.

The utility of a spatial framework within which reference sites are selected and bioassessment is undertaken is confirmed by these results. The importance of additional factors such as substratum that influence macroinvertebrate assemblages, is highlighted by the number of river type variables, at the scale of site and habitat, that were identified as important discriminators of macroinvertebrate assemblages in both the composite classification and biotope-specific classifications.

E4.6 Variability in macroinvertebrate assemblages within a region

The final chapter (Chapter 7) draws together aspects from all preceding ones, by examining spatial and temporal variability of macroinvertebrate assemblages within the most spatially and temporally variable group of sites identified in Chapter 3, namely upland sites of the Fynbos bioregion of the Western Cape. The degree of dissimilarity was a minimum of 47%, even when differences in the availability of biotopes, i.e. separating sites with- and without-vegetation, were included. Results confirmed that differences between sites in the two subregions, namely mountain streams and foothill-cobble beds, were not significant, although upland sites did form distinct Groups, particularly when mountain stream sites were considered in isolation.

Of importance from a bioassessment perspective, SASS Scores calculated for these upland sites were less variable than the macroinvertebrate assemblages and did not preclude the detection of disturbance at monitoring sites. Biological bands derived for data interpretation that utilised the relationship between ASPT and SASS4 Score provided a means whereby variability resulting from differences in the availability of biotopes and seasonal differences could be taken into account. Examination of the relative frequency of occurrence of taxa within each biological band revealed three different trends in response to increased disturbance. One group of taxa, many of which were high-scoring, sensitive taxa characteristic of minimally-disturbed upland sites, and many of which showed a preference for the stones-in-current biotope, decreased as disturbance increased. A second group of taxa, including several tolerant and low-scoring taxa such as Muscidae and Oligochaetes, increased in response to disturbance. A third group of taxa remained relatively unaffected by increased disturbance and included several hemipterans, dragonflies and damselflies.

Development of biocriteria is an important process in the effective protection of aquatic ecosystems and the confidence with which a judgement of biological condition is made depends on the soundness and scientific validity of the bioassessment tool (e.g. the biotic index) and the reference condition defined.

E4.7 Incorporation of abundance into SASS

SASS is a qualitative index and does not include abundance as part of the index. Rather, abundance is used as a descriptive aid for data interpretation. Examination of data in this study showed that there is a highly significant linear correlation between unweighted and weighted SASS Scores. This indicates that the inclusion of rank abundances did not alter the assessment of disturbance appreciably. The key difference was a broadening of the SASS4 Score range, particularly of the upper limit, suggesting that greater resolution may be attained between minimally disturbed sites and mildly disturbed sites, i.e. biological bands A and B. The adherence to the current practice of using the rank abundance estimates as additional descriptive and interpretive tools of the macroinvertebrate assemblage at a site is probably sufficient for interpreting bioassessment data.

E5. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study has shown that spatial and temporal heterogeneity are features of South African river systems. For effective management of these lotic systems it seems clear that intrinsic spatial and temporal heterogeneity and variability need to be understood and incorporated within the context of bioassessment. On the basis of the results of this study, it is possible to partition spatial variability such that defining reference conditions based on several similar reference sites is feasible. Adopting a regional framework, within which reference sites are selected and reference conditions defined, facilitates initial partitioning of variability resulting from differences at the regional and subregional levels.

Further spatial partitioning is necessary at the habitat level, specifically separation of SASS-biotopes during the bioassessment and analysis phase. In this way, differences in the availability of SASS-biotopes between reference and monitoring sites may be taken into account, and subsequent results will thus reflect conditions other than those resulting from habitat differences. Temporal variability, whilst not as obvious as biotope

differences, needs to be considered when defining reference conditions, with certain taxa more common in one or other season. The importance of seasonal differences was shown to vary between geographic regions. Temporal variability did not, however, curtail the detection of disturbance at monitoring sites.

Notwithstanding the spatial and temporal variability, and the identification of environmental variables at all scales acting on and influencing macroinvertebrate distributions, it is possible to define a reference condition for macroinvertebrates. This study has shown that a reference condition comprised of biocriteria in the form of SASS scores and expected SASS-taxa allows the identification of disturbed sites.

Recommendations for future research and management aspects are provided below.

- Further testing of the utility of regional classifications would be useful since the limited data for the Western Cape prevented rigorous testing of regional classifications. It would be advantageous to repeat the analyses once additional reference-site data have been collected.
- Biotope-preferences, in particular, are based on correlative data, and whilst preferences were apparent in many taxa, it would be useful to test these preferences experimentally or expand the number of biotope-specific assessments taking into account the hydraulic conditions, specifically whether the biotope is in- or out- of current. Further consideration needs to be given to these differences and the possibility of limiting bioassessment to fewer, more specific biotope types, which have comparable hydraulic characteristics.
- Aquatic vegetation, i.e. *Isolepis* spp., in upland sites of the Western Cape, appears to provide an important habitat for aquatic organisms. The distribution of *Isolepis* in this region and information on the utilisation, including seasonal importance, of *Isolepis* by aquatic organisms would be very useful, particularly given the pressures exerted on Western Cape rivers with regards to flow regulation and water abstraction.
- In South Africa knowledge of the life histories of aquatic organisms is severely limited. Such information would provide valuable insight into observed seasonal

variability and enable greater understanding of temporal heterogeneity in lotic systems.

- There is a clear need to expand the geographical range of reference sites and to initiate a long-term programme aimed specifically at defining reference conditions. Experience elsewhere demonstrates the importance of national co-operation and the participation of multiple departments and organisations in the water sector.
- Regional experts, who are familiar with the region, provide an excellent starting point for identification of river types and potential reference sites.
- The development of predictive models as in the United Kingdom and Australia is strongly recommended for South Africa.
- By ensuring that all biomonitoring practitioners adhere to the standard sampling protocol, which includes the collection of a subset of environmental variables and separate biotope-group sampling, we will be ensured of an extensive and useful dataset in the future.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

TERMS OF REFERENCE

The overall focus of the project was the derivation of ecological reference conditions for aquatic invertebrates and the examination of factors affecting the utility of such reference conditions within a national biomonitoring programme. Initially the report was to focus on the Western Cape, but was subsequently expanded to include both the Western Cape and Mpumalanga, on the basis that the comparison between the two regions was also of importance. Because sites were not selected and data not collected for the express purpose of the aims listed below, the number and location of reference sites and the frequency of sampling was not optimal. In particular, the frequency of data collection varied substantially and different sub-sets of data have thus been selected to address specific aims in this study. The specific aims of the projects and details of the presentation of results are given below.

Aims of the project

1. To test the protocol developed during an (IWQS, DWAF) project (Dallas 2000b) by applying it to another region, i.e. the Western Cape.

   The protocol has been tested throughout the study and specific comment is made in chapter 9 on management implications and recommendations.

2. To examine the spatial variability of macroinvertebrate assemblages between and within different ecoregions/bioregions, subregions and river types, i.e. testing the validity of ecoregions/bioregions, subregions and river types as units for defining homogenous regions and to discuss this variability in relation to establishing reference conditions.

   The utility of the spatial framework, at the regional and subregional levels, has been tested for both the Western Cape and Mpumalanga - Chapter 3. Factors at the level of river type, which contribute to defining homogenous regions, have been identified for Mpumalanga (Chapter 6) and the Western Cape (Chapter 7).

3. To examine the utilisation of SASS biotopes/habitats by macroinvertebrates and discuss implications with respect to the influence of biotope availability on Reference Condition SASS Scores and expected reference communities.

   Biotope availability and the utilisation of SASS-biotopes by macroinvertebrate taxa are examined in Chapter 4. The effect of biotope availability on the identification of environmental predictors is also assessed (Chapter 6).

4. To examine temporal variability of macroinvertebrate assemblages and discuss implications for establishing reference conditions.

   Temporal variability of macroinvertebrate assemblages and implications for aquatic bioassessment is addressed in Chapter 5.

5. To derive ecological reference conditions for macroinvertebrates for specific river types of the Western Cape.

   The variability of macroinvertebrate assemblages at upland sites of the Western Cape has been examined and reference conditions for this region have been defined (Chapter 6). The scarcity of suitable data for lowland sites and sites within other areas within the Western Cape prevented derivation of reference conditions for other river types.

6. To examine methodological aspects of SASS4 with a view to incorporating abundances and verifying sensitivity/tolerance scores and discuss implications for establishing reference conditions.

   The incorporation of abundance estimates is explored in Appendix B, in particular the effect of abundances on SASS Scores and the detection of disturbance. Methods for verifying sensitivity/tolerance scores have not been included on the basis that the most recent version of SASS5 included several modifications to these scores, again on the basis of the experience of SASS practitioners.

ACKNOWLEDGEMENTS

This report is the amalgamation of components of three research projects undertaken whilst a member of the Freshwater Research Unit, Department of Zoology, University of Cape Town and Southern Waters Ecological Research and Consulting. I gratefully acknowledge the funding received from the Water Research Commission (WRC) and the Institute of Water Quality Studies (IWQS), Department of Water Affairs & Forestry.

Enormous thanks to my colleague, Dr Jenny Day, whose encouragement, friendship and insight have and continue to inspire me. Thanks to Dr Steve Mitchell, of the Water Research Commission, for his unfailing support of my research and for his encouragement to apply for funds for this project. Thanks also to my many friends and colleagues in the Freshwater Research Unit and Southern Waters, who have supported me in my research through the years.

Thanks to Liz Day, Penny Scott and Jonathan Musikanth for assistance with field work in the Western Cape and to Charlene Coulsen who provided invaluable assistance throughout the WRC projects. Data for the Mpumalanga region were the result of a collaborative research project funded by IWQS. I thank the following for their assistance with field work: Christa Thirion, Annelise Gerber and Mary-Jean Gabriel (IWQS), Colleen Todd and Petro Cilliers (Environmentek), Jacques Venter (Kruger National Park) and Gerhard Schultz (Mpumalanga Parks Board).

CHAPTER 1. INTRODUCTION

1.1 INTRODUCTION

The protection of water resources in order to ensure their long term sustainability and the utilisation of these resources in the most efficient and effective manner, within the constraints set by the requirements for their protection (Anonymous 2001), are the key interdependent components of the South African National Water Act (Act No 36 of 1998). This integrated approach to resource protection requires that measurable and verifiable resource quality objectives (RQOs), that clearly define acceptable levels of protection for water resources, be established. The RQOs have four components: requirements for water quantity (water level and flow), requirements for water quality (chemical, physical and biological characteristics of the water), requirements for habitat integrity (of instream and riparian habitats), and requirements for biotic integrity (health, assemblage structure and distribution) (Anonymous 2001). These components highlight the complexity and interactive nature of aquatic ecosystems. An alteration in any one of the independent components, namely water quantity, water quality or habitat integrity will invariably lead to a change in biotic integrity. Biological assessment, or bioassessment, is a tool that integrates the effects of these components. Its utility in assessing environmental condition, in particular water quality and general river condition, and in defining reference conditions for macroinvertebrates in river ecosystems, forms the basis of this report.

1.2 BIOASSESSMENT

Changes in environmental conditions can be identified using either a "bottom-up" or a "top-down" approach (Scrimgeour & Wicklum 1996). A "bottom-up" approach typically relies on data produced from simple laboratory systems (i.e. eco-toxicological), often at small temporal and spatial scales, to model changes in natural systems. This approach is thought to suffer from low environmental realism, although it can be effective if the underlying mechanisms of environmental change are known (Scrimgeour & Wicklum 1996). In the "top-down" approach, changes at the level of assemblage and ecosystem are directly assessed in the natural environment followed by identification of their causes. Cairns *et al.* (1993) suggested that development of top-down assessment methods should

result in successful environmental management. Two major categories of top-down endpoints are monitored in ecosystems, namely biotic structural components and ecosystem processes or functions. Of these, structural changes are thought to be more responsive to ecosystem stress or disturbance than functional ones (Howarth 1991 cited by Scrimgeour & Wicklum 1996). Characterising the response of an ecosystem to disturbance can be achieved using biological or ecological indicators and is termed instream biological response monitoring or bioassessment (Roux *et al*. 1999). Disturbance is defined as "any relatively discrete event in time that disrupts ecosystem, assemblage or population structure and changes resources, substratum availability, or the physical environment (White & Pickett 1985).

Bioassessment may be defined as the utilization of one or more components of the biota to assess the effect of a change in another component such as water quality. It is the process of determining whether human activity has altered the biological properties of an ecosystem (Hawkins & Norris 2000). Potential components of the biota that may be used include periphyton (Barbour *et al.* 1999), macroinvertebrates (Reynoldson *et al.* 1997, Barbour *et al.* 1999, Dallas 2000), fish (Karr 1981, Kleynhans 1999) and riparian vegetation (Kemper 1999). Bioassessment provides a time- and constituent-integrated assessment of the ecological or biological integrity of the system under consideration. Biological integrity is the ability of an aquatic ecosystem 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 & Dudley 1981). Bioassessment has been acclaimed as a more sensitive and reliable measure of environmental conditions than either physical or chemical measurements (Warren 1971) and using biota as indicators of disturbance in an ecosystem has proven successful (Rosenberg & Resh 1993).

Traditionally, physico-chemical monitoring formed the backbone of water quality monitoring in South Africa (DWA 1986) and elsewhere (e.g. Barbour *et al*. 1996), and control of surface water quality has been through the control of effluent discharges. 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, i.e. unless sample collections are continuous over time, pulsed releases of effluents that result in an alteration of water quality may not be recorded. 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, and 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.

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. 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. For example, changes in pH are particularly significant in altering the toxicity of a variety of chemical constituents, including trace metals (Dallas & Day 1993). These subtle magnifying and reducing effects would not necessarily be revealed by routine physico-chemical monitoring.

The traditional physico-chemical evaluations of water quality have been largely inadequate (Warren 1971, Barbour *et al.* 1996), as have the use of physico-chemical standards to protect the aquatic environment from, for example, downstream effects of wastewater treatment works in South Africa (Dickens & Graham 1998). While focusing 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, have also been overlooked (Harris 1995 cited by Schofield & Davies 1996). Organisms, however, because they are dependent on the medium in which they live, i.e. the water body, are sensitive to all alterations to the water body by, for example, pollution or habitat alteration, and alterations will be reflected in the biotic assemblage. The biota therefore act as indicators of the overall ecological condition of the aquatic ecosystem, by acting as continuous monitors of the water they inhabit (Hawkes 1979), thereby enabling long-term analysis of both regular and intermittent discharges, variable

concentrations of pollutants, single and multiple pollutants, and synergistic or antagonistic effects. The biota, however, whilst indicating that a water body is impacted, do not provide insight into the cause of the problem. For this reason, Hawkes (1997) suggests that bioassessment, which produces biological data, and physico-chemical monitoring, which produces physical and chemical data, are really complementary. He suggests it would not be useful to correlate the two assessments, as bioassessment information is probably of greatest value when it does not confirm the chemical data, thus revealing the effect of other physical or chemical factors. Reynoldson & Metcalfe-Smith (1992) suggest that biological systems should be the standard for monitoring, assessment, and target formulation, and that the role of chemistry and physics is most important in the identification of factors causing impairment and the selection of appropriate remedial actions.

There is general consensus that benthic macroinvertebrates are amongst the most sensitive components of aquatic ecosystems and they have been widely used in bioassessment. Briefly, as summarised by Rosenberg & Resh (1993), macroinvertebrates are ubiquitous and diverse, and are therefore affected by a variety of disturbances in many different types of aquatic habitats. Sensitivity to stress varies with species and the large number of species within an assemblage offers a spectrum of responses to environmental stresses. In their aquatic phase, macroinvertebrates are largely non-mobile and are thus representative of the location being sampled, which allows effective spatial analyses of disturbance. They have relatively long life cycles compared to other groups (e.g. planktonic organisms), which allows elucidation of temporal changes caused by disturbances. A major limitation, however, of using macroinvertebrates in bioassessment is their heterogeneous 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.

1.3 SPATIAL AND TEMPORAL VARIABILITY IN MACROINVERTEBRATE ASSEMBLAGES

Lotic systems are naturally heterogeneous (Poff & Ward 1990, Cooper *et al.* 1997, Palmer & Poff 1997, Townsend *et al.* 1997). Heterogeneity, defined as variability in a process or

pattern over space or time (Palmer & Poff 1997), may occur at multiple spatial and temporal scales for both biotic and abiotic factors, and influences both pattern and process in ecological systems (Townsend 1989, Palmer & Poff 1997). Differences in factors such as flow-rate, stream size, temperature, substrate and resource availability among sites lead to spatial variability in the macroinvertebrate assemblage. Seasonal variability of such factors at a site may lead to temporal variability in macroinvertebrate assemblages. A stream may therefore be viewed as a mosaic of patches characterised by different environmental conditions (Pringle *et al.* 1988), having an ensuing patchy distribution of macroinvertebrates. Discrete substrate patches may exist, for example, due to differences in the velocity at which particles are mobilized, and the consequent sorting of bed materials by size (Frissel *et al.* 1986). Patch size and patch boundaries perceived by individual organisms and by individuals over time, vary significantly among organisms (Pringle *et al.* 1988) depending on the size and ecological requirements of the organism.

The extent of spatial and temporal variability in macroinvertebrate assemblages in South Africa, and in particular the implications of this variability for bioassessment and defining reference conditions, are examined in this report. Briefly, a reference condition is the condition that is representative of a group of minimally-disturbed sites, i.e. reference site, organised by selected physical, chemical and biological characteristics (Reynoldson *et al*. 1997) and that enables the degree of degradation or deviation from natural conditions to be ascertained. Two key questions addressed in this report are:

1) to what extent is spatial heterogeneity a feature of lotic ecosystems in South Africa, and is it possible to partition intrinsic spatial variability in such a way that defining reference conditions based on several similar reference sites is feasible?
2) to what extent is temporal heterogeneity a feature of lotic ecosystems in South Africa, and is it possible to account for intrinsic temporal variation in macroinvertebrate assemblages such that an anthropogenic disturbance at a monitoring site may be detected when comparing it to a derived reference condition?

1.3.1 Spatial heterogeneity and variability

Factors leading to spatial heterogeneity of macroinvertebrate assemblages in lotic environments are varied and occur and act at different spatial scales. The nested hierarchical relationship (Frissel *et al.* 1986) between factors potentially affecting biotic

assemblages suggests that processes higher up in the hierarchy affect processes lower in the hierarchy, i.e. regional catchment characteristics constrain local structure (Lammert & Allan 1999). Thus, an event or disturbance that causes a shift in a large-scale system will change the capacity of all the lower-level systems (Frissel *et al.* 1986). The hierarchical nature of factors which affect the structure and dynamics of river channels and thus macroinvertebrate assemblages was recognised by Rowntree & Wadeson (1999), who adapted a model proposed by Frissel *et al*. (1986) for managing rivers in South Africa. Rowntree & Wadeson's (1999) geomorphological framework incorporates six nested levels ranging from catchment, to geomorphological zone, stream segment, channel reach, morphological unit, and hydraulic biotope (Rowntree *et al.* 1998). The geomorphological framework, together with factors acting at each level, are discussed in turn.

Catchment factors such as geology (Richard *et al*.1997) affect water chemistry, whilst climate determines the hydrological type, and geomorphology the channel type, substratum composition and erosion potential. Hynes (1975) aptly described the influence of catchment level factors on riverine ecosystems and processes with: "In every respect, the valley rules the stream". Geological differences, for example, can produce different stream water chemistry (Day & King 1995) that may strongly influence biotic assemblages (Richard *et al.* 1996, 1997). In addition to these abiotic factors, natural biogeographic differences in the distribution of riverine biota may lead to biotic differences between rivers and/or sites. Rivers are isolated geographic entities and as such they form important foci for speciation and therefore for the development of biological diversity (Davies & Day 1998). Heterogeneity of macroinvertebrate assemblages at the level of catchment may, therefore, be a reflection of both abiotic factors such as geology and climate, together with biogeographic differences and evolutionary histories of individual taxa.

The geomorphological zone, stream segment and channel reach all relate to the longitudinal profile of a river as it flows from source to sea. Gradual changes in environmental factors such as altitude (Jacobsen *et al.* 1997), water temperature (Hawkins *et al.* 1997), flow-rate (Statzner & Higler 1986) and food resources along the longitudinal profile exert a direct control on the population dynamics of macroinvertebrates and other organisms, resulting in characteristic biological communities and ecological river zonation (Hawkes 1975). These longitudinal changes led to the formulation of the River Continuum Concept (RCC, Vannote *et al.* 1980) that views all rivers as possessing continuous

gradients of physical and chemical conditions, including width, depth, velocity, flow volume, temperature and eutropy gain, that are progressively and continuously modified downstream from source to sea. The unidirectional nature of river systems means that water-flow, solutes, detritus, sediment and organisms are constantly delivered from upstream to downstream (Cooper *et al*. 1998). Within the context of the RCC, energy input and organic matter transport, storage and use by macroinvertebrate functional feeding groups, are thought to be regulated largely by fluvial geomorphic processes (Vannote *et al.* 1980). This leads to the formation of species assemblages characteristic of particular reaches of a river. Important to the RCC is that activities upstream have an effect downstream, i.e. it is a continuum. Within the RCC, the concept of functional feeding groups has proved to be quite contentious, with differences in the applicability of this concept arising between northern and southern hemisphere river systems. In the Western Cape of South Africa, open-canopied fynbos streams, protracted leaf-fall throughout spring and summer, and functional feeding constraints imposed by different life stages of aquatic organisms, have led researchers to question the validity of this concept in such systems (Davies & Day 1998). Of the abiotic conditions associated with longitudinal river zonation, the physical characteristics of flow, or stream hydraulics, were considered by Statzner & Higler (1986) to be the overriding factors governing zonation of macroinvertebrates in streams and rivers. Hawkins *et al.* (1997) have shown that water temperature, which varies along both elevational and latitudinal gradients, is also an important determinant of biotic patterns.

Both the morphological unit, which relates to channel morphology, and the hydraulic biotope, act at the level of the site and the habitat of macroinvertebrate assemblages, as well as at the level of individual taxa within these assemblages. Hydraulic biotope is defined as a spatially distinct instream flow environment determined by the hydraulic and substrate characteristics associated with each morphological unit (Rowntree & Wadeson 1999). The level of habitat is not limited to hydraulic biotopes and other terms, such as "mesohabitat" (Armitage *et al.* 1995), "SASS-biotope" (South African Scoring System; Chutter 1998, Dallas 1997) and "substrate-type" (Collier 1995) have also been used to describe this level. For each, the term habitat or biotope describes the environment in which an aquatic organism lives and incorporates aspects such as the substrate-type, hydraulic and chemical conditions. Since taxa often have specific substrate or hydraulic requirements, habitat-level factors may account for much local variation in

macroinvertebrate assemblages. For example, Tennant (1976 cited by Tharme 1996) considered wetted perimeter, depth and velocity to be important physical habitat parameters for the wellbeing of aquatic organisms. The structure of the substratum, which is inseparably linked to variations in flow (Resh & Rosenberg 1984), may also be related to the presence or absence of individual aquatic organisms since substratum structure may restrict or enhance an insect's ability to adhere, cling or burrow, affects its ability to escape from predators, be protected from current or disturbance, construct cases, or deposit eggs. Predation, in particular the type of predator, has also been shown to affect the variation of prey such as mayfly and caddisfly densities at the habitat-level, i.e. within-riffles (Crowl *et al.* 1997).

In addition to isolated effects of factors at each level of the hierarchy, it is often a combination of environmental factors at various levels in the hierarchy that affects macroinvertebrate distribution and abundance. There also appears to be some scale dependence to this spatial heterogeneity and variability, with species assemblages appearing predictable and governed by large-scale patterns in hydrology and geology at the largest spatial scales, whilst site-based studies tend to reveal high variability and appear to be governed by local physical and biological factors (Wiley *et al*. 1997). For example, instream habitat structure and organic matter inputs are determined primarily by local conditions such as vegetation cover at a site, whereas nutrient supply, sediment delivery, hydrology and channel characteristics are influenced by regional conditions, including landscape features and land use/cover at some distance upstream and lateral to stream sites (Allan *et al*. 1997). As such, human alteration of the landscape affects the riverine ecosystem via multiple processes operating over different spatial scales (Allan *et al*. 1997). Understanding the intrinsic spatial variability of aquatic biota, together with the anthropogenic modification of biotic assemblages, is therefore complex.

In the context of bioassessment, spatial heterogeneity of macroinvertebrate assemblages is often taken into account by partitioning areas into relatively homogeneous regions, i.e. regional classification. Bioassessment and aquatic resource management is then conducted within the context of the established regional frameworks. There has been much debate on the validity of these regional classifications and whilst they have been applied throughout the world for bioassessment purposes (e.g. Harding *et al.* 1997, Johnson 2000, Marchant *et al.* 2000, Rabeni & Noisy 2000), until recently, there has been no rigorous evaluation of

how well these classifications perform (Hawkins & Norris 2000). For a regional classification to be deemed useful and ecologically sound, it needs to improve our understanding of the system, to explain and order natural variability, to provide a framework for sampling and management, to allow the extrapolation of site-specific information, and to lend a measure of predictability of ecosystem response to land-use practices (Bryce & Clarke 1996). Ultimately, the ability to detect anthropogenic disturbance is a direct function of how well regional classifications partition natural variation among sites (Hawkins & Vinson 2000). Can spatial variability thus be partitioned sufficiently, such that, when comparing a monitoring or test site to a reference site or reference condition, an impact resulting from, for example an effluent discharge, is detected?

1.3.2 Temporal variability

Lotic systems exhibit daily and seasonal periodicity in factors such as discharge and temperature and seasonal and year-to-year variability in macroinvertebrate assemblages have been linked to precipitation events and stream hydrographs (McEravy *et al.* 1989). Temperature is known to be an important mechanism affecting the growth and distribution of stream insects (Hawkins *et al*. 1997). Many organisms are adapted to changes in temperature and discharge, and have life history stages such as emergence, feeding and growth that are cued into them. The phenology of individual species within an assemblage will alter the observed composition of the assemblage at different times of the year (Linke *et al*. 1999). Water temperature, in addition to being a climatically-driven variable, is affected by local factors such as channel morphology and riparian shading, and summer maximum temperatures, in particular, may limit the occurrence of certain species (Hawkins *et al.* 1997). Seasonal variability may change longitudinally down a river, as noted by King (1981) in a study of the Eerste River, South Africa, in which upland sites had a single macroinvertebrate assemblage, whilst lowland sites had a distinct winter and summer assemblage. This spatial-temporal interaction was also observed by Pearson & Franklin (1968, cited by Linke *et al.* 1999) who noted that several mayfly species tended to occur downstream earlier in the year and upstream in later months.

Given that the ultimate objective of bioassessment is to evaluate the effect of human activities on biological resources (Fore *et al*. 1996), it is necessary to be able to identify which observed

differences in macroinvertebrate assemblages stem from natural or intrinsic heterogeneity and variability in the system, and which are the result of an anthropogenic effect. The goal, therefore, is to decide whether or not a site exposed to stress is disturbed, while minimising both Type I (sites fail which should have passed) and Type II (sites passed which should have failed) errors of analysis (Bailey 1996 cited by Linke *et al.* 1999). Passing or failing may be based on established biocriteria such as biotic index scores appropriate to the region or suites of reference sites. A site would fail if the biotic index score was less than the expected or reference score, and would pass if the score exceeded the reference score. Of the two types of errors, Type II, i.e. failure to detect an environmental effect, is fairly common in environmental monitoring (Carlisle & Clements 1999). One approach followed to facilitate the detection of anthropogenic effects is the use of minimally-disturbed sites, termed reference sites, with which monitoring or test sites are compared. This approach, termed the Reference Condition Approach, is used to generate a range of expected reference conditions for macroinvertebrate assemblages and/or biotic indices.

1.4 BIOTIC INDICES AND ECOLOGICAL REFERENCE CONDITIONS

Biotic indices are numerical indices, which use one or more components of the biota to provide a measure of the biological condition of a site. Biotic indices based on macroinvertebrate assemblages have proven to be useful measures of stream or ecosystem "health" and are widely applied today (Hellawell 1986, Rosenberg & Resh 1993), with many countries beginning to rely on biological assessments as their primary measure of the ecological health of surface waters (Gerritsen *et al.* 2000). Ecosystem or river health, whilst being a useful and widely understood concept, is difficult to describe in precise, scientific terms (Schofield & Davies 1996). Schofield & Davies (1996) have taken it to mean the degree of similarity to a minimally-disturbed river of the same type, particularly in terms of its biological diversity and ecological functioning, and it is this definition that is adopted in this report.

One of the advantages of biotic indices is that they formalise what any good biologist, familiar with local biota, knows about the biological condition of a stream and they communicate biological condition to policy makers and concerned citizens, thus providing a scientific basis for management decisions that affect aquatic resources (Fore *et al.* 1996). Historically, biotic indices have often been calculated *a posteriori* from quantitative

macroinvertebrate sampling (e.g. Chutter 1972, Hilsenhoff 1988). However, labour and time constraints associated with such quantitative sampling prompted the development of qualitative rapid bioassessment methods such as the BMWP system (Biological Monitoring Working Party, e.g. Wright 1995), the Australian SIGNAL biotic index (Stream Invertebrate Grade Number Average Level, Chessman 1995) and SASS (South African Scoring System, Chutter 1998). Not only do these rapid bioassessment methods utilise simplified data interpretation methods via the generation of biotic indices, but several also reduce the time needed to process samples, either by being field-based (e.g. SASS), by limiting the number or organisms identified, i.e. fixed-count method (e.g. SIGNAL), or by limiting taxonomic resolution to that of family or higher (e.g. SASS, SIGNAL).

Whilst there is still much debate about the potential loss of information that may occur when biotic indices are used (Brown 1997), for example, by omitting abundances from the index calculation for SASS4, they have been used effectively to reveal the effects of many different anthropogenic impacts. Biotic effects on riverine macroinvertebrate assemblages that have been effectively assessed using biotic indices include the effects on receiving water bodies of organic pollution (Cao *et al.* 1997b) via discharges from sewage treatment works (e.g. Chessman 1994, Wright *et al.* 1995), wastewater discharges (e.g. Chessman *et al.* 1997, Dickens & Graham 1998) and trout farm effluent (e.g. Loch *et al*. 1996, Brown 1997), the effects of mixed diffuse runoff such as urban storm water runoff (e.g. Chessman *et al.* 1997), the effects of agriculture (e.g. Quinn *et al.* 1997), afforestation (e.g. Quinn *et al*. 1997, Rothrock *et al*. 1998), metal pollution (e.g. Carlisle & Clements 1999) and experimental insecticide treatments (Wallace *et al.* 1996).

In South Africa, riverine macroinvertebrates are one 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 of aquatic ecosystems in South Africa. Briefly, SASS is a scoring system 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 *et al*. 1995, Dallas 1997). Data interpretation is based on two calculated values, namely SASS4 Score, which is the sum of the sensitivity/tolerance scores for taxa present at a site, and average score per taxon (ASPT), which is SASS4 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, Chutter 1998).

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. Chutter 1998) to complex predictive models, which relate environmental variables to biotic communities (e.g. Wright 1995, Smith *et al.* 1999). 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. This "expected" condition is referred to as the reference condition. Bioassessment is generally applied within the context of ecological reference conditions, which represent an expected, realistic and scientifically-authentic ecological benchmark with which bioassessment information is compared.

1.4.1 What are 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 enable the degree of degradation or deviation from natural conditions to be ascertained, and thereby serve as a foundation for developing biological criteria for the protection of aquatic ecosystems. In a regionally varied landscape, this means identifying biotic patterns that vary with normal geomorphic variations of the landscape, as well as alterations caused by anthropogenic influences (Richards *et al*. 1997).

A reference condition is usually derived from a suite of similar reference sites, termed a regional reference condition, although single site-specific reference conditions are sometimes also used. Site-specific 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, and are needed when there are concerns with specific point sources. A typical example of a "paired" scenario would be upstream and downstream of a sewage treatment works discharge point where point-source effluent is discharged into the receiving water body.

Regional reference conditions are necessary because pristine sites, particularly in the lower reaches of rivers, generally no longer exist, and near-pristine sites are often scarce. Inferences need to be made from minimally-disturbed sites to those impacted by human activity. Reynoldson & Wright (2000) describe a three-staged process for selecting regional reference sites. The first stage involves the adoption or definition of a stratification process such as ecoregions or stream order, so that the full range of reference conditions is represented. The second stage is the incorporation of local knowledge and the selection of sites using information on the extent to which a site has been disturbed. Criteria used to assess the level of disturbance often include a qualitative assessment of land-use, water quality impacts, modifications to discharge and physical alterations to the channel. Studies have shown that site selection is an iterative process involving initial site selection as outlined above, followed by a "ground-truthing" or site validation phase, and data collection and analysis phase (e.g. Dallas 2000b, Reynoldson & Wright 2000). Final selection of reference sites is conducted after examination of the data (Reynoldson & Wright 2000).

Defining reference conditions for suites of reference sites necessitates a classification system such that reference sites are grouped into homogeneous entities, with biological attributes from sites within a homogeneous entity being more similar to each other than to sites within a different homogeneous entity. In the case of reference conditions based on macroinvertebrates, this would imply that the macroinvertebrate assemblages at reference sites within a homogeneous group are more similar to one another than to macroinvertebrates at reference sites in a different homogeneous group. In this way, classification systems attempt to partition spatial variability characteristic of lotic systems, thereby producing a more efficient monitoring and assessment programme. Classification of sites into groups or classes within which ecological expectations are similar is considered integral to the use of regional reference sites (Gerritsen *et al*. 2000). There are essentially two approaches for classification of reference sites, a regional and a multivariate approach.

1.4.2 Approaches for deriving ecological reference conditions for riverine macroinvertebrates

The two approaches for classifying reference sites are fundamentally different even though

they begin with the same premise and require the same data (Reynoldson *et al*. 1997). The regional approach classifies reference sites *a priori*, based on geographic and physical attributes. Geographic regions, termed ecoregions, are predefined largely using mapped landscape characteristics such as climate, physiography, geology, soils and vegetation (Omernik 1987). This approach assumes that monitoring or test site characteristics match the chosen regional (e.g. ecoregional) reference sites (Reynoldson *et al*. 1997). Naturally occurring biotic assemblages, as components of the ecosystem, would be *expected* to differ among, for example, ecoregions, but to be relatively similar within a given ecoregion. The ecoregion concept thus provides a geographic framework for management of aquatic ecosystems and their components. Within an ecoregion, additional qualifiers such as stream size, hydrologic regime, elevation, and natural riparian vegetation need to be considered for further partitioning variability of macroinvertebrate assemblages within an ecoregion (Barbour *et al.* 1999). Metrics such as measures of richness (e.g. total number of taxa, number of Ephemeroptera taxa), composition [e.g. Ephemeroptera: Plecoptera: Trichoptera (EPT) ratio], tolerance/intolerance (e.g. % tolerant taxa, % dominant taxa), feeding (e.g. % filterers, % grazers/scrapers, etc.] and habit (e.g. % clingers) or indices [e.g. SASS4 Scores, Average Score Per Taxon (ASPT)] are then interpreted within the homogeneous regions. A metric is a calculated term or enumeration representing some aspect of biological assemblage structure, function, or other measurable characteristic that changes in some predictable way with increased human influence (Fausch *et al.* 1990 cited by Barbour *et al.* 1995, Gibson 1994 cited by Barbour *et al.* 1995). The regional approach is widely used in the United States (e.g. Gerritsen *et al.* 2000, Rabeni & Doisy 2000).

The multivariate approach classifies reference sites *a posteriori* using multivariate analysis of macroinvertebrate fauna (Reynoldson *et al*. 1997). It makes no *a priori* assumptions about the similarity of macroinvertebrate assemblages at different sites. Rather, faunal data are used to group sites that have similar taxonomic composition, thus providing an objective way of grouping reference sites with similar macroinvertebrate assemblages. Groups of sites do not necessarily conform to geographic stratification (Gerritsen *et al.* 2000). 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 (Reynoldson *et al*. 1997). A predicted or "expected" macroinvertebrate assemblage is compared with the actual assemblage and the ratio of observed/expected (*O/E*) families is used as a measure of ecological condition (Wright *et al*. 1993, Parsons & Norris 1996). The expected BMWP

(Wright 2000) or SIGNAL scores (Chessman 1995) for a monitoring site may then be calculated based on the expected taxa. Both the United Kingdom (Wright *et al*. 1993) and Australia (Smith *et al*. 1999) have adopted the multivariate approach within their bioassessment programmes, respectively RIVPACS (River Invertebrate Prediction and Classification System) and AusRivAS (Australian River Assessment System).

There has been much debate on the relative scientific validity of each approach and studies give support to both the ecoregion approach (e.g. Rabeni & Doisy 2000, Feminella 2000) and the multivariate approach (e.g. Marchant *et al.* 2000, Sanden & Johnson 2000, Van Sickle & Hughes 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 analysis of the biological data (e.g. Gerritsen *et al.* 2000, Johnson 2000). Johnson's (2000) study, of littoral macroinvertebrate assemblages from 363 lakes in Sweden, lends support to using ecoregions as a spatial framework for resource management. He suggests, however, that the low congruence between some ecoregion boundaries and the biota, indicate that another form of partitioning at the level of catchment or ecosystem-type is needed to further reduce spatial variability.

1.4.3 The South African approach

A regional approach has been adopted in South Africa, whereby a hierarchical spatial classification scheme sub-divides the country in a logical and ecologically-meaningful way so that variation between rivers in the country is best accounted for (Eekhout *et al*. 1996). The development of this spatial framework attempts to take biotic differences, resulting from climatological, geological, geomorphological and biogeographic differences amongst rivers, into account. The adoption of a regional approach rather than the more data-intensive multivariate approach is also the result of limited monetary and human resources within South Africa. However, as Eekhout *et al*. (1996) have stressed, the adoption of a regional approach does not preclude the eventual transference to a multivariate approach, whereas the opposite is true.

The three-tiered hierarchical spatial framework (Figure 1.1) developed for the classification of South African rivers includes an ecoregion level I or bioregion, a subregion (level II) and a river-type (level III). Level I, i.e. ecoregions or bioregions, is

currently in a state of flux with two different classifications being mooted. Ecoregions (Kleynhans *et al*. 1998a) are based on a top-down classification of South African rivers using landscape variables such as physiography, climate, geology and soils, together with potential natural vegetation (i.e. vegetation types that would have occurred were it not for the major anthropogenic transformations). Bioregions are based on biophysical conditions, derived by examining the biogeographic distribution patterns of riverine macroinvertebrates, fish and riparian vegetation (Eekhout *et al.* 1997) and physical characteristics of the rivers (Brown *et al*. 1996). The validity of each of these level I classifications has not yet been tested. The level II, subregional classification reflects broad geomorphological characteristics and distribution patterns of components of the biota. Rivers are longitudinally divided into the following zones: source zone, mountain headwater stream, mountain stream, foothill-cobble bed, foothill-gravel bed and lowland sand bed or lowland floodplain (Wadeson 1999). Three other geomorphological zones associated with a rejuvenated profile, namely upland flood plain, rejuvenated bedrock fall/cascade and rejuvenated foothills, were also proposed. Level III of the hierarchy aims to account for variation among rivers within a subregion or geomorphological zone and factors such as river size, hydrological type (ephemeral, seasonal or perennial), geomorphological characteristics (channel type, substratum composition) and other chemical and biological factors are considered. River size, for example, has been strongly related to taxonomic diversity of invertebrate assemblages with small streams (1^{st} order) being less taxonomically diverse than larger streams (4^{th} or 5^{th} order) (Minshall *et al.* 1985).

1.5 INTERPRETING BIOASSESSMENT DATA USING ECOLOGICAL REFERENCE CONDITIONS

Management action depends on the knowledge that a certain impact causes an aquatic assemblage or ecosystem to respond in some way that is outside the natural range of variation (Roux *et al.* 1999) and the ultimate objective of any bioassessment programme is to facilitate the detection of disturbance at a site as reflected by one or more components of the biota. Reference conditions facilitate this by defining what is expected at a site and provide a means of comparing observed conditions with expected conditions. This is a complex task and one that requires careful consideration of factors that may potentially affect data interpretation. Any reference condition is also likely to be a dynamic one, changing as our ecological understanding of the system grows (Meyer 1997).

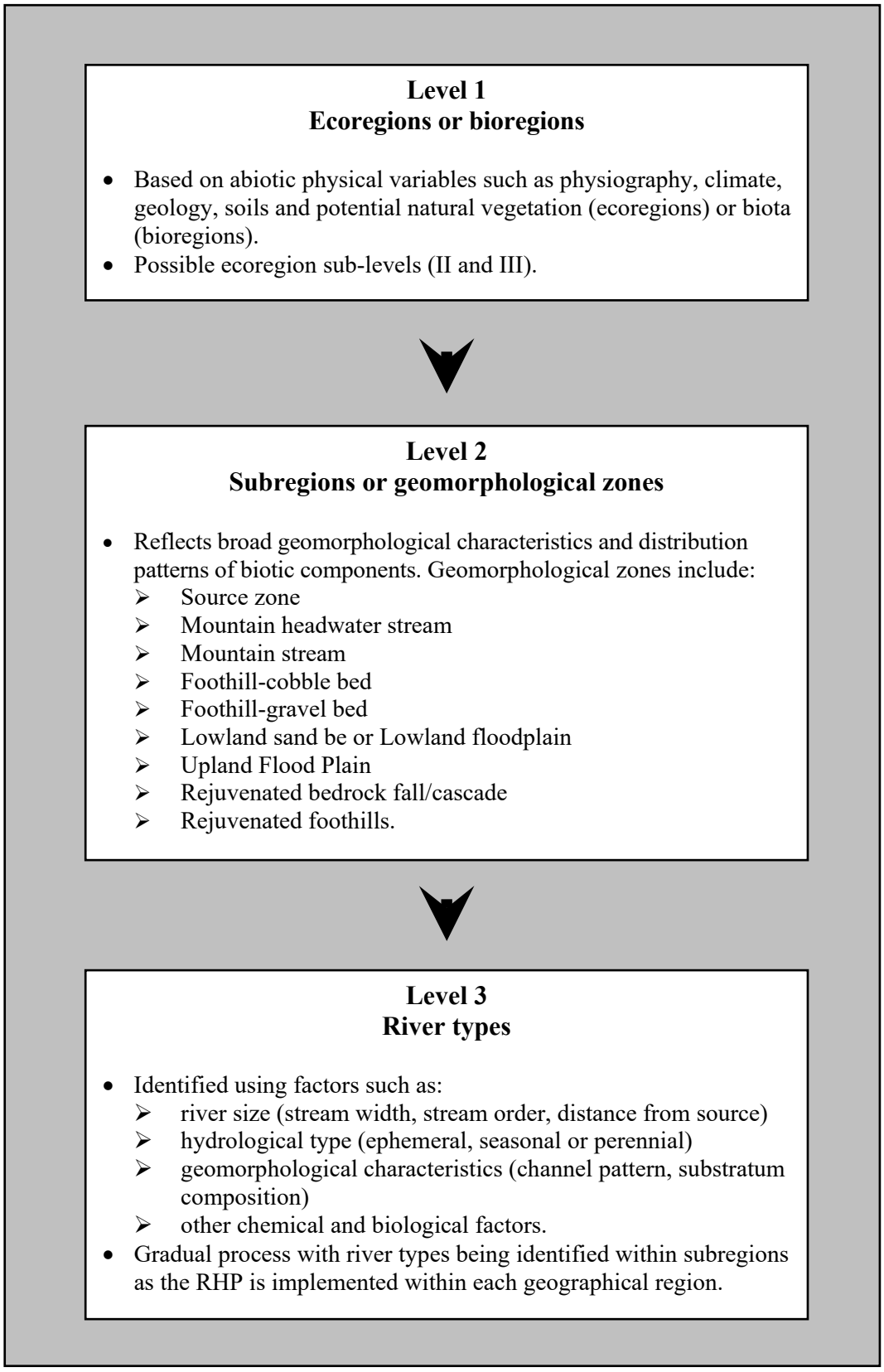


Figure 1.1 A three-tiered hierarchical spatial framework indicating the components incorporated at each level and, in the case of subregions, the different subregions.

Guidelines or biocriteria for the interpretation of environmental conditions with respect to established reference conditions have been formulated by several authors (e.g. Hughes 1995, Minns 1995) and, as already mentioned, range from simple interpretative tables to more complex predictive models. Minns (1995) proposed the following rule: "If any ecosystem is to retain the inherent capacity to return to its original state given the removal of all human alterations and stresses, any degree of change greater than 50% relative to the original state, is unlikely to be tolerated". Similarly, Hughes (1995) suggests that: (1) 90% of the reference condition is still high quality and perhaps within the range of natural and measurable variability, (2) 75% of the reference condition is still acceptable, (3) 50% - 75% of the reference condition could be considered marginal, and (4) less than 50% of the reference condition is unacceptable. These ranges are not based on actual data, but approximate the expected conditions in terms of deviation from reference. The reference condition is that derived for the particular suite of reference sites and is often based on calculated metrics or metrics combined into a composite index such as the Stream Condition Index used in Florida (Barbour *et al.* 1996). The sensitivity of the reference condition approach can be increased by modelling and explaining variation in the assemblage descriptor, e.g. number of taxa, among reference sites, and then using the predictive model to refine the expectation of the descriptor's value at a test or monitoring site (Bailey *et al*. 1998).

In predictive modelling systems such as RIVPACS (Wright *et al.* 1993, Wright 1995) and AusRivAS (Furse 2000, Simpson & Norris 2000), the use of "biological banding" systems with different bands representing different biological conditions, serves to simplify data interpretation and to aid management decisions. The severity of any environmental impact is assessed based on how much the number of taxa observed (O) deviates from the number expected (E), i.e. reference condition, calculated as the O/E ratio (Reynoldson *et al*. 1997). The ratios of the Observed/Expected (O/E) taxa and O/E ASPT are calculated and biological bands or ranges (X, A, B, C and D), which represent different levels of biological condition, are derived (Furse 2000, Simpson & Norris 2000). In this way, the calculation of O/E ratios at a monitoring site enables it to be assigned to a biological class.

The classes and band widths are based on percentiles calculated from groups of reference sites (Table 1.2) and are thus based on actual data that allow the intrinsic spatial and temporal variability to be incorporated.

Table 1.2 Division of O/E SASS4 Scores, O/E taxa and O/E ASPT, into five biological classes or "bands" for reporting the biological condition in South African rivers (Modified from the RIVPACS and AusRivAS banding system, Furse 2000, Simpson & Norris 2000).

Biological Class	Description	O/E SASS4 Score, O/E Taxa, O/E ASPT
X	**Richer than reference**: O/E greater than 90th percentile of reference sites	More taxa found than expected. SASS4 Score and ASPT greater than expected. Potential biodiversity "hot spot".
A	**Reference:** O/E within range of central 85% of reference sites (i.e. 5th to 90th percentiles)	SASS4 Score, number of taxa and ASPT within range of 85% of reference sites.
B	**Below reference:** O/E below 5th percentile of reference sites. Band width equal to median minus the 5th percentile.	Fewer taxa than expected. SASS4 Score and ASPT lower than expected. Potential impairment of water quality and/or habitat with loss of pollution-sensitive taxa.
C	**Well below reference:** O/E below Band B, same width as Band B.	Many fewer taxa than expected. SASS4 Score and ASPT much lower than expected. Substantial impairment of water quality and/or habitat. Major loss of pollution-sensitive taxa.
D	**Impoverished:** O/E below Band C to zero.	Few of the expected taxa remain. Severe impairment. Remaining taxa hardy and pollution-tolerant.

If variability within a group of reference sites is high the band width will be greater than if the variability within a group of reference sites was low. The inclusion of a band X in the system of Furse (2000) and Simpson & Norris (2000), allows sites of exceptional biodiversity to be identified, in that these sites will have O/E ratios greater than those of the reference site. For data interpretation, the final biological class given to a site is the median of the two or three individual bands, except when the band for ASPT is lowest, in which case the ASPT band takes precedence (Wright *et al.* 1993). Thus, if O/E SASS4 Score and O/E Taxa at a site are class B, but O/E ASPT is a class C, then the final biological class assigned to the site would be a class C. This rule has been devised because of the greater reliability of ASPT and because over-sampling and biotope availability are known to affect SASS Score (Wright *et al.* 1993, Dallas 1997).

1.6 A PROTOCOL FOR DERIVING ECOLOGICAL REFERENCE CONDITIONS FOR RIVERINE MACROINVERTEBRATES

A protocol for has been developed as a guide for biomonitoring practitioners in their endeavours to derive ecological reference conditions for riverine macroinvertebrates (Dallas 2000b). It was developed using data collected in the northern region of South Africa (Mpumalanga and Northern Province) and has not yet been tested in other regions in South Africa. The protocol developed, shown as a flow diagram (Figure 1.2), adopts a regional reference condition approach which also incorporates separate analyses of macroinvertebrate assemblages in an attempt to verify the spatial framework and to factor in potential variability resulting from physical, seasonal and habitat/biotope factors. Briefly, the processes are as follows. Details are available in Dallas (2000b).

1. Identification of homogeneous regions

A three-tiered hierarchical spatial framework has been developed in an attempt to identify homogeneous regions within which biomonitoring can be undertaken.

- *Level 1: Bioregions or ecoregions:* Bioregions (Brown *et al.* 1996) represent broad historical distribution patterns of riverine macroinvertebrates, fish and riparian vegetation (Eekhout *et al.* 1997), and which have been modified using local knowledge. Ecoregions are based on factors such physiography, climate, geology, soils and potential natural vegetation. At present, both level 1's are used since the suitability of one or the other with respect to biomonitoring and the RHP has not yet been established.
- *Level 2: Subregions or geomorphological zones* reflect broad geomorphological characteristics and distribution patterns of components of the biota. Rivers are longitudinally divided into the following zones: Source zone, Mountain headwater stream, Mountain stream, Foothill-cobble bed, Foothill-gravel bed and Lowland sand bed or Lowland floodplain (Wadeson 1999). Three other geomorphological zones associated with a rejuvenated profile, namely Upland Flood Plain, Rejuvenated bedrock fall/cascade and Rejuvenated foothills, were also proposed.
- *Level 3: River types* are identified using factors such as river size (e.g. stream width, stream order etc.), hydrological type (ephemeral, seasonal or perennial), geomorphological characteristics (channel pattern, substratum composition) and other chemical and biological factors.

Differentiation into Levels 1 and 2, i.e. ecoregions and subregions, is a map-based desktop exercise, whilst Level 3, i.e. river types, is undertaken at the ground truthing and data analysis stage.

2. Selection of " minimally-disturbed " sites

"Minimally-disturbed" or "least-impacted" sites, i.e. sites exposed to minimal anthropogenic influences, are identified using local knowledge, land-use maps and existing biomonitoring information.

3. Preliminary site screening and ground truthing

This phase involves assessing each site in the field. The geomorphological zones are confirmed and anthropogenic influences are checked by examining the surrounding land-use, channel, bed and bank modifications, and present status. Potential Level 3 river type factors are identified.

4. Sampling macroinvertebrates using SASS

SASS4 sampling is undertaken using the appropriate SASS protocol (Chutter 1998). For the purposes of deriving reference conditions, it is recommended that sampling be conducted in three seasons and that biotope-groups are sampled separately (i.e. stones-in-current/stones-out-of-current; marginal and aquatic vegetation; gravel/sand/mud). An assessment of the habitat is undertaken simultaneously (e.g. IHAS).

5. Measurement of environmental variables

Selected environmental variables are measured, including catchment (e.g. longitude, latitude, altitude, distance from source and stream order), site (channel pattern, stream width, habitat depths, geological type, vegetation type and canopy cover), habitat (substratum richness, composition and dominance, the percentage of each substratum type, percentage embeddedness, the number and combination of biotopes, the percentage of each biotope present, and the percentage cover of algae and macrophytes), and water chemistry variables (pH, temperature, conductivity, turbidity, dissolved oxygen and nutrients).

6. Classification of reference sites

Reference sites are classified into groups of sites on the basis of the similarity of their macroinvertebrate assemblages. Macroinvertebrate data from each of three seasons and all

three biotope-groups are combined for the analysis.

7. Identification of environmental variables which best discriminate between Reference Groups

Environmental variables are identified which best discriminated between groups of sites, termed "Groups". These variables are used to characterise each of the Groups in terms of catchment, site, habitat and water chemistry variables.

8. Verification of homogeneous regions

The validity of the spatial framework is examined by comparing the Groups with the identified homogenous regions.

9. Isolation of river type factors contributing to Group classification

Specific river type factors such as substratum type, which were considered significant in differentiating between Groups, were identified.

10. Assessment of the influence of biotope availability and sampling season

Comparing SASS Scores or macroinvertebrate assemblages from sites, with different biotopes available or which have been sampled in different seasons, may lead to misinterpretations. For this reason it is advised that the potential effect of both biotope availability and sampling season on macroinvertebrate assemblages and SASS Scores, be examined. Separate- versus combined-biotope sampling and single- versus multiple-season sampling is examined so that erroneous interpretation with respect to water quality or river health can be avoided.

11. Characterisation of Groups of sites

Each identified Group is characterised in terms of environmental variables, SASS Scores, expected SASS taxa and biotope considerations.

12. Comparison of monitoring site with reference condition

Following the standard sampling protocol, monitoring site data is compared with the appropriate reference condition. *Observed* (monitoring site) to *Expected* (reference condition) ratios are calculated and site is assigned to a biological band based on the *O/E* ratio.

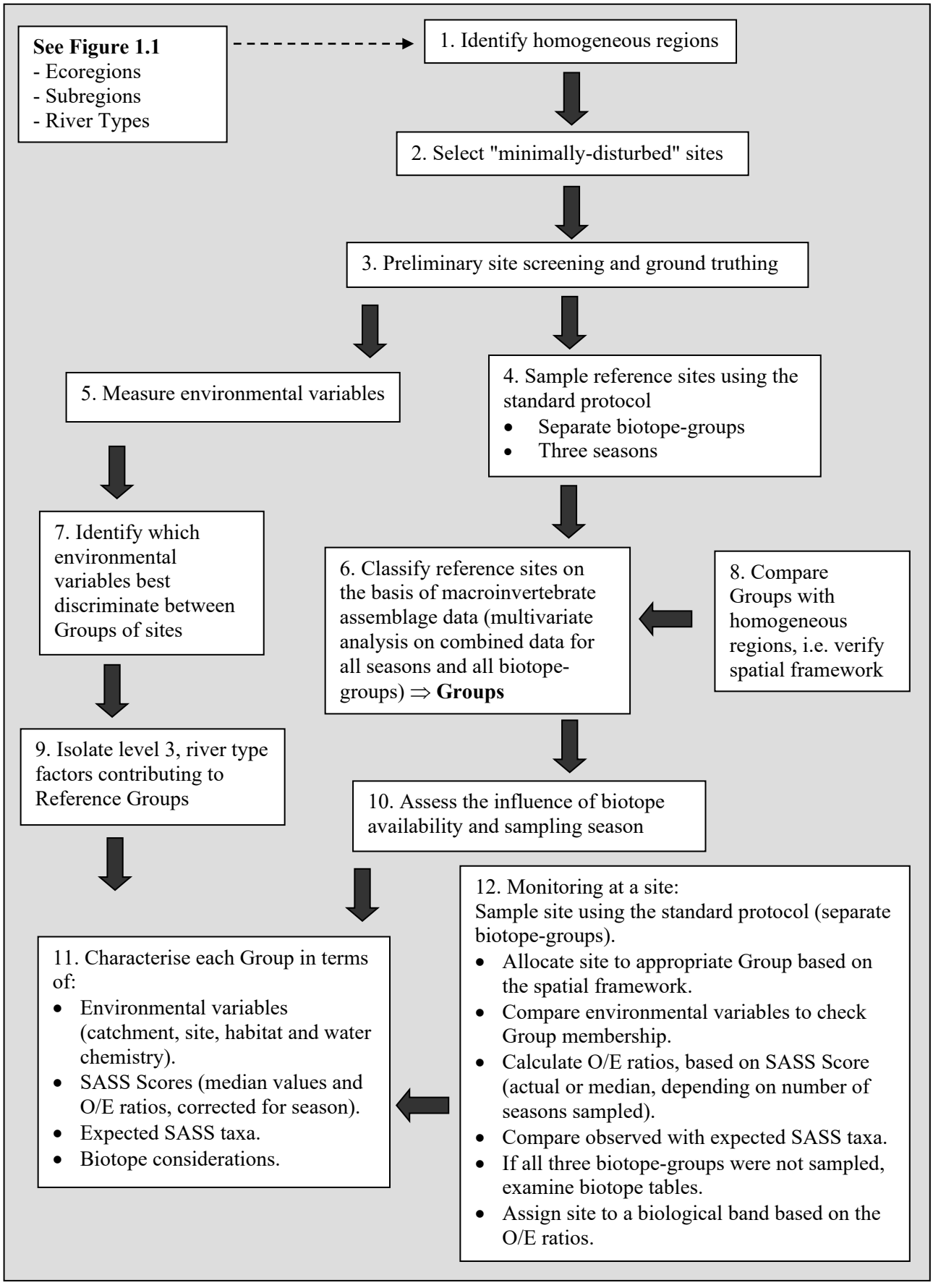


Figure 1.2 Suggested protocol for deriving ecological reference conditions for riverine macroinvertebrates and its use within the RHP (Dallas 2000b).

1.7 OBJECTIVES AND STRUCTURE OF THIS REPORT

This report is based on data collected in the Western Cape during two previous Water Research Commission (WRC)-funded projects. The first focused on the "effects of water quality variables on riverine ecosystems" (Dallas & Day 1993, Dallas *et al.* 1995) and the second on "the development of tools for assessing regional water quality guidelines" (Dallas *et al.* 1998). During these projects, research into and development of the key bioassessment tool used in South Africa, namely SASS4, was undertaken (Dallas 1995, Dallas 1997). A subsequent project, commissioned by the Department of Water Affairs and Forestry (DWAF), focused on the "derivation of ecological reference conditions for riverine macroinvertebrates" (Dallas 2000a, 2000b, Dallas & Fowler 2000, Fowler *et al.* 2000). This report, therefore, represents an amalgamation and analysis of data from each of these project, addressing specific objectives related to aquatic bioassessment and defining ecological reference conditions for riverine macroinvertebrates. Because sites were not selected and data not collected for the express purpose of these aims, the number and location of reference sites and the frequency of sampling was not optimal. In particular, the frequency of data collection varied substantially and different sub-sets of data have thus been selected to address specific aims in this study.

Aims of the project

- To test the protocol developed during an Institute for Water Quality Studies (IWQS, DWAF) project (Dallas 2000b) by applying it to another region, i.e. the Western Cape.
- To examine the spatial variability of macroinvertebrate assemblages between and within different ecoregions/bioregions, subregions and river-types, i.e. testing the validity of ecoregions/bioregions, subregions and river-types as units for defining homogenous regions and to discuss this variability in relation to establishing reference conditions.
- To examine the utilisation of SASS biotopes/habitats by macroinvertebrates and discuss implications with respect to the influence of biotope availability on Reference Condition SASS Scores and expected reference communities

- To examine temporal variability of macroinvertebrate assemblages and discuss implications for establishing reference conditions.
- To derive ecological reference conditions for macroinvertebrates for specific river types of the Western Cape.
- To examine methodological aspects of SASS4 with a view to incorporating abundances and verifying sensitivity/tolerance scores and to discuss implications for establishing reference conditions.

CHAPTER 2. MATERIALS AND METHODS

2.1 STUDY AREAS

Data presented in this report were collected from two geographic regions (Table 2.1) in South Africa: the Western Cape (Figure 2.1) and Mpumalanga (Figure 2.2). The two regions are separated by approximately 1300 km. The river name, site code, ecoregion level I (Kleynhans *et al.* 1998a), ecoregion level II (Kleynhans *et al.* 1998b), bioregion (Brown *et al.* 1996), subregion and latitude and longitude co-ordinates for each site are given in Table 2.1. Sub-sets of data were used for analysis of particular aspects within each chapter, details of which are provided in the relevant chapter.

Study sites were selected to represent minimally-disturbed or least-impacted conditions and are thus considered reference sites. These sites were exposed to minimal anthropogenic influences and criteria used to assess the level of disturbance included a qualitative assessment at the site of land-use, water quality impacts, flow modifications and physical alterations to the channel. Other factors, such as the variety of suitable biotopes for sampling, site accessibility and safety during sampling operations, were considered. Experience has shown that the selection of reference sites is an iterative process, with some potential reference sites discarded at the "ground-truthing" phase, and others excluded on the basis of subsequent data analysis. In such instances, it does not necessarily imply that the site is disturbed, although this may indeed be the case, rather, the outlying site may be representative of a different suite of reference sites. Monitoring sites, in addition to reference sites, were selected and sampled to represent a range of disturbance.

It should be reiterated that data used in this report were not collected specifically for this study, but rather collected during the course of three research projects undertaken between 1994 and 2000. The number and location of reference sites selected and the frequency of sampling was therefore not optimal. In Mpumalanga reference sites were selected and sampled within the context of a study aimed at defining ecological reference conditions for rivers in this region. This dataset therefore comprises a range of sites covering a broad geographic region at which sampling was temporally replicated in a structured manner. Sites in the Western Cape were selected and sampled with objectives, other than defining

reference conditions, in mind. In particular, the frequency of data collection varied substantially and different sub-sets of data have thus been selected to address specific aspects of the study. Data from both Mpumalanga and the Western Cape, have, however, provided an opportunity to explore the extent to which spatial and temporal heterogeneity are a feature of lotic systems in both the Western Cape and Mpumalanga. The data provide a means of assessing the implications of the intrinsic variability of lotic systems on defining reference conditions for macroinvertebrates.

2.1.1 Western Cape

The Western Cape has a Mediterranean climate with hot, dry summers and cool, rainy winters (Walton 1994). The mountains generally comprise hard, resistant, quartzitic sandstones of the Table Mountain Group (Figure 2.3, Vegter 1995) and water flowing over such strata is characteristically acidic and low in nutrients and dissolved solids. The lower lying regions often fall within the Malmesbury or Bokkeveld Groups, comprising largely shales. Water flowing over these formations tends to be higher in dissolved solids. River water in the Western Cape region is considered to be sodium- and chloride-dominated (Day & King 1995). The upper catchments are often dominated by sclerophyllous fynbos vegetation (Figure 2.3), which leaches out the humic substances that give many rivers their distinctive dark brown colour. Potential natural vegetation in the lowland regions is largely renosterveld (Low & Rebelo 1996), although much of this vegetation has been removed through agricultural activities. The climatic, geological, geomorphological and vegetation characteristics of the Western Cape have contributed to the high degree of endemism in the aquatic invertebrate fauna of this region (Harrison & Agnew 1962). The acid stream fauna of the upper catchments largely belong to the old Element, commonly called the palaeoendemics and referred to as the South Temperate Gondwanian fauna (Harrison 1978), and are essentially restricted to perennial systems in high rainfall areas.

In the Western Cape, selection of reference sites in upper catchments was comparatively easy, but became progressively more difficult in the lower reaches of the catchment, where the cumulative effects of all upstream and adjacent disturbances are experienced, and in some instances reference sites represent "best-available" conditions. Where possible, sites were selected in protected areas such as nature reserves or in areas with restricted access such as forestry reserves. Most lowland disturbances were linked to agricultural practices,

and some downstream, minimally-disturbed reaches may have been affected by upstream activities. Forty reference sites distributed on 28 rivers and forty disturbed sites, representing test or monitoring sites, distributed on 22 rivers were sampled with variable frequency between 1994 and 1996. An additional reference site and additional monitoring sites were sampled in 2000/1, together with repetitive sampling of a few reference and monitoring sites. Details pertaining to the sampling frequency of each site are provided in Appendix A. The monitoring sites and replicated assessments were used in data validation (Chapter 7).

2.1.2 Mpumalanga

Mpumalanga spans two climatic regions, the Plateau Slopes that has warm, wet summers and cool, dry winters and the Subtropical Lowveld that has hot, wet summers and warm, dry winters (Walton 1994). Study sites were distributed over three distinct physiographic regions: the high interior plateau, characterised by cool temperatures (10-18ºC) and high rainfall (400 to 1000 mm per year); the escarpment zone with temperatures ranging from 10 to 22ºC and rainfall from 600 to 1200 mm per year; and the low-lying, drier region, with an annual average temperature of 22ºC and drier conditions with rainfall between 400-600 mm per year. These regions also broadly correspond to three vegetation biomes, namely grasslands, found at higher altitudes on plateaus and slopes, patches of afromontane forest on the escarpment, and savanna or bushveld, which is dominant in the lower plains (Figure 2.4). River water in this region is dominated by calcium, magnesium and bicarbonate (Day & King 1995) and reflects fairly complex geological formations (Figure 2.4). The invertebrate fauna is part of the Pan-Ethiopian Afrotropical group (Harrison 1978), and comprises three sub-groups: widespread, hardy species, often associated with marginal vegetation habitats; highveld, temperate species characteristic of the elevated "highveld" or central highland regions of Mpumalanga; and tropical or warm stenothermal species, which has extended southwards from Central Africa into the low-lying regions of Mpumalanga (Harrison 1965b).

In Mpumalanga, upper-catchment areas were largely affected by afforestation, whilst several lowland sites were situated in the protected reserve of the Kruger National Park. Some of these sites were, however, still exposed to upstream disturbance, particularly disturbances resulting from agricultural and forestry practices. Seventy-four potential

reference sites were initially assessed and screened for anthropogenic influences. Of these, fifty-seven reference sites, distributed on 34 rivers, were sampled in May, July and September 1999.

2.2 SAMPLING METHODS

2.2.1 Benthic macroinvertebrates: SASS4 sampling

Benthic macroinvertebrates were sampled using the qualitative rapid bioassessment method, SASS4 (South African Scoring System). A kick net (300x300 mm frame, 950 µm-mesh) was held immediately downstream of the area to be sampled. All available SASS-biotopes, namely stones-in-current (SIC), stones-out-of-current (SOOC), aquatic or instream vegetation (AQV), marginal vegetation (MV), gravel (G), sand (S) and mud (M), were sampled, either separately or per biotope-group, i.e. by combining particular biotopes as follows: SIC + SOOC, AQV + MV and G + S + M. These combined SASS-biotope groups are referred to as SASS biotope-groups. SIC were kicked for approximately two minutes if all were loose and for five minutes if some were immovable. Loose substratum was agitated and dislodged organisms were collected downstream in the net. The SOOC biotope was sampled by kicking an area approximately 1 m^2 area and dislodged organisms collected by sweeping the net over the stones. Aquatic and marginal vegetation were swept for approximately 2 metres, and gravel, sand and mud were stirred and swept for 30 seconds. The contents of the net were tipped into a large sorting tray, debris was removed, and organisms were identified to the SASS taxonomic level, mostly family, and their abundance groupings recorded (A: 1-10, B: 11-100, C: 101-1000, D: > 1000 individuals). All taxa identified in a SASS sample are referred to as SASS-taxa. Once the sample(s) has been collected, the tray was searched for the shorter of either 20 minutes or until five minutes had passed since an additional family had been found. If SASS biotope-groups, i.e. SIC/SOOC, AQV/MV and GSM, were sampled separately, identification was carried out for each biotope-group.

The family-level of taxonomic resolution is used because the SASS method is designed to be a rapid, field-based method, so that identifications to genus or species is not feasible. Taxonomy at the levels of genus and species is in a state of flux in South Africa and many taxa have yet to be described. Wright *et al.* (1998a) showed a strong correlation between the number of BMWP (equivalent to SASS) families and the number of species using data from

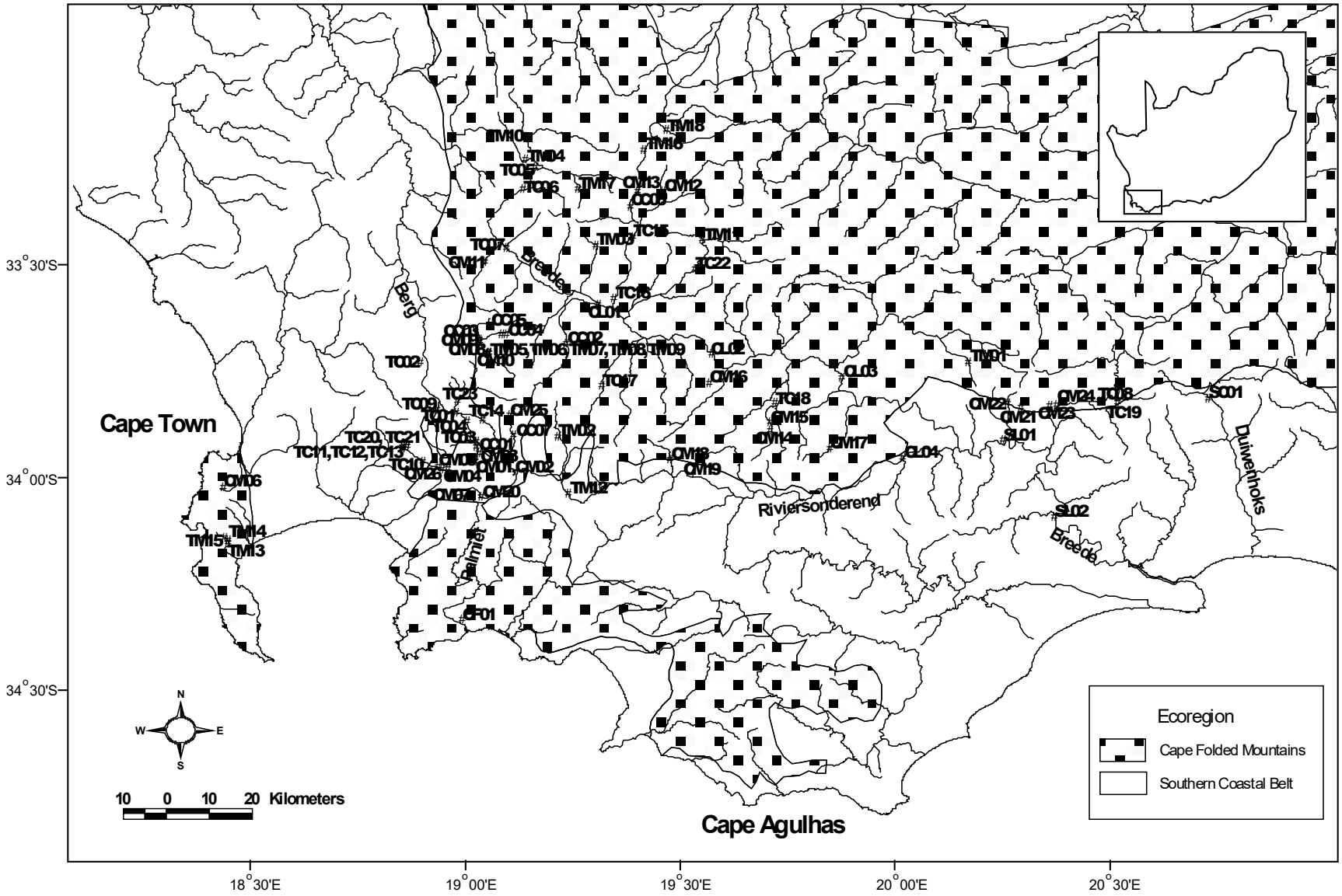


Figure 2.1 **Location of study sites in the Western Cape. Reference sites are coded with a primary code, indicating ecoregion (C = Cape Fold Mountains, S = Southern Coastal) and secondary code, indicating subregion [M = mountain stream, C = foothill-cobble bed, F = rejuvenated foothill and L = Lowland floodplain]. Monitoring sites are prefaced with a "T", i.e. test sites.**

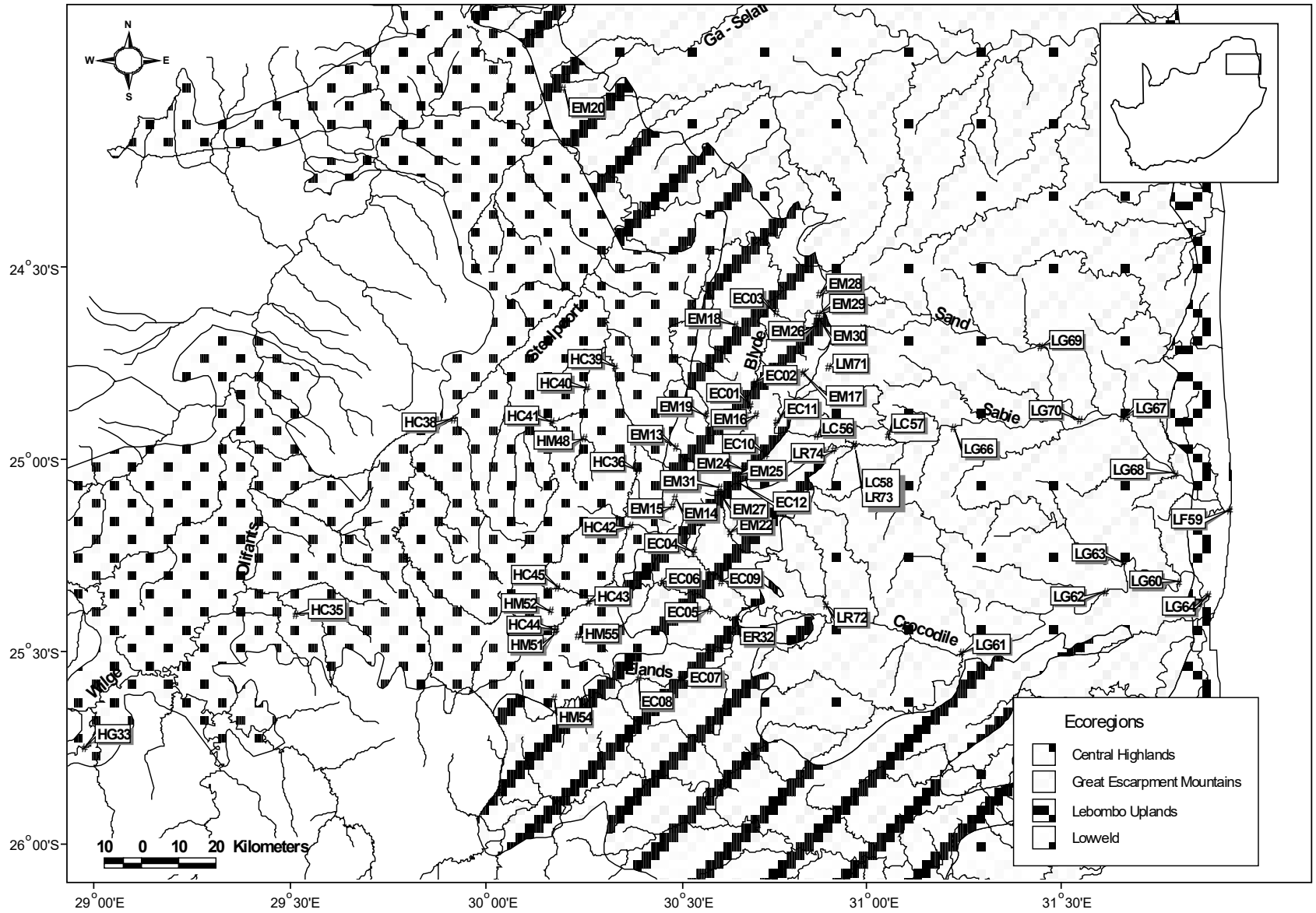


Figure 2.2 **Location of study sites in Mpumalanga. Reference sites are coded with a primary code, indicating ecoregion (E = Great Escarpment Mountain, H = Central Highlands, L = Lowveld) and secondary code, indicating subregion [M = mountain stream, C = foothill-cobble bed, G = foothill-gravel bed, R = rejuvenated cascade, F = rejuvenated foothill].**

Table 2.1. List of sites assessed during this study. Region (WC = Western Cape, MPU = Mpumalanga), **River name, Site code** (Reference site coding has been standardised using a primary and secondary code. Primary codes are based on ecoregion Level I, whilst secondary codes are based on subregions within which the site falls. Monitoring or test sites prefaced with a "T" followed by the subregion code in which they occur), **Ecoregion Level I** (C = Cape Fold Mountains, S = Southern Coastal, E = Great Escarpment Mountains, H = Central Highlands, L = Lowveld and U = Lebombo Uplands), **Ecoregion Level II** (Mpumalanga only), **Bioregion** (F = Fynbos, S = Southern Coastal, N = Northern Uplands, B = Bushveld Basin, L = Lowveld), **Subregion** (M = mountain stream, C = foothill-cobble bed, G = foothill-gravel bed, L = lowland floodplain, R = rejuvenated cascade, F = rejuvenated Foothill) and **GIS co-ordinates** (latitude and longitude). Codes in parenthesis relate to sites reassigned in Chapter 3 (Section 3.4.3).

Region	River Name	Site Code	Ecoregion Level I	Ecoregion Level II	Bioregion	Subregion	GIS latitude	GIS longitude
WC	Assegaaibosch	CM01	C	-	F	M	-33.96944	19.07778
WC	Berg	CM02	C	-	F	M	-33.97778	19.06667
WC	Berg	CM03	C	-	F	M	-33.98611	19.06806
WC	Eerste	CM04	C	-	F	M	-33.99444	18.99444
WC	Lang	CM05	C	-	F	M	-33.98750	18.97222
WC	Window Stream	CM06	C	-	F	M	-33.97778	18.42778
WC	Palmiet	CM07	C	-	F	M	-34.05833	19.04167
WC	Elandspad	CM08	C	-	F	M	-33.76111	19.12778
WC	Elandspad	CM09	C	-	F	M	-33.73333	19.11500
WC	Kraalstroom	CM10	C	-	F	M	-33.76111	19.13333
WC	Wit	CM11	C	-	F	M	-33.56667	19.15000
WC	Rooiels	CM12	C	-	F	M	-33.45833	19.61667
WC	Unspecified	CM13	C	-	F	M	-33.45278	19.55278
WC	Houtbaais	CM14	C	-	F	M	-33.99167	19.81667
WC	Houtbaais	CM15	C	-	F	M	-33.97500	19.81944
WC	Rietvlei	CM16	C	-	F	M	-33.87778	19.67917
WC	Boesmans	CM17	C	-	F	M	-34.04306	19.96389
WC	Baviaans	CM18	C	-	F	M	-34.02917	19.55833
WC	Boesmanskloof	CM19	C	-	F	M	-34.04083	19.62500
WC	Riviersonderend	CM20	C	-	F	M	-34.06389	19.07083
WC	Duiwelsbos	CM21	C	-	F	M	-33.99861	20.45833
WC	Hermitage	CM22	C	-	F	M	-33.98750	20.42500
WC	Meulkloof	CM23	C	-	F	M	-34.00333	20.52861
WC	Grootkloof	CM24	C	-	F	M	-34.00139	20.54944
WC	Perdekloof	CM25	C	-	F	M	-33.89722	19.16806
WC	Swartboskloof	CM26	C	-	F	M	-33.99444	18.99444
WC	Berg	CC01	C	-	F	C	-33.95556	19.07361
WC	Holsloot	CC02	C	-	F	C	-33.75833	19.32917
WC	Molenaars	CC03	C	-	F	C	-33.73056	19.11250
WC	Molenaars	CC04	C	-	F	C	-33.72500	19.18333
WC	Molenaars	CC05	C	-	F	C	-33.72333	19.17028
WC	Sanddriftskloof	CC06	C	-	F	C	-33.48333	19.52917
WC	Dutoits	CC07	C	-	F	C	-33.94167	19.17083
WC	Duiwenshoek	SC01	S	-	S	C	-34.02083	20.93333
WC	Palmiet	CF01	C	-	F	F	-34.31900	18.98500
WC	Breede	CL01	C	-	F	L	-33.68417	19.42194
WC	Breede	CL02	C	-	F	L	-33.81667	19.69167
WC	Breede	CL03	C	-	F	L	-33.89583	20.01250
WC	Riviersonderend	CL04	C	-	F	L	-34.07917	20.14583
WC	Breede	SL01	S	-	F	L	-34.05000	20.40417
WC	Breede	SL02	S	-	F	L	-34.24028	20.51250

Region	River Name	Site Code	Ecoregion Level I	Ecoregion Level II	Bioregion	Subregion	GIS latitude	GIS longitude
WC	Dwarriega	TM01	C	-	F	M	-33.89167	20.33750
WC	Elandskloof	TM02	C	-	F	M	-33.95417	19.28111
WC	Hartebees	TM03	C	-	F	M	-33.55833	19.43333
WC	Koekoedou	TM04	C	-	F	M	-33.35889	19.29000
WC	Kraalstroom	TM05	C	-	F	M	-33.76111	19.13333
WC	Kraalstroom	TM06	C	-	F	M	-33.76111	19.13333
WC	Kraalstroom	TM07	C	-	F	M	-33.76111	19.13333
WC	Kraalstroom	TM08	C	-	F	M	-33.76111	19.13333
WC	Kraalstroom	TM09	C	-	F	M	-33.76111	19.13333
WC	Modder	TM10	C	-	F	M	-33.31167	19.28361
WC	Raaswater	TM11	C	-	F	M	-33.56989	19.69992
WC	Riviersonderend	TM12	S	-	F	M	-34.07750	19.29083
WC	Silvermine	TM13	C	-	F	M	-34.09481	18.42200
WC	Silvermine	TM14	C	-	F	M	-34.08400	18.41500
WC	Silvermine	TM15	C	-	F	M	-34.09114	18.42200
WC	Spekriviersкloof	TM16	C	-	F	M	-33.36667	19.58056
WC	Vals	TM17	C	-	F	M	-33.43472	19.40472
WC	Valsgat	TM18	C	-	F	M	-33.32917	19.64167
WC	Berg	TC01	S	-	F	C	-33.87917	19.03333
WC	Berg	TC02	S	-	F	C	-33.76389	18.95833
WC	Berg	TC03	S	-	F	C	-33.94306	19.07500
WC	Berg	TC04	S	-	F	C	-33.90000	19.04444
WC	Breede	TC05	C	-	F	C	-33.37917	19.30417
WC	Breede	TC06	C	-	F	C	-33.42083	19.26667
WC	Breede	TC07	C	-	F	C	-33.54111	19.20694
WC	Buffelsjag	TC08	C	-	F	C	-34.00417	20.65833
WC	Dwars	TC09	S	-	F	C	-33.86806	18.98611
WC	Eerste	TC10	S	-	F	C	-33.97222	18.93472
WC	Eerste	TC11	S	--	F	C	-33.94028	18.89167
WC	Eerste	TC12	S	-	F	C	-33.93889	18.88889
WC	Eerste	TC13	S	-	F	C	-33.93889	18.88750
WC	Franschhoek	TC14	S	-	F	C	-33.90139	19.08889
WC	Hex	TC15	C	-	F	C	-33.54839	19.52672
WC	Hex	TC16	C	-	F	C	-33.67500	19.46389
WC	Hoeks	TC17	C	-	F	C	-33.85833	19.40833
WC	Keisers	TC18	C	-	F	C	-33.93333	19.83750
WC	Kruis	TC19	C	-	F	C	-34.00833	20.70278
WC	Lanzerac	TC20	S	-	F	C	-33.93611	18.90000
WC	Lanzerac	TC21	S	-	F	C	-33.93611	18.90000
WC	Nuy	TC22	C	-	F	C	-33.63056	19.67500
WC	Wemmers	TC23	S	-	F	C	-33.85417	19.03889
MPU	Blyde	EC01	E	E 3	N	C	-24.93298	30.74823
MPU	Blyde	EC02	E	E 3	N	C	-24.87775	30.76038
MPU	Treu	EC03	E	E 3	N	C	-24.70900	30.81800
MPU	Blystaanspruit	EC04	E	E 3	N	C	-25.28752	30.59537
MPU	Crocodile	EC05	E	E 2	N	C	-25.43000	30.64000
MPU	Crocodile	EC06	E	E 3	N	C	-25.36198	30.51085
MPU	Elands	EC07	E	E 2	N	C	-25.59900	30.56000
MPU	Elands	EC08	E	E 1 (2)	N	C	-25.59735	30.44694
MPU	Houtbosloop	EC09	E	E 3	N	C	-25.36085	30.66873
MPU	Klein-Sabie	EC10	E	E 3	N	C	-25.05358	30.79130
MPU	Mac-Mac	EC11	E	E 4	N	C	-24.97330	30.81650
MPU	Sabie	EC12	E	E 3	N	C	-25.12100	30.71700

Region	River Name	Site Code	Ecoregion Level I	Ecoregion Level II	Bioregion	Subregion	GIS latitude	GIS longitude
MPU	Spekboom	EM13	E	E 3	N	M	-25.03360	30.54815
MPU	Sterkspruit	EM14	E	E 3	N	M	-25.15830	30.54610
MPU	Unspecified	EM15	E	E 3	N	M	-25.17694	30.54028
MPU	Grootfonteinspruit	EM16	E	E 3	N	M	-24.95433	30.76328
MPU	Heddle	EM17	E	E 3	N	M	-24.85367	30.89008
MPU	Kgwete	EM18	E	E 3	N	M	-24.73903	30.71078
MPU	Ohrigstad	EM19	E	E 3	N	M	-24.95400	30.63100
MPU	Ga-Selati	EM20	E	E 3	N	M	-24.16100	30.25400
MPU	Nelspruit	EM22	E	E 3	N	M	-25.24355	30.69408
MPU	Unspecified	EM24	E	E 3	N	M	-25.08517	30.72875
MPU	Lone Creek	EM25	E	E 3	N	M	-25.10413	30.71333
MPU	Mohlomobe	EM26	E	E 4	N	M	-24.74000	30.92100
MPU	Sabie	EM27	E	E 3	N	M	-25.14700	30.66800
MPU	Unspecified	EM28	E	E 4	N	M	-24.66252	30.93277
MPU	Sand	EM29	E	E 4	N	M	-24.71148	30.93017
MPU	Unspecified	EM30	E	E 4	N	M	-24.72502	30.93418
MPU	Unspecified	EM31	E	E 3	N	M	-25.13258	30.66647
MPU	Crocodile	ER32	E	E 2	N	R	-25.44900	30.71000
MPU	Wilge	HG33	H	H 9	N	G	-25.75100	28.95800
MPU	Selon	HC35	H	H 11	B (N)	C	-25.43373	29.52858
MPU	Dorps	HC36	H	H 11	N	C	-25.08917	30.44889
MPU	Klip	HC38	H	H 12	N	C	-24.96242	29.95533
MPU	Spekboom	HC39	H	H 12	N	C	-24.83877	30.38900
MPU	Waterval	HC40	H	H 12	N	C	-24.89133	30.31068
MPU	Waterval	HC41	H	H 11	N	C	-24.97008	30.21780
MPU	Alexanderspruit	HC42	H	H 11	N	C	-25.22558	30.42723
MPU	Crocodile	HC43	H	H 11	N	C	-25.40900	30.31600
MPU	Elandsfonteinspruit	HC44	H	H 11	N	C	-25.47900	30.22700
MPU	Lunsklip	HC45	H	H 11	N	C	-25.37400	30.23000
MPU	Unspecified	HM48	H	H 11	N	M	-25.01300	30.30638
MPU	Elandsfonteinspruit	HM51	H	H 11	N	M	-25.48233	30.22523
MPU	Kareekraalspruit	HM52	H	H 11	N	M	-25.43100	30.21100
MPU	Tautesloop	HM54	H	H 11	N	M	-25.64300	30.22000
MPU	Wilgekraalspruit	HM55	H	H 11	N	M	-25.49230	30.28573
MPU	Mac-Mac	LC56	L	L 5	N	C	-25.00800	30.92600
MPU	Marite	LC57	L	L 5	L	C	-25.00813	31.11465
MPU	Sabie	LC58	L	L 6	N	C	-25.03000	31.02700
MPU	Sabie	LF59	U (L)	U 1 (L 7)	L	F	-25.18500	32.03100
MPU	Crocodile	LG60	L	L 7	L	G	-25.36100	31.89500
MPU	Crocodile	LG61	L	L 5	L	G	-25.53600	31.31200
MPU	Crocodile	LG62	L	L 6	L	G	-25.38600	31.70000
MPU	Crocodile	LG63	L	L 6	L	G	-25.31500	31.74900
MPU	Crocodile	LG64	U (L)	U 1 (L 7)	L	G	-25.39100	31.97600
MPU	Sabie	LG66	L	L 6	L	G	-24.98889	31.28940
MPU	Sabie	LG67	L	L 6	L	G	-24.96300	31.74300
MPU	Sabie	LG68	L	L 7	L	G	-25.09900	31.88600
MPU	Sand	LG69	L	L 6	L	G	-24.79100	31.52300
MPU	Sand	LG70	L	L 6	L	G	-24.96700	31.62700
MPU	Maritsane	LM71	L	L 5	N	M	-24.84000	30.95600
MPU	Nelspruit	LR72	L	L 5	N	R	-25.42128	30.95155
MPU	Mac-Mac	LR73	L	L 5	N	R	-25.03000	31.02600
MPU	Sabie	LR74	L	L 5	N	R	-25.04043	30.97095

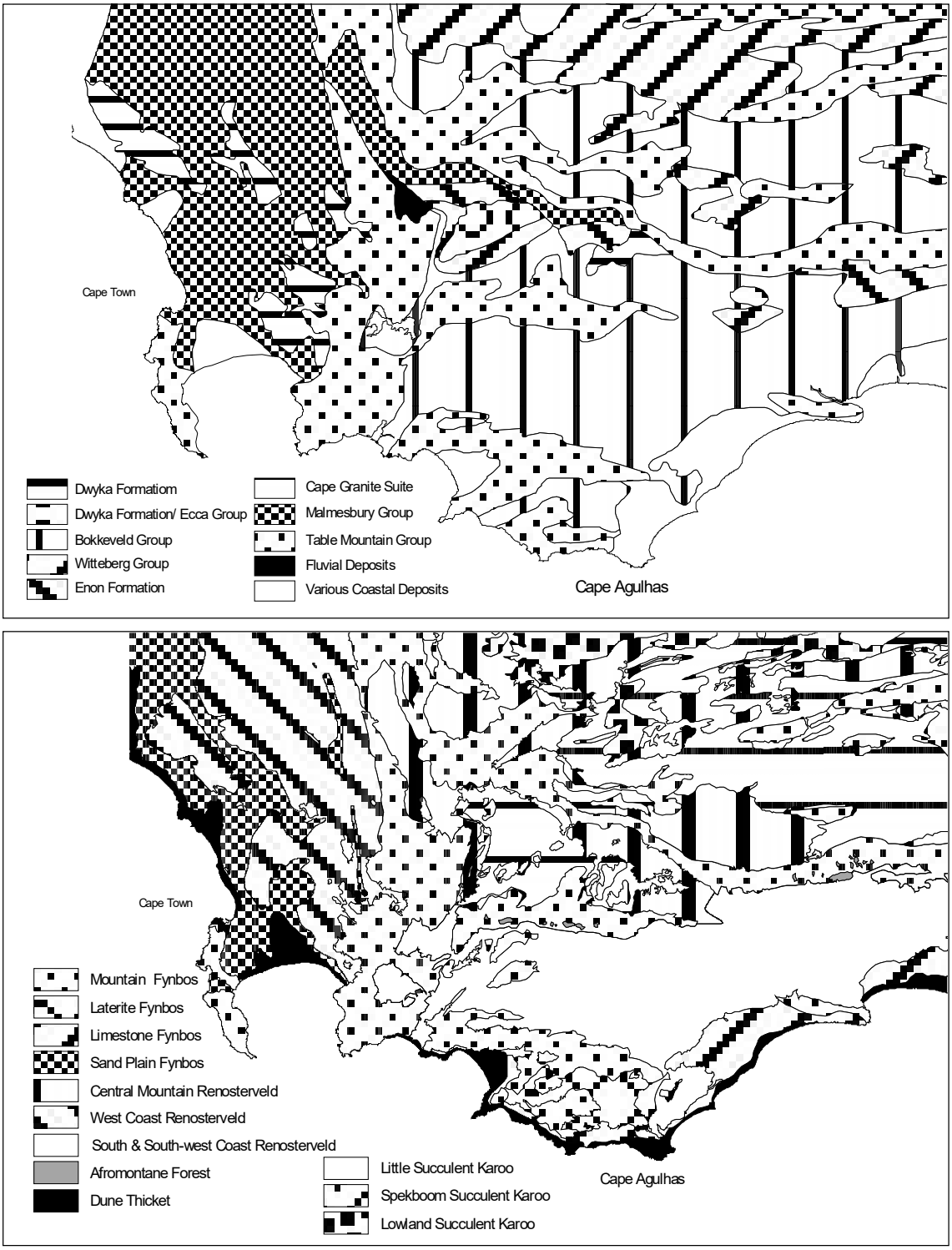


Figure 2.3 Geological map with simplified lithostratigraphy (from Vegter 1995) and potential natural vegetation (from Low & Rebelo 1996) for the Western Cape region.

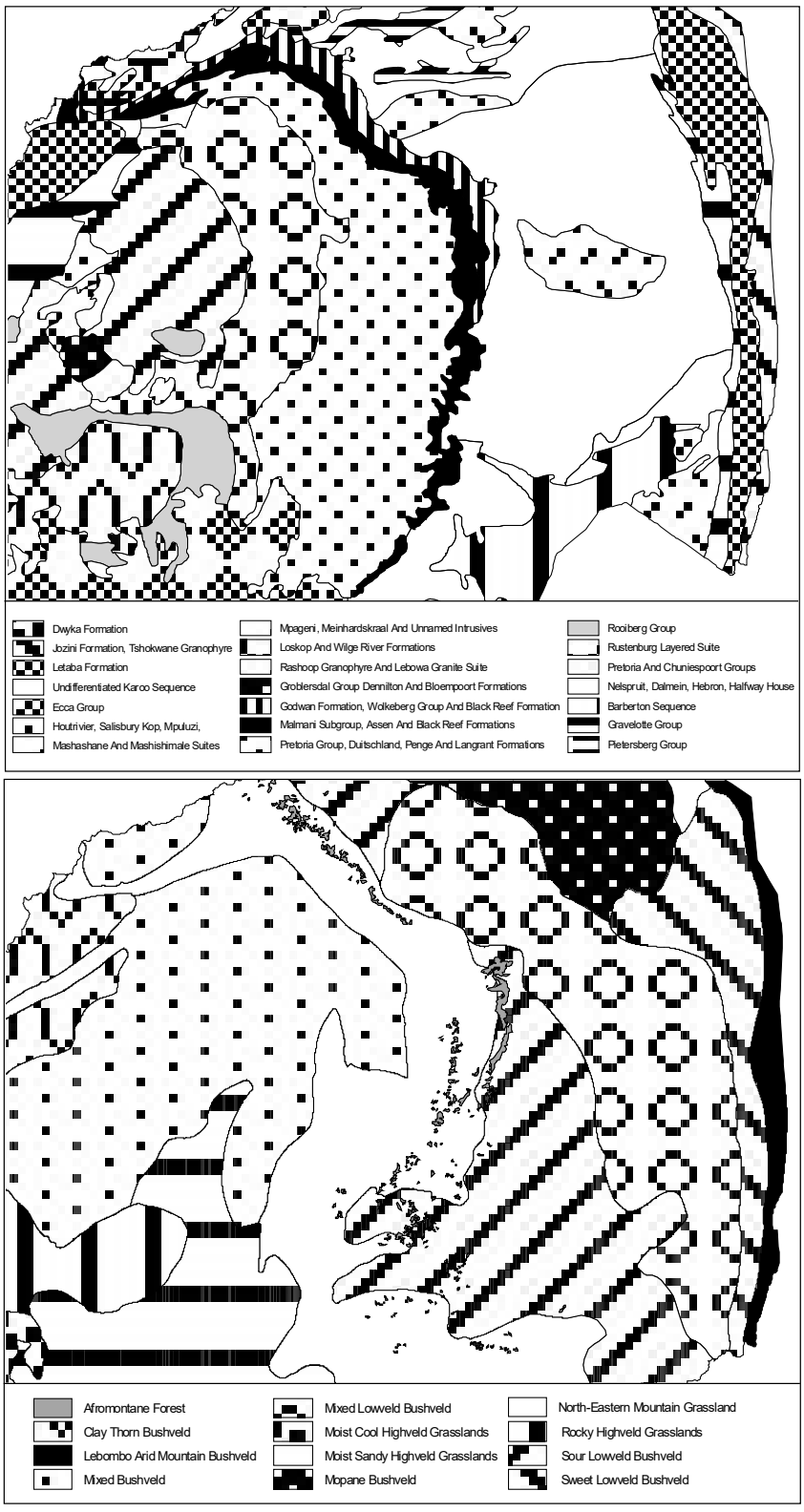


Figure 2.4 Geological map with simplified lithostratigraphy (from Vegter 1995) and potential natural vegetation (from Low & Rebelo 1996) for the Mpumalanga region.

614 sites in the United Kingdom. Species- and family-level data also produced similar longitudinal distribution patterns of macroinvertebrate assemblages down the Rhone River, France (Bournard *et al.* 1996). Marchant *et al.*'s (1995) findings, which showed that family-level identification provided adequate discrimination from which to group sites based on their macroinvertebrate assemblages, led the Australian River Assessment Scheme (AusRivAS) programme to use family-level taxonomic data (Smith *et al*. 1999). Guerold (2000), however, cautioned on the use of family-level data since this taxonomic level led to underestimation of all indices tested, including the Shannon's diversity index, Margalef's diversity index and the I-Simpson's index. For example, the Shannon diversity index at species-level was always higher than index values from genus or family-level. He suggested though that, if the purpose of a study was to detect an impact of a disturbance on macroinvertebrate assemblages, then determination to family-level may be adequate. Wells (1992), however, in a study on the Sabie-Sand river system in South Africa, found, that there was no significant difference in diversity (Shannon's diversity index) between genus and family level. In South Africa, moreover, given the uncertainty of the taxonomy of many invertebrate groups, species or genus-level identifications may be less consistent and accurate with a higher probability of error than family-level identifications (Voshell *et al.* 1997). SASS has been shown to differentiate adequately between sites with different water quality in a way comparable to a protocol involving intensive box-sampling and laboratory sorting (Dallas 1995). On the basis of these studies, and the limitations of the field-based sampling strategy, family level data were considered to be adequate for the purposes of bioassessment used in this study.

SASS was developed for use in riverine ecosystems (Chutter 1998) and is based on the Biological Monitoring Working Party (BMWP) method of the United Kingdom. Each macroinvertebrate taxon, mostly at family level, is given a score based on its sensitivity/tolerance to water quality impairment. These scores range from 1 for a tolerant taxon, to 15 for a sensitive taxon. In SASS4 (Version 4), the version used in this study, two families have been assigned a sliding scale of scoring because of the presence of both tolerant and sensitive species within them. These are Baetidae (Ephemeroptera) and Hydropsychidae (Trichoptera) and scores range from 4, for one type, 6 for two types and 12 for three types. A third sliding scale is incorporated for the cased-caddisfly trichopterans which are grouped together and scored of the basis of the number of types of cases present in the sample, as follows: one type scores 8, two types score 15 and three types score 20. A SASS- taxon

therefore refers to each taxon or "type" that has a distinct sensitivity/tolerance score. Examples of SASS-taxa are Oligochaeta, Baetidae 3 Types, Leptophlebiidae and Trichoptera (cased caddis 1 Type). Once a site has been sampled and each taxon recorded, the scores are summed to give a SASS4 Score, the number of taxa is counted, and an Average Score per Taxon (ASPT) value is calculated by dividing SASS4 Score by the number of taxa.

Recently, a new version of SASS has been developed, namely SASS5 (Version 5). The sensitivity/tolerance scores of certain of the taxa have been adjusted to better reflect their known tolerance to water quality impairment using empirical SASS4 data collected over the last six years. A few additional taxa have also been added and cased-caddisfly trichopterans are now included as separate families rather than as a group-type, i.e. families such as Barbarochthonidae, Leptoceridae, and Sericostomatidae are no longer lumped together and recorded as "Trichoptera (cased caddis 3 Types)".

2.2.2 Physical attributes and chemical constituents

A variety of physical attributes and chemical constituents of the water were measured at each site. Instruments and analytical methods varied to some extent within and between geographic regions. In the Western Cape in 1994 and consistently in Mpumalanga, *in situ* measurements were made of temperature using a mercury thermometer (accurate to ± 0.5 ºC), conductivity using a Crison CDTM-523 conductivity meter (accurate to 0.01 mS cm^{-1} and with a built-in temperature compensation of 25 ºC) and pH using a Crison pH/mv meter 506 (accurate to 0.01 pH unit). In the Western Cape in 1995 *in situ* measurements were conducted using a Grant YSI Water Quality Data logger 3800. Temperature (accurate to ± 0.4 ºC), conductivity (accurate to 3% between 0 and 2 000 mS m^{-1} and 4% between 2 000 and 10 000 mS m^{-1} and with a built-in temperature compensation of 25 ºC), pH (accuracy to ± 0. 2 pH unit), turbidity (accurate to ± 6% or 2NTU), dissolved oxygen (accuracy ± 2% of reading) were measured.

In the Western Cape, water samples for chemical analyses were collected from rapidly flowing areas, filtered on site (Whatman 45 µm GF/F filter papers) and frozen within 24 hours. In Mpumalanga, sampled water was not filtered and was preserved with mercury chloride on site. All filtered water, except that for analysis of ammonium, was bottled in polythene vials that had been pre-cleaned in 5% Extran^{R} solution (phosphate-free), and rinsed

in deionised and then double-distilled water. Samples for analysis of ammonium were stored in glass vials that had been pre-washed in HCl. Details of the analytical methods used for each variable are as follows:

Total dissolved solids: The concentration of total dissolved solids (TDS, mg l^{-1}) was determined by evaporating 800 ml of filtered water from pre-weighed pyrex glass beakers at 60ºC. Weighing was done on a Sartorius precision laboratory balance accurate to 1 mg.

Anions and cations: The concentrations of the anions sulphate and chloride were measured by means of ion exchange chromatography using an HPIC-AS4A anion exchange separator column, with a carbonate/ bicarbonate buffer eluent. Results were expressed in mg l^{-1}, accuracy ± 0.005 mg l^{-1}. The concentrations of the cations potassium, sodium, calcium and magnesium were also measured by means of ion exchange chromatography using an HPIC-AS4A cation exchange separator column, with an appropriate eluent. Results were expressed in mg l^{-1}, accuracy ± 0.005 mg l^{-1}.

Total alkalinity: Total alkalinity was measured by titrating the sample with 0.005M HCl (methyl orange indicator) according to the method prescribed by Golterman *et al*. (1978). Standardisation was against NaOH, titrated with 0.005M oxalic acid (phenolphthalein indicator). Results were obtained as mg l^{-1} $CaCO_3$, and expressed as meq l^{-1}. Accuracy is estimated at 2-10%.

Nutrients: The concentration of the nutrients: ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^-- N), nitrite nitrogen (NO_2^-- N) and soluble reactive phosphorus (SRP; PO_4^{3-}- P) were determined using a Technikon Auto Analyser (AA11). The principles of the method are outlined in Mostert (1983). Results are expressed in mg l^{-1} of the nutrient atom. For nitrite and nitrate, the detection limit is 1 μg l^{-1}.

2.2.3 Additional site characteristics

Several other biotic and abiotic factors thought to be important in either the interpretation of macroinvertebrate data or in characterising a site, were assessed. These included details of the ecoregion (Kleynhans *et al*.1998a), bioregion (*Brown et al.*1996) and subregion in which each site occurred. The distance from source, altitude, stream order, geological-type

(Vegter 1995), vegetation-type (Low & Rebelo 1996), hydrological-type (perennial, seasonal or ephemeral) and rainfall region of each site were recorded.

Catchment and land-use, water quality impacts and channel condition were assessed and used as a guide for identifying reference sites. Catchment and land-use included features regarding the condition of the local catchment and land-use within the catchment. The presence and extent of each identified land-use type "within" and "beyond" a five metre perimeter was recorded. Since water quality impacts are linked to land-use, each identified land-use was also rated in terms of the potential impact on water quality in the receiving water body. In-channel and bank modifications were noted and the extent of their impact upstream and downstream of the site was rated. Present status, which refers to the number and severity of anthropogenic disturbances on a river and the damage they potentially inflict on the system, was assessed using a modified site-based method of Kemper & Kleynhans (1998). This assessment attempts to quantify instream and riparian zone disturbances at a site, and includes abiotic factors, such as the presence of weirs and dams, the extent of water abstraction, pollution and dumping of rubble, and biotic factors, such as the presence of alien plants and animals. Aspects considered in this assessment are those regarded as primary causes of degradation of a river ecosystem.

Site- and habitat-level measurements were made of the stream dimensions and substratum composition. The macro-channel, active channel and water surface widths, left and right bank heights, and minimum, maximum and average depths of the available deep- and shallow-water biotopes were recorded. The relative percentage contribution of each substratum type (bedrock, boulder, cobble, pebble, gravel, sand and silt/mud) was recorded. Details of the biotopes sampled, both at the resolution of SASS-biotope, e.g. SIC, SOOC, AQV, etc., and at the specific-biotope level, e.g. cobble riffle, bedrock run, marginal vegetation-in-current, sand-in-backwater, etc., were recorded.

2.3 DATA ANALYSIS

2.3.1 Biological data

Multivariate procedures

Multivariate procedures were selected for analyses of macroinvertebrate assemblage-based data gathered in this study. In contrast to univariate analyses [e.g. Analysis of Variance (ANOVA), regression], multivariate procedures consider each taxonomic group/family to be a variable and the presence/absence or abundance of each taxonomic group/family to be an attribute of a site or time (Norris & Georges 1993). Subtle changes in the taxon composition across sites or in abundance of particular taxon across sites are not inherently masked by the need to summarize the combined characteristics of a site into a single value (Norris & Georges 1993). Multivariate procedures are therefore more likely to facilitate the detection of spatial and temporal trends in biotic assemblage data. All multivariate analyses were performed using the *Primer Version 5* software package for windows. The multivariate procedure followed is given below.

The data matrix consists of *p* rows (taxonomic groups) and *n* columns (samples). The data are binary, i.e. the presence or absence of each taxonomic group is given for each sample. For binary data, the presence or absence of taxa occurring at low densities assumes a larger role than the numerically abundant taxa, and a large number of taxa contribute to the discrimination of sites. Binary data have been shown to distinguish adequately between minimally-disturbed sites, which have rich faunal assemblages (Furse *et al*. 1994), and between minimally-impacted and disturbed sites (Wright *et al.* 1994), although Thorne *et al.* (1999) considered abundance to be important in distinguishing sites that have a poor fauna, i.e. moderately and severely disturbed sites. At such sites, differences in the patterns of dominance of a few taxa, seems to be important. Since multivariate analyses in this report are based on data from minimally-disturbed sites, the use of binary data should be adequate. For classification and ordination of macroinvertebrate data, taxa present at less than 5% of the sites assessed were generally excluded from classifications. Unlike studies focusing on biodiversity and conservation, where rare taxa are important, bioassessment aims to establish the ecological condition or health of a river, using invertebrates as indicators. It is assumed that rare taxa contribute little information to studies designed to detect differences in assemblage composition (Barbour & Gerritsen 1996), particularly within the context of

bioassessment. This issue is however often debated, with Fore *et al*. (1996) arguing that, whilst the presence of rare taxa in the data matrix means lots of zeros, which can degrade a statistical solution, the presence of rare taxa in the field indicates near-pristine conditions capable of supporting these (often) sensitive taxa. However, this is not considered the case in the present study, since many of the rare taxa that were present at less than 5% of the sites and which were excluded from analyses, were moderately to very pollution-tolerant taxa, suggesting that rarity did not reflect the extent to which a site was "pristine".

The Bray-Curtis coefficient has been recommended for community structure analysis of biological data on (*PRIMER Version 5*). This measure of similarity is suited to presence-absence data (Moss *et al.* 1999). The Bray-Curtis measure has the form (Field *et al.* 1982):

$$\delta_{jk} = \frac{\sum_{i=1}^{s} | _{ij} - Y_{ik} |}{\sum_{i=1}^{s} (Y_{ij} + Y_{ik})}$$

where Y_{ij} = score for the *i*th species in the *j*th sample; Y_{ik} = score for the *i*th species in the *k*th sample; δ_{jk} = dissimilarity between the *j*th and *k*th samples summed over all *s* species. δ_{jk} ranges from 0 (identical scores for all species) to 1 (no species in common) and is the complement of the similarity S_{jk}: $S_{jk} = 1 - \delta_{jk}$ Comparison of each sample with every other sample using this measure of similarity/dissimilarity leads to a triangular matrix, which can then be used in cluster and ordination analyses. According to Clarke & Warwick (1994) the dissimilarity coefficient is a more natural starting point than the similarity coefficient in constructing ordinations, in which dissimilarities (δ) between parts of samples are turned into distances (d) between sample locations on a "map". A large dissimilarity indicates a greater distance.

SIMPER analysis (Clarke & Warwick 1994) was performed in order to identify the taxa most responsible for the differences between groups of sites identified in the cluster and ordination analyses. Since the data are binary, i.e. presence/absence only, it was not possible to use average abundances, so those taxa contributing to the similarity within a group of sites or the dissimilarity between groups of sites were simply identified.

Cluster analysis (or classification)

Cluster analysis aims to find "natural groupings" of samples such that samples within a group are more similar to each other than to samples in different groups (Clarke & Warwick 1994). Hierarchical agglomerative clustering, using group-average linking, was used on the data matrix, to produce a dendrogram. 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).

Ordination of samples by multi-dimensional scaling (MDS)

MDS produces an ordination of *n* samples in a specified number of dimensions. 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 & Warwick 1994). The distance between samples attempts to match dissimilarities in community structure: nearby points have similar communities and distant points have dissimilar ones. The advantage of using MDS over other ordination procedures such as Principle Components Analysis is its ability to handle, with comparative ease, missing data, replication and data of non-uniform reliability for which it is desirable to give unequal weights to the dissimilarities in seeking the "best" map (Field *et al.* 1982). The calculation of the stress value provides a good means of assessing the reliability of the MDS ordination. A stress value of < 0.05 gives an excellent representation with minimal prospect of misinterpretation (Clarke & Warwick 1994). A stress value of < 0.1 corresponds to a good ordination with less prospect of a misleading interpretation. A stress value of < 0.2 gives a useful two-dimensional picture although conclusions should not be based only on the ordination, which should be complemented by an alternative technique (e.g. cluster analysis). The ordination can also be run in a three dimensional scale to determine the stress values in three dimensions.

All results presented in this study are based on the results of both cluster and ordination analyses.

Analysis of similarity (ANOSIM)

ANOSIM is a non-parametric procedure that is applied to the Bray-Curtis similarity matrix underlying the classification or ordination of samples (Clarke & Warwick 1994). It allows the testing of the null hypothesis that there is no significant difference between groups,

which are specified *a priori* to analysis, based on hypotheses. One-way ANOSIM was used to test whether or not there were significant differences in assemblage structure amongst homogeneous regions (Chapter 3), biotope-groups (Chapter 4) and amongst seasons (Chapter 5). The ANOSIM tests were performed on presence/absence transformed data, analysed using the Bray-Curtis measurement of similarity. In ANOSIM, a Global R value of approximately zero indicates that the null hypothesis is true and that similarities between- and within-groups are roughly the same (Clarke & Warwick 1994).

Classification Strength

Classification strength (CS) was assessed by comparing the mean of all between-class similarities (*Bbar*) with the overall weighted mean of within-class similarities (*Wbar*) using *MEANSIM6* (Van Sickle 1997, Van Sickle & Hughes 2000). CS = *Wbar* - *Bbar*. The strength of each classification was illustrated using a dendrogram format, with the dendrogram node plotted as *Bbar*. One branch is drawn for each class, with its end plotted at W, for that class (Van Sickle 1997). The longer the branch, the greater the increase in % similarity. A strong classification is therefore one that has a low *Bbar* and long branches, i.e. high W. To test the hypothesis that there is no class structure, the p-value is estimated as the proportion of random reassignments having *M* smaller than M_{obs}, where $M_{obs} = B/W$. The null hypothesis was rejected if the estimated p was < 0.05.

Univariate procedures

Univariate procedures were used to examine differences in the three metrics calculated in SASS, namely SASS4 Score, number of taxa and ASPT. Since data were often not normally distributed, a non-parametric equivalent of ANOVA, namely the Kruskal-Wallis Test, which compares median values, was used. Individual pairs of faunal samples were compared using the non-parametric Kolmogorov-Smirnov Test. The results of all analyses were considered significant at $p < 0.05$. Univariate analyses were performed using the *Statistica Version 5* software package for windows.

2.3.2 Environmental variables, including physical attributes and chemical constituents

Discriminant Function Analyses

Environmental variables were considered in relation to macroinvertebrate assemblages,

specifically, how different environmental factors affect the spatial distribution of macroinvertebrates. Discriminant Function Analyses (DFA, *Statistica 5.5 for Windows*) was used for this purpose. It facilitates the identification of the environmental variables that best explain faunal groups as determined via cluster and ordination analysis. Prior to DFA, variables were analysed using a non-parametric ANOVA (Kruskal-Wallis) using faunal group membership as the factor variable. Environmental variables that showed significant differences ($p < 0.05$) among faunal groups were chosen for further analyses. Stepwise DFA was used to select an optimum subset of physical and chemical variables (Chapters 6, 7). DFA assumes that variables are independent and normally distributed. Data which did not conform to normality were $\log_{10}(x)$ transformed prior to DFA. Ratios were calculated for monovalent: divalent cations as $[Na^+]+[K^+]/[Na^+]+[K^+]+[Ca^{2+}]+[Mg^{2+}]$ and major anions as $[Cl^-]/[Cl^-]+[HCO_3^-]$ ratios, where [ ] means "concentration of", and included in the analyses of Chapter 7.

CHAPTER 3. SPATIAL VARIABILITY IN MACROINVERTEBRATE ASSEMBLAGES AT THE REGIONAL AND SUBREGIONAL LEVELS

3.1 INTRODUCTION

River ecosystems are longitudinal systems that integrate the characteristics of the catchments they drain. They exhibit a high degree of spatial and temporal variability, particularly in semi-arid environments such as large areas of South Africa (Eekhout *et al.* 1997). Spatial variability of macroinvertebrate assemblages is a widely studied characteristic of lotic environments (e.g. Hawkes 1975, Statzner & Higler 1986, Hawkins *et al.* 1997) and, in the context of bioassessment, spatial heterogeneity is often taken into account by partitioning areas into relatively homogeneous regions, i.e. regional classification. Classification is broadly defined as a process in which a set of objects, systems or ideas (entities) are divided into a number of discrete groups on the basis of some measure of their similarity or differences with respect to one or more pre-defined criteria (Eekhout *et al.* 1997). A primary goal of many classification systems is to provide a spatial framework, such as ecoregions (Omerick 1987, Kleynhans *et al.* 1998a), within which aquatic resource management, including bioassessment, is conducted. The ecoregion concept hypothesises that contiguous land-forms with similar geology, soils, vegetation and climate are likely to possess similar biotic assemblages (Omernik 1987). The underlying assumptions are that natural variation is predictable among systems within the same ecoregion where environmental features are similar; and that by stratifying natural variation into spatially explicit, homogeneous ecoregions, one can detect responses to disturbance at one site by comparing it to a reference site in the same ecoregion (Omernik & Bailey 1997). Until recently, there has been no rigorous evaluation of the ability of ecoregions to partition spatial variability (Hawkins & Norris 2000).

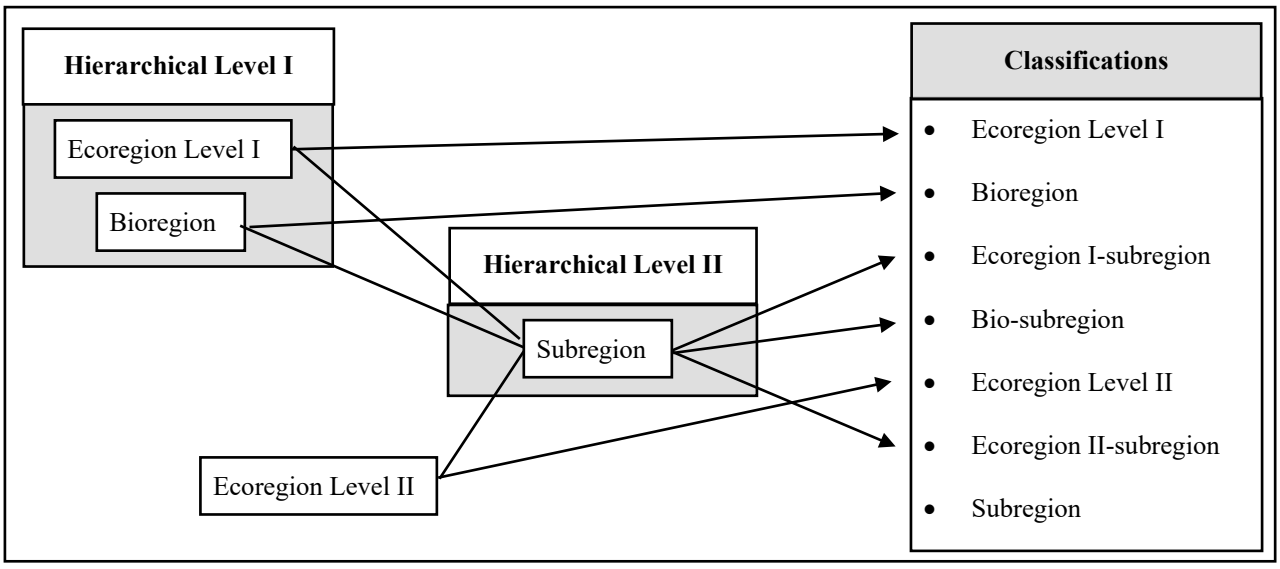
Considerable work has been undertaken on the classification of South African rivers (King *et al.* 1992), including classifications based on physico-chemical factors (Noble & Hemens 1978), flow patterns (Joubert & Hurly 1994), water chemistry (Day *et al*. 1998), biogeography (Eekhout *et al*. 1997, Brown *et al.* 1996), landscape features such as physiography, climate and geology (Allanson *et al.* 1990, Kleynhans *et al*. 1998a) and

combinations thereof (Harrison 1959). The classifications currently considered as most suitable for use as a framework within which bioassessment is conducted are those based on biogeography, termed bioregions, and landscape features, termed ecoregions. These bio-and ecoregions represent the upper level of a three-tiered, hierarchical spatial framework (Figure 3.1) developed in South Africa and are aimed at partitioning spatial variability at the regional or catchment level. Bioregions are based on biophysical conditions and are derived by examining the biogeographic distribution patterns of riverine macroinvertebrates, fish and riparian vegetation (Eekhout *et al.* 1997) together with the physical characteristics of the rivers (Brown *et al*. 1996). Ecoregions (Kleynhans *et al*. 1998a) are based on a top-down classification of rivers using mapped landscape characteristics including physiography, climate, geology, soils and potential natural vegetation. A second ecoregional level, which represents an intermediate level between levels I and II in the spatial hierarchy, has also been proposed for parts of South Africa (Kleynhans *et al*. 1998b). The second level of the hierarchical framework is the subregional classification and it reflects broad geomorphological characteristics and longitudinal distribution patterns of components of the biota. The third level of the hierarchy attempts to account for variation among rivers within a subregion or geomorphological zone and factors such as river size, hydrological type (ephemeral, seasonal or perennial) and geomorphological characteristics (channel type, substratum composition) are considered. By incorporating three spatial scales it is anticipated that spatial variability at the level of catchment, river, site and habitat, may be incorporated and partitioned such that variability of macroinvertebrate assemblages within an identified homogeneous group of sites is minimised. Ecological reference conditions based on macroinvertebrates within this homogeneous group should, therefore, enable a disturbance at a monitoring site to be detected.

The aim of this chapter is to assess the performance of different classification systems by gauging their classification strengths, i.e. the degree to which classifications minimise within-class biotic similarity relative to between-class biotic similarity (Hawkins & Norris 2000, Van Sickle & Hughes 2000). Levels I and II of the proposed South African hierarchical spatial framework (Brown *et al*. 1996, Kleynhans *et al*. 1998a) are examined and the classifications tested include ecoregion level I, bioregions, ecoregion Level II, ecoregion Level I combined with subregions, termed eco-subregion, and bioregion combined with subregions, termed bio-subregion, ecoregion Level II combined with subregions, and subregion (Figure 3.1). The

hypothesis that spatial variability in macroinvertebrate assemblages may be partitioned *a priori* using geographic delineaters is tested. The following specific questions are addressed: 1) do macroinvertebrate assemblages differ amongst ecoregions, bioregions, eco-subregions, bio-subregions and subregions? and 2) which spatial classification system is most effective at partitioning variability in macroinvertebrate assemblages? Following the procedure outlined by Hawkins & Norris (2000), the performance of each of the *a priori* classifications was judged by comparing their classification strengths to an objectively derived standard determined by *a posteriori* clustering of sites into groups based on their biotic composition. The spatial variability in macroinvertebrate assemblages and, in particular the ability of classification systems to partition this variability, is discussed in relation to aquatic bioassessment and the establishment of reference sites within identified homogeneous regions.

Figure 3.1 Diagram illustrating the various classifications indicating levels I and II of the hierarchical spatial framework adopted in South Africa.



3.2 STUDY AREA

Ninety-eight sites, situated on 66 rivers, were sampled (Table 3.1). Of these, 34 sites were situated on 26 rivers in the Western Cape region and 64 were on 39 rivers in Mpumalanga. Some Western Cape sites were sampled on two or three occasions within spring and have been included in analyses on a per-assessment basis. Only minimally-impacted sites, with respect to anthropogenic disturbance, were selected in both regions so that effects resulting

from impaired water quality could be avoided. In lowland rivers, identification of minimally-impacted sites was difficult and those sites identified represent the best-attainable condition, i.e. the best available within the lower reaches of the rivers. Sites in the Western Cape were assessed with variable frequency during 1994 and 1995 (see Appendix A), whilst sites in Mpumalanga were each assessed on three occasions (May, July and September) in 1999. The distribution of sites amongst the different classification classes is tabulated in Table 3.2. Abbreviations have been standardised such that classes derived by combining classifications are the combination of the two classifications, i.e. the Fynbos (F) bioregion class and the mountain stream (M) subregion class is given as FM for the classification which combines bioregions and subregions into bio-subregions.

Table 3.1 Sites assessed during this study, indicating river and geographic region. The codes for sites on each river are given in parenthesis and relate to Table 2.1 and Figures 2.1 and 2.2 in Chapter 2.

Geographic Region	River (site codes)
Western Cape	Assegaaibosch (CM01), Baviaans (CM18), Berg (CM02, CC01), Boesmanskloof (CM20), Boesmans (CM17), Breede (CL01, CL02, CL03, SL01, SL02), Duiwelsbos (CM21), Duiwenshoek (SL01), Dutoits (CC07), Eerste (CM04), Elandspad (CM09), Grootkloof (CM24), Hermitage (CM22), Holsloot (CC02), Houtbaais (CM14, CM15), Lang (CM05), Meulkloof (CM23), Molenaars (CC05), Palmiet (CM07, CF01), Rietvlei (CM16), Riviersonderend (CM20, CL04), Rooiels (CM12), Sanddriftskloof (CC06), Unspecified (CM13), Window Stream (CM06), Wit (CM11)
Mpumalanga	Alexanderspruit (HC42), Blyde (EC01, EC02), Blystaanspruit (EC04), Crocodile (EC05, EC06, ER32, HC43, LG60, LG61, LG62, LG63, LG64), Dorps (HC36), Elands (EC07, EC08) Elandsfonteinspruit (HC44, HM51), Ga-Selati (EM20), Grootfonteinspruit (EM16), Heddle (EM17), Houtbosloop (EC09), Kareekraalspruit (HM52), Kgwete (EM18), Klein-Sabie (EC10), Klip (HC38), Lone Creek (EM25), Lunsklip (HC45), Mac-Mac (EC11, LC56, LR73), Marite (LC57), Maritsane (LM71), Mohlombe (EM26), Nelspruit (EM22, LR72), Ohrigstad (EM19), Sabie (EC12, EM27, LC58, LF59, LG66, LG67, LG68, LR74), Sand (EM29, LG69, LG70), Selon (HC35), Spekboom (EM13, HC39), Sterkspruit (EM14), Tautesloop (HM54), Treu (EC03), Unspecified (EM15, EM24, EM28, EM30, EM31, HM48), Waterval (HC40, HC41), Wilge (HG33) and Wilgekraalspruit (HM55)

Table 3.2 Distribution of sites amongst the different classification classes. Each classification class constitutes a grouping of sites based on the particular spatial classification (Ecoregion Level I, Bioregion, Ecoregion Level II and Subregion) or combination of spatial classifications (EcoregionI-subregion, Bio-subregion and Ecoregion II-subregion) (see figure 3.1). Where level I of the spatial hierarchy (Ecoregion Level I or Bioregion) has been combined with subregion, i.e. level II, coding is a combination of the two codes. The number of individual sites in each classification may be calculated from Table 2.1 in Chapter 2.

Classification	Geographic Region	
	Western Cape	**Mpumalanga**
Ecoregion Level I	Cape Fold Mountains (C), Southern Coastal (S)	Great Escarpment Mountains (E), Central Highlands (H), Lowveld (L) and Lebombo Uplands (U)
Bioregion	Fynbos (F), Southern Coastal (S)	Northern Uplands (N), Bushveld Basin (B) and Lowveld (L)
Ecoregion I-subregion	CM, CC, CL, CF and SL	EM, EC, ER, HM, HC, HG, LM, LC, LG, LF and LR
Bio-subregion	FM, FC, FL, FF, SC and SL	NM, NC, NR, BC, LC, LG and LF
Ecoregion Level II	Unknown	E1, E2, E3, E4, H9, H11, H12, L5, L6, L7
Ecoregion II-subregion	Unknown	E1C, E2C, E2R, E3M, E3C, E4M, E4C, H9G, H11M H11C, H12C, L5M, L5C, L5G, L5R, L6C, L6G, L7G
Subregion	mountain stream (M), foothill-cobble bed (C), lowland (L) and rejuvenated foothill (F)	M, C, foothill-gravel bed (G), rejuvenated cascade (R) and F

3.3 MATERIALS AND METHODS

3.3.1 Benthic macroinvertebrates: SASS4 sampling

Benthic macroinvertebrates were sampled using the qualitative rapid bioassessment method, SASS4. A detailed description of the method is given in Chapter 2.

3.3.2 Data analysis

Macroinvertebrate assemblage analysis

Cluster analysis and non-metric multidimensional scaling (MDS) were used to examine similarities amongst sites based on macroinvertebrate assemblage composition (Clark & Warwick 1994). Analysis of Western Cape and Mpumalanga data together, and subsequent within-region analysis for the Western Cape was based on macroinvertebrate data collected from all available biotope-groups in spring. Subsequent within-region analysis for Mpumalanga was based on composite macroinvertebrate data generated from invertebrates collected in three seasons (autumn, winter and spring) and from all available biotope-groups. Classification of sites based on two or three seasons rather than one season is often recommended as it is considered a more robust means of classifying sites since temporal variation is reduced (Turak *et al.* 1999). Taxa present at less than 5% of sites were considered to be rare taxa and were excluded from the classifications. All data were presence/absence transformed (*PRIMER Version 5*) and the Bray-Curtis coefficient was used on these transformed data. Hierarchical agglomerative clustering, using group-average linking, was used on the data matrix. Ordination of samples by MDS was undertaken, and stress values used to assess the reliability of the MDS ordination. Sites that did not group with other sites during the preliminary analyses were considered to be outliers and were excluded from the final classifications. The distinguishing taxa responsible for the similarity within group of sites and the dissimilarity amongst groups of sites were established using SIMPER (*PRIMER Version 5*). Those taxa responsible for 90% within-group similarity were examined. Spatial ordination of each regional classification was examined by overlaying regional classes on the macroinvertebrate ordination.

Classification strength

One-way Analysis of Similarities (ANOSIM) was used to test whether or not there were significant differences in macroinvertebrate assemblages amongst classification classes of the various regional classifications. Classification strength of each regional classification was also assessed by comparing the mean of all between-class similarities (*Bbar*) with the overall weighted mean of within-class similarities (*Wbar*) using *MEANSIM6* (Van Sickle 1997, Van Sickle & Hughes 2000).

SASS4 Scores, Number of Taxa and ASPT

SASS scores for classification classes within each regional classification and each group of sites based on macroinvertebrate assemblages were compared statistically using the non-parametric Kruskal-Wallis Test. Individual pairs of biotope-groups were compared using the non-parametric Kolmogorov-Smirnov Test. The results of all analyses were considered significant at $p < 0.05$.

3.4 RESULTS

3.4.1 Analysis of macroinvertebrate assemblages

Macroinvertebrate assemblages clustered largely by geographical region and formed five groups. A "group" is the term used to describe a group of sites that have similar macroinvertebrate assemblages. It represents the objectively derived standard determined by *a posteriori* classification of sites into groups based on their biotic composition. Group 1 comprised Western Cape (WC) sites and were approximately 65% dissimilar from Group 5, comprising Mpumalanga (MPU) sites (Figure 3.2, MDS: 3D-stress = 0.17). One WC site (CL03) grouped with MPU sites, one WC site (CL01) clustered with two MPU sites (LG63 and LG68) and the remaining lowland WC sites (SL01, SL03 and CL04) clustered together. The WC site (SL02) and MPU site (EM17) were outliers. The Global R value of the ANOSIM analysis indicated that macroinvertebrate assemblages from WC were significantly different from those of MPU (Global $R = 0.771$, $p < 0.001$).

Taxa contributing to the similarity in macroinvertebrate assemblages within each Group are tabulated on the basis of those contributing to the first 50% similarity and those contributing the next 40% (Table 3.3). The division has been included since many of the taxa are present in all groups, but their importance in defining within-group similarity varies. Taxa responsible for the within-group similarity were relatively distinct, with Group 1 comprising 17 taxa, including several sensitive ones and two families endemic to the Western Cape (Table 3.3). Group 2, comprising the rejuvenated foothill site and a mountain stream site, included some of the same sensitive taxa, although several were absent and additional ones included. Only 9 taxa were identified in Group 3, several of which were hemipterans. Taxa from Group 4, comprising lowland WC sites, included the afro-tropical family, Tricorythidae, crabs and shrimps (Natantia), with the notable absence

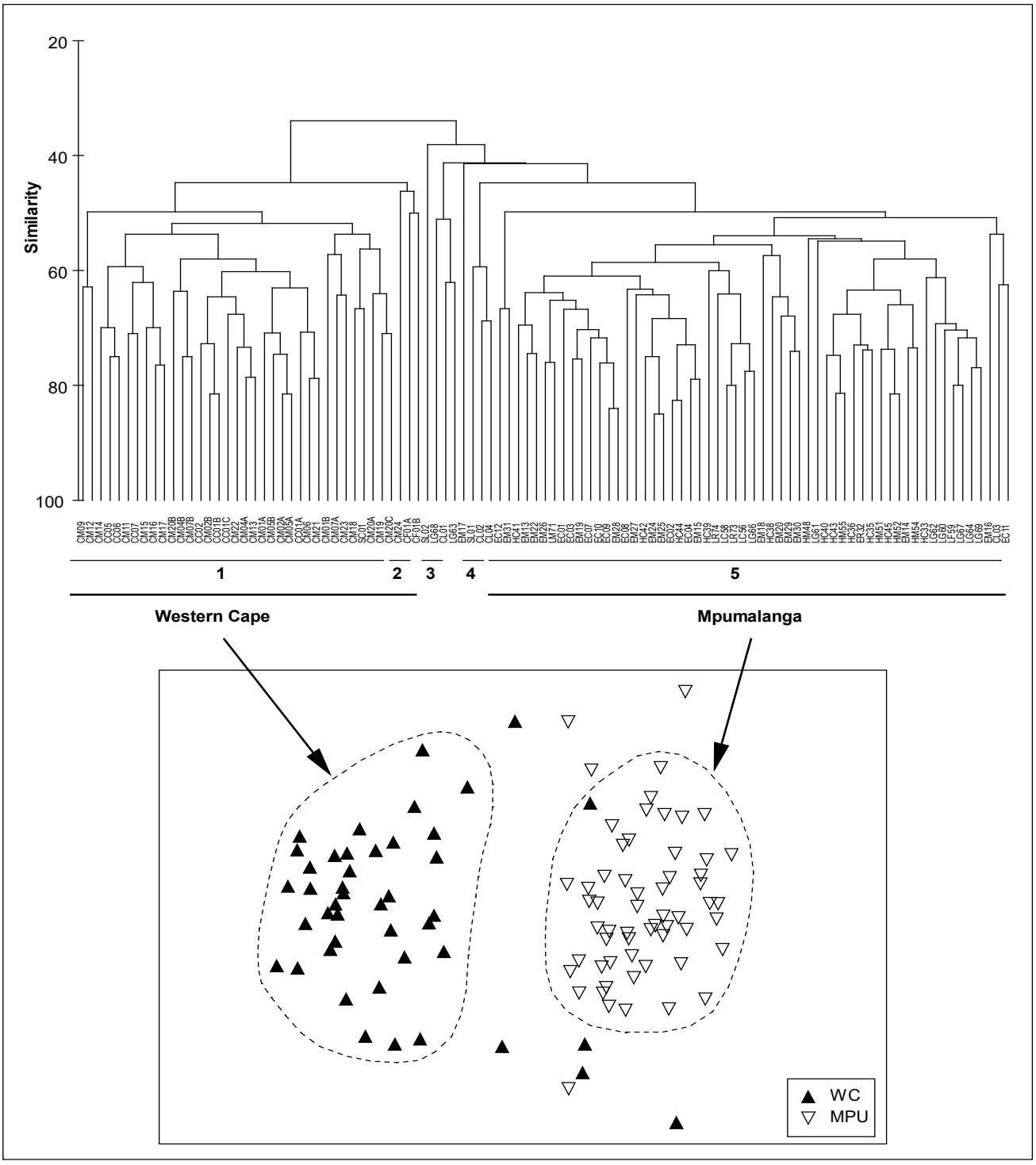


Figure 3.2 Dendrogram and MDS ordination showing the classification of sites based on taxa recorded in spring. Codes: primary: C = Cape Fold Mountains, S = Southern Coastal, E = Great Escarpment Mountain, H = Central Highland, L = Lowveld ecoregion; secondary: M = mountain stream, C = foothill-cobble bed, G = foothill-gravel bed, R = rejuvenated cascade, F = rejuvenated foothill and L = Lowland floodplain. Western Cape sites assessed more than once have been coded to distinguish sampling year: A = Sept 1994, B = Nov 1994, C = Oct/Nov 1995.

Table 3.3 Taxa contributing to within-group similarity of five Groups identified from sites in the Western Cape and Mpumalanga. Those taxa contributing to the first 50% of the similarity are indicated by ♦; the remaining taxa contributing to the next 40% (i.e. 90% in total) of the similarity are indicated by □.

Group	**1**	**2**	**3**	**4**	**5**
Average similarity	**55.4%**	**48.7%**	**56.0%**	**65.1%**	**56.3%**
Number of distinguishing taxa	*17*	*10*	*9*	*13*	*29*
Notonemouridae	□				
Perlidae					□
Baetidae 3 Types	□	♦	♦	♦	♦
Caenidae		□	□		♦
Teloganodidae	♦	♦			
Heptageniidae	□				□
Leptophlebiidae	♦			♦	♦
Tricorythidae				□	♦
Elmidae/Dryopidae	♦	♦		□	♦
Gyrinidae	□			♦	□
Helodidae Larvae	□				
Hydraenidae	□				
Limnichnidae					
Psephenidae					□
Corydalidae	□	□			
Hydropsychidae 1 Type				□	□
Hydropsychidae 2 Types	□				□
Hydropsychidae 3 Types					
Hydroptilidae				♦	
Philopotamidae	□				
Case Caddis 1 Type			□		
Case Caddis 3 Types	□	□			
Athericidae	□				□
Ceratopogonidae					□
Chironomidae	♦	□	□	□	♦
Simuliidae	♦	♦	□	♦	♦
Tabanidae					□
Tipulidae	□				□
Corixidae			♦		□
Naucoridae			♦		□
Veliidae		□	□		□
Aeshnidae					□
Coenagrionidae					□
Gomphidae			♦		♦
Libellulidae		□			□
Oligochaeta					□
Hydrachnellae					□
Brachyura (Crabs)				□	□
Natantia (Shrimps)				□	
Planariidae					♦
Ancylidae				□	□
Sphaeriidae				□	

of several taxa characteristic of upland sites. Twenty-nine taxa characterised Group 5, which consisted predominantly of Mpumalanga sites, including several taxa largely restricted to the more northern regions of South Africa. Several families of hemipterans and odonates were included in this group.

In the Western Cape, macroinvertebrate assemblages formed three Groups, with Group 1 separating further into four sub-groups (A-D). Group 1 comprised 23 of the 24 mountain stream sites and all the foothill-cobble bed sites; Group 2 comprised one mountain stream site and the rejuvenated foothill site; and Group 3 comprised six lowland sites (Figure 3.3, MDS: 3-D stress = 0.17). Group 3 was 65% dissimilar from Groups 1 and 2, and Group 2 was 57% dissimilar from Group 1. Within-class variability of Group 1 was high. Group 1 comprised several sensitive taxa characteristic of upland sites (Table 3.4), Group 2 had several sensitive taxa present although some were absent, whilst Group 3, was characterised by several lowland taxa including crustaceans and molluscs, which were not identified as important within the other groups. Taxa responsible for further separating group 1 sites into four sub-groups (Table 3.4) included notonemourid stoneflies, heptageneid mayflies, gyrinid and limnichnid beetles, ecnomid caddisflies and athericid and blephariceridipterans.

In Mpumalanga, cluster and ordination analysis of composite macroinvertebrate data resulted in separation into five Groups (Figure 3.4, MDS 3-D Stress = 0.16). Sites identified as outliers, and thus excluded from the final classification, included EM14, EM16, EM17, EM31 and HM48. Groups 1, 2 and 3 comprised mostly upper-catchment sites within the mountain stream and foothill-cobble bed subregions. Group 4 consisted of sites from several subregions. Group 5, which was approximately 40% dissimilar for other groups, comprised mostly sites in the foothill-gravel bed subregion. Two sub-groups, 3A and 5A were apparent within groups 3 and 5 respectively.

Taxa contributing to the similarity in macroinvertebrate assemblages within each Group are tabulated on the basis of those contributing to the first 50% similarity and those contributing to the next 40% (Table 3.5). Several taxa characterised only selected Groups, whilst others contributed to within-group similarity of all Groups. Notably in Groups 3 and 4 were Perlidae, Helodidae in Groups 1 and 3, Psephenidae in Groups 1, 2 and 3, Philopotamidae in Groups 2 and 4, Trichoptera (cased caddis 3 Types) in Group 5, several

hemipteran and odonate families in Group 2 and 5, and Natantia in Group 5.

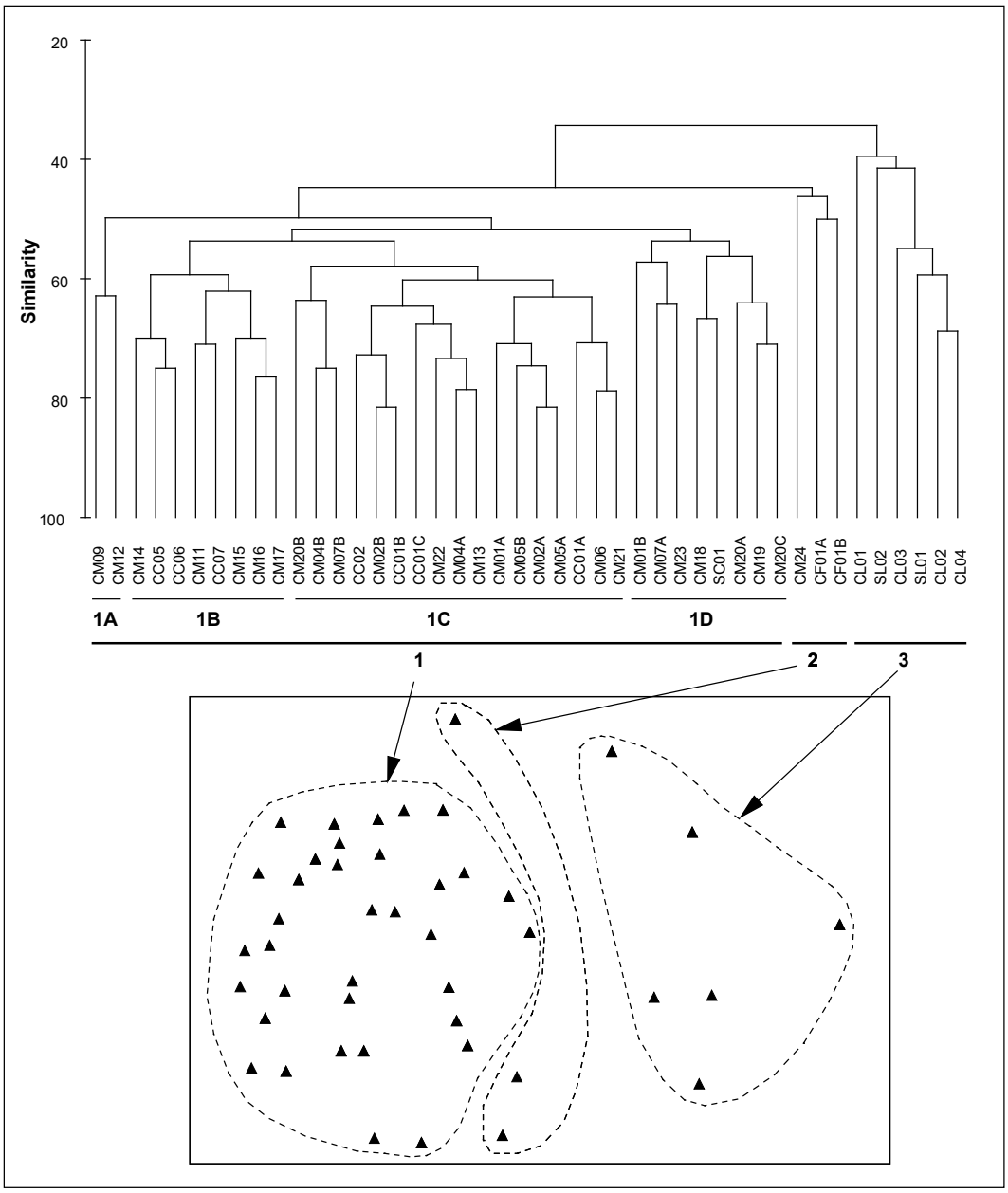


Figure 3.3 Dendrogram and MDS ordination showing the classification of sites in the Western Cape (WC). Codes: primary: C = Cape Fold Mountains, S = Southern Coastal; secondary: M = mountain stream, C = foothill-cobble bed, F = rejuvenated foothill and L = Lowland floodplain. Sites assessed more than once have been coded to distinguish sampling year: A = Sept 1994, B = Nov 1994, C = Oct/Nov 1995.

Table 3.4 Taxa contributing to within-group similarity of three main Groups and four sub-groups identified from sites in the Western Cape. Those taxa contributing to the first 50% of the similarity are indicated by ♦; the remaining taxa contributing to the next 40% (i.e. 90% in total) of the similarity are indicated by □.

	Groups			Sub-groups of Group 1			
	1	**2**	**3**	**1A**	**1B**	**1C**	**1D**
Average similarity	**55.1%**	**47.5%**	**47.7%**	**52.7**	**64.5**	**62.1**	**56.7**
Number of distinguishing taxa	*17*	*10*	*16*	*14*	*12*	*15*	*14*
Notonemouridae	□				□	□	□
Baetidae 2 Types							□
Baetidae 3 Types	□	♦	♦	□		□	
Caenidae		□	♦	□			
Teloganodidae	♦	♦		♦	♦	♦	□
Heptageniidae	□					□	
Leptophlebiidae	♦		♦	♦	♦	♦	♦
Tricorythidae			□				
Elmidae/Dryopidae	♦	♦	□	□	♦	□	♦
Gyrinidae	□		□				♦
Helodidae Larvae	□			□	□	♦	
Hydraenidae	□					□	□
Limnichnidae							□
Corydalidae	□	□			♦	♦	□
Ecnomidae				□			
Hydropsychidae 1 Type				□	♦		
Hydropsychidae 2 Types	□		□				□
Hydroptilidae			□				
Philopotamidae	□			□	□		
Case Caddis 2 Types						□	
Case Caddis 3 Types	□	□		♦	□	□	♦
Athericidae	□						□
Blephariceridae						□	
Chironomidae	♦	□	♦	♦	□	□	♦
Simuliidae	♦	♦	♦	♦	♦	♦	♦
Tipulidae	□				□	□	
Veliidae		□					
Chlorolestidae				□			
Gomphidae				□			
Libellulidae		□					
Oligochaeta			□				
Brachyura (Crabs)			□				
Natantia (Shrimps)			□				
Ancylidae			□				
Lymneidae			□				
Sphaeriidae			□				

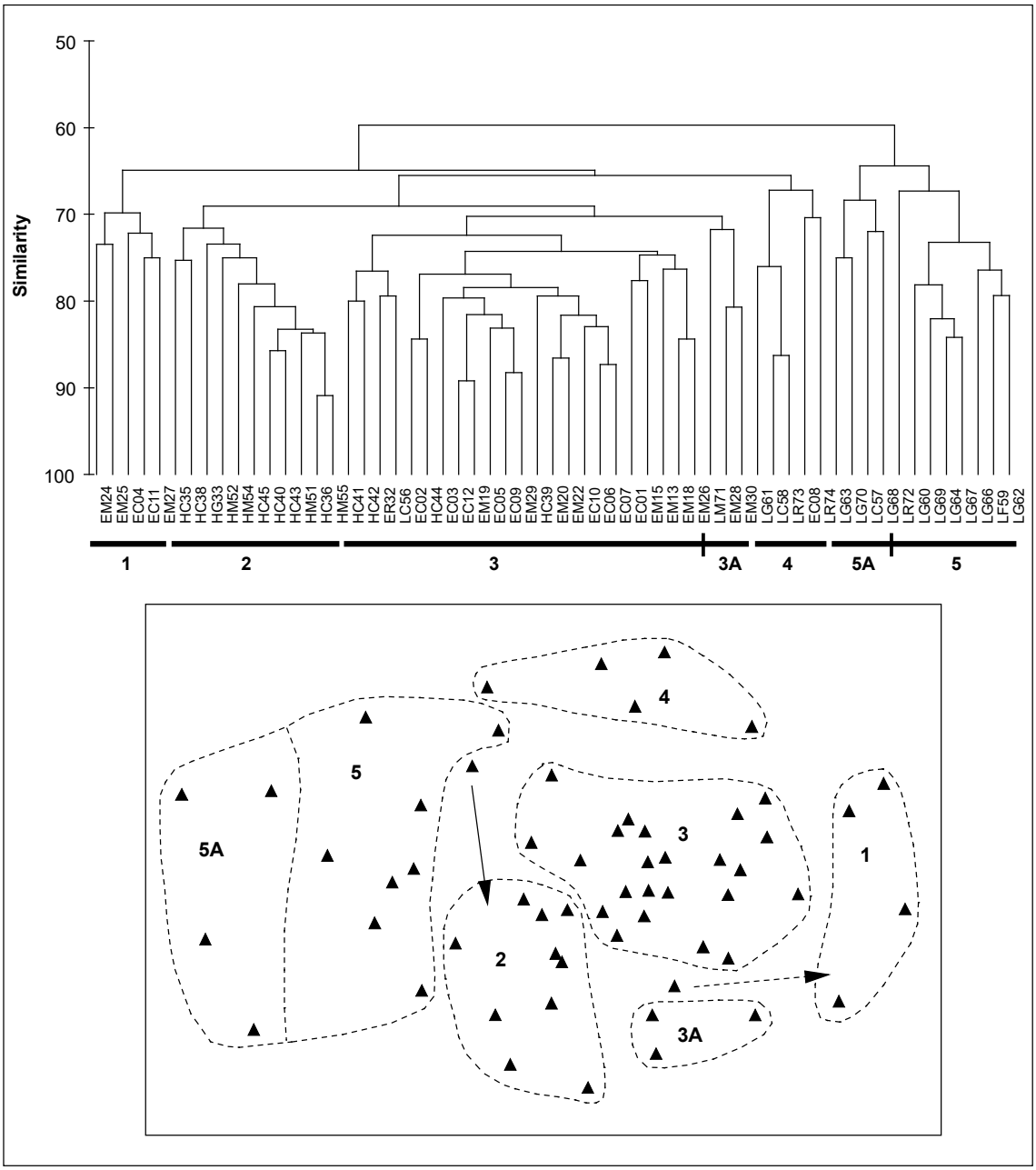


Figure 3.4 **Dendrogram and MDS ordination showing the classification of sites in Mpumalanga based on macroinvertebrate taxa recorded in three seasons (autumn, winter and spring). Codes: primary: E = Great Escarpment Mountain, H = Central Highland, L = Lowveld ecoregion; secondary: M = mountain stream, C = foothill-cobble bed, G = foothill-gravel bed, R = rejuvenated cascade and F = rejuvenated foothill.**

Table 3.5 Taxa contributing to within-group similarity of five main Groups identified from sites in Mpumalanga. Those taxa contributing to the first 50% of the similarity are indicated by ♦; the remaining taxa contributing to the next 40% (i.e. 90% in total) of the similarity are indicated by □.

Group	**1**	**2**	**3**	**4**	**5**
Average similarity	**71.2**	**76.2**	**74.5**	**71.2**	**68.8**
Number of distinguishing taxa	*21*	*35*	*29*	*22*	*30*
Perlidae			□	□	
Baetidae 3 Types	□	□	♦	♦	♦
Caenidae	□	□	♦	□	□
Heptageniidae		□	□	♦	♦
Leptophlebiidae	♦	♦	♦	□	□
Tricorythidae	♦	♦	♦	♦	□
Dytiscidae		□			♦
Elmidae/Dryopidae	♦	♦	♦	♦	♦
Gyrinidae			♦	♦	□
Helodidae Larvae	□		□		
Hydrophilidae		□			□
Psephenidae	□	□	□		
Hydropsychidae 1 Type					□
Hydropsychidae 2 Types		♦	□		
Hydropsychidae 3 Types	□			□	
Hydroptilidae		□			
Philopotamidae		□		□	
Psychomyiidae	♦		□		
Case Caddis 3 Types					□
Athericidae	♦		♦	♦	□
Ceratopogonidae	□	□	□	□	□
Chironomidae	□	♦	♦		♦
Culicidae		□			
Simuliidae	♦	♦	♦	♦	♦
Tabanidae	□	♦	□		□
Tipulidae	♦	□	□	□	
Belastomatidae					□
Corixidae		♦		□	♦
Gerridae		□	□		□
Naucoridae					♦
Notonectidae					□
Pleidae		□			
Veliidae	♦	♦	♦	□	♦
Aeshnidae		♦	□		
Calopterygidae		□			
Chlorocyphidae		□	□	□	
Coenagrionidae		♦	♦		□
Corduliidae					□
Gomphidae		♦	♦	♦	♦
Libellulidae		□	□		♦
Oligochaeta	□	□	□	♦	□

Group	1	2	3	4	5
Hydrachnellae	♦	♦		□	□
Brachyura (Crabs)	□	♦	♦		
Natantia (Shrimps)					□
Planariidae	♦	♦	□	♦	
Ancylidae	□	□	□	□	
Planorbidae		□			
Sphaeriidae		♦			□

3.4.2 Spatial ordination of regional classifications

Macroinvertebrate faunas of the Western Cape exhibited spatial differences with a general separation of lowland sites from those in mountain stream and foothill-cobble bed subregions (Figure 3.5). Macroinvertebrate faunas of Mpumalanga showed a certain degree of concordance with regional classifications (Figure 3.6). Sites within the foothill-gravel bed subregion of the Lowveld eco- and bio-regions consistently separated from sites within the mountain stream and foothill-cobble subregions of the Great Escarpment and Central Highland ecoregions, and Northern Upland bioregion. A single site (HG33) in the foothill-gravel bed subregion of the Central Highlands ecoregion clustered near the other foothill-gravel bed sites. There was limited separation of sites within the Great Escarpment Mountain and Central Highland ecoregions.

3.4.3 Relative classification strength

In the Western Cape, ANOSIM results revealed that macroinvertebrate assemblages from classification classes (e.g. Cape Fold Mountain ecoregion, Fynbos bioregion) within all classifications were significantly different with the exception of the bioregional classification (Table 3.6). The Southern Coastal (S) bioregion was, however, only represented by a single site (SC01). Ecoregion level I had the highest Global R value indicating that this classification had a high within-class similarity and low between-class similarity. Examination of pair-wise results of individual classes within each classification (Table 3.6) revealed that this was largely a reflection of subregional differences, with all upper-catchment sites within the mountain stream and foothill-cobble bed subregions, significantly different from lowland sites. The scarcity of sites in the Southern Coastal ecoregion and bioregion, and lowland subregion, however, limits interpretation of results.

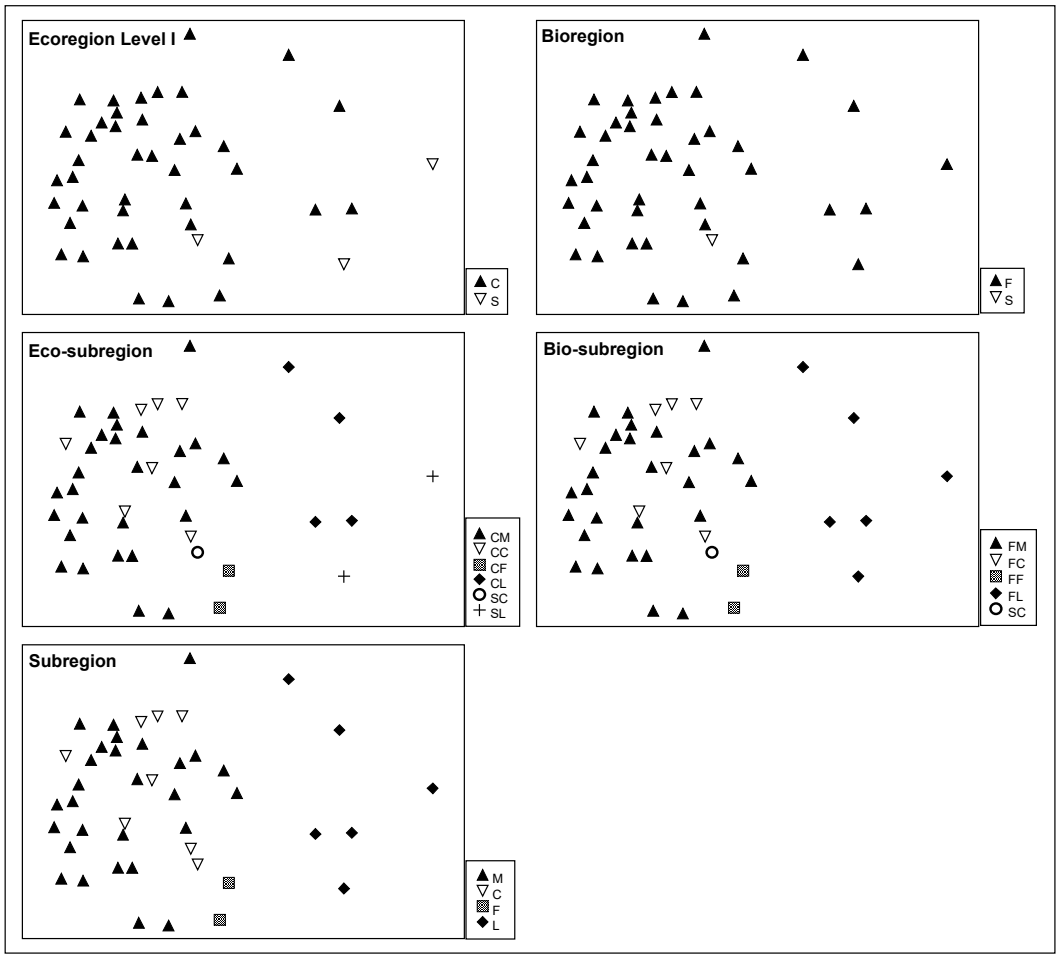


Figure 3.5 Regional patterns in macroinvertebrate distributions on a MDS ordination for the Western Cape coded for five classification systems. Codes: primary: C = Cape Fold Mountains, S = Southern Coastal, F = Fynbos; secondary: M = mountain stream, C = foothill-cobble bed, F = rejuvenated foothill and L = Lowland floodplain, and combinations thereof.

On the basis of these ANOSIM results, certain classification classes were combined for calculation of classification strength. At the eco-subregion level, SC was combined with CC, and at the bio-subregion level, SC was combined with FC. Calculation of classification strength showed that, of the regional classifications, ecoregions had the highest CS, followed by bio-subregions and subregions. In all classifications the hypothesis that there is no class structure was rejected (10 000 permutations, $p < 0.0001$) and macroinvertebrate assemblages were therefore considered more homogenous within than between regions (Figure 3.7). Mean between-class similarity of sites in the mountain stream and foothill-cobble bed subregions was 55%, and thus represented natural

candidates for aggregation (Van Sickle 1997). When these sites were combined such that sites in the mountain stream and foothill-cobble bed subregions were classed together, the classification strength of the resultant classification proved to be slightly higher than that generated through the *a posteriori* classification of sites based on the macroinvertebrate assemblages.

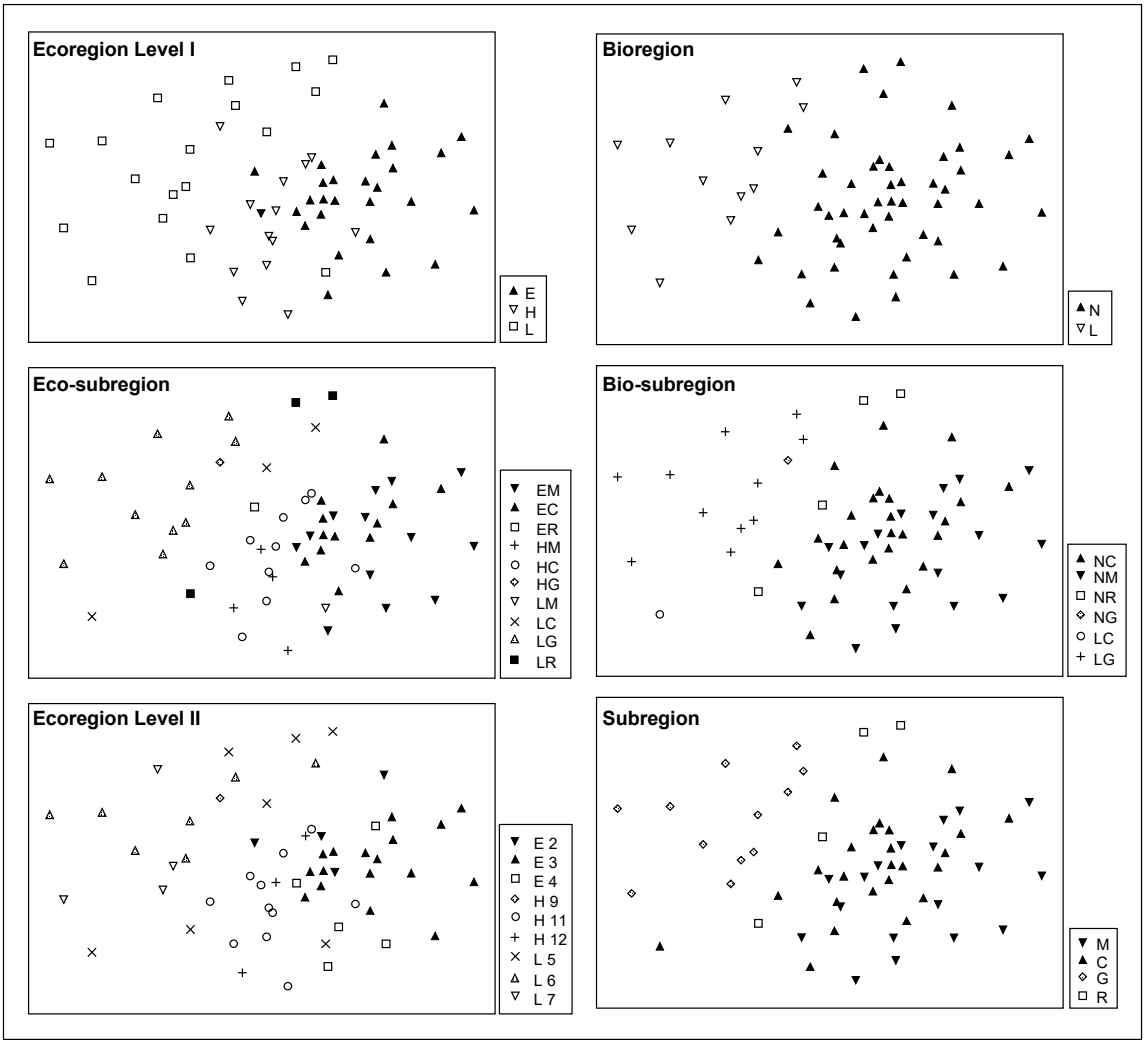


Figure 3.6 Regional patterns in macroinvertebrate distributions on a MDS ordination for Mpumalanga coded for six classification systems. Codes: primary: E = Great Escarpment Mountain, H = Central Highlands, L = Lowveld; N = Northern Uplands; secondary: M = mountain stream, C = foothill-cobble bed, G = foothill-gravel bed and R = rejuvenated cascade, and combinations thereof.

Table 3.6 Analysis of Similarity (ANOSIM) for each classification applied to Western Cape data. The Global R value is given and significant differences amongst individual classification classes, as determined by pair-wise tests, are indicated with shading ($p < 0.05$). Classes in the same column are not significantly different from one another. Codes: primary: C = Cape Fold Mountains, S = Southern Coastal, F = Fynbos; secondary: M = mountain stream, C = foothill-cobble bed, F = rejuvenated foothill and L = Lowland floodplain, and combinations thereof. The number of sites (n) within each classification class are shown.

Classification	Global R	Class	n	Pair-wise differences		
Ecoregion Level I	0.544	C	41			
		S	3			
Bioregion	-0.014	F	43			
		S	1			
Ecoregion I-subregion	0.419	CM	28			
		CC	7			
		CF	2			
		CL	4			
		SC	1			
		SL	2			
Bio-subregion	0.414	FM	28			
		FC	7			
		FF	2			
		FL	6			
		SC	1			
Subregion	0.416	M	28			
		C	8			
		L	6			
		F	2			

In Mpumalanga, preliminary ANOSIM results revealed that sites in the Lebombo Uplands (LU) ecoregion were not significantly different to sites in the Lowveld (L) ecoregion (see Table 2.1). Sites in LU were therefore combined with L sites for subsequent ecoregion analysis and with LG for eco-subregional analysis. Similarly, on the basis of ANOSIM, one rejuvenated foothill (F) site (LF59) was not significantly different from the foothill-gravel bed (G) sites, and LF59 was therefore considered as a G site for all ecoregional analysis (Table 2.1). A single site occurred in the Bushveld Basin (B) bioregion (HC35), but was shown to not be significantly different from sites in the Northern Uplands (N) bioregion (ANOSIM) and was therefore combined with N sites in the bioregional analysis. At ecoregional level II, a single site (EC08) in GEM1 was not significantly different from GEM2 (ANOSIM) and was thus considered as a GEM2 site in the classifications. Details

of these reallocations are provided in Chapter 2, Table 2.1, with new classes given in parenthesis.

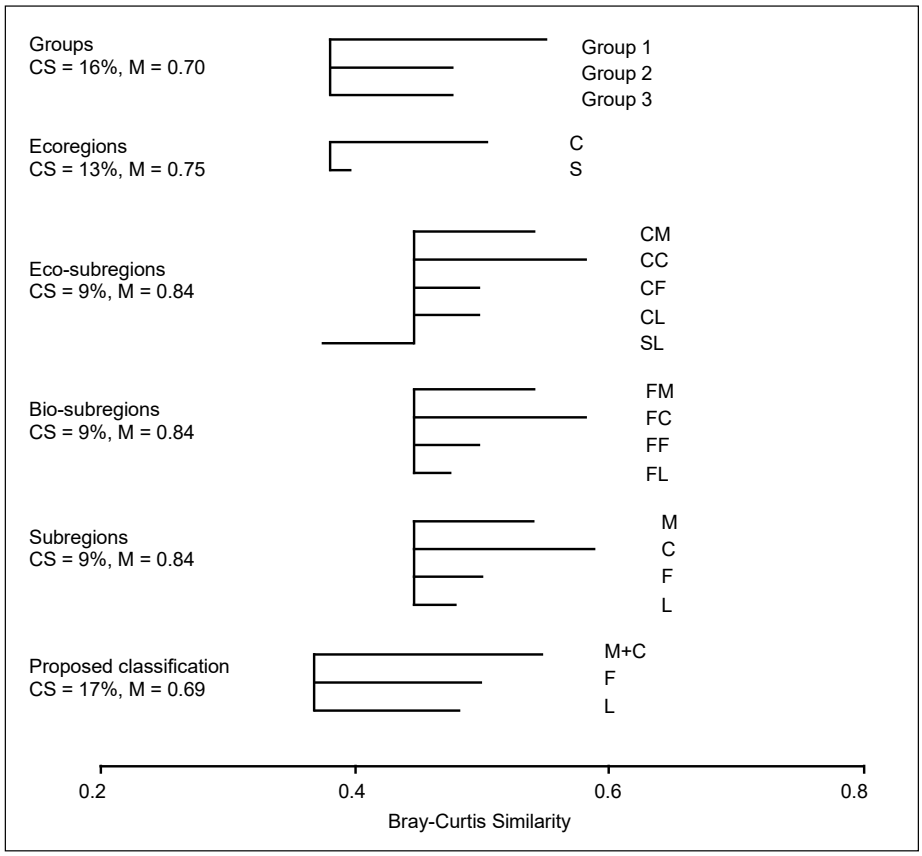


Figure 3.7 **Mean similarity dendrograms of five alternate classifications for macroinvertebrate assemblages of the Western Cape. A sixth classification which combines M and C subregion is proposed. The vertical lines represent the mean between-class similarity (*Bbar*) and the horizontal lines terminate at the mean within-class similarity (W_1). M = *Bbar/Wbar*, where *Wbar* is the overall weighted mean of all within-class similarities. CS (classification strength) = *Wbar-Bbar*. Codes: primary: C = Cape Fold Mountains, S = Southern Coastal, F = Fynbos; secondary: M = mountain stream, C = foothill-cobble bed, F = rejuvenated foothill, L = lowland, and combinations thereof.**

ANOSIM results revealed that macroinvertebrate assemblages from classes within all classifications were significantly different from each other, as indicated by the Global R values (Table 3.7). Bioregions had the highest Global R value (0.622) suggesting that this classification was most successful at increasing within-class similarity and decreasing between-class similarity. Eco-subregional classification had the second highest Global R value (0.520). The results of the classification strength analysis supported the observation that, of the regional classifications, bioregions was the strongest, followed by ecoregions.

In all classifications the hypothesis that there is no class structure was rejected (10 000 permutations, $p < 0.0001$) and macroinvertebrate assemblages were therefore considered more homogeneous within than between regions (Figure 3.8). Comparing these classifications with other studies (Van Sickle & Hughes 2000) on the basis of the M-ratio, however, suggests that all classifications, including the one based on groups, are fairly weak. Classification strength increases progressively as M-ratio decreases from 1.0 to 0. The M-ratios in these analyses were all greater than or equal to 0.86. In some instance, the overall weighted mean of within-class similarities (*Wbar*) is less than the between-class similarities (*Bbar*) suggesting that macroinvertebrates assemblages from sites within the particular class are exceedingly variable. Examples include the rejuvenated cascade sites in the Lowveld ecoregion and sites within L5C of the ecoregion level II classification.

3.4.4 SASS4 Scores, Number of Taxa and ASPT

In the Western Cape, SASS4 Score and ASPT varied significantly among Groups, eco-subregions, bio-subregions, subregions and proposed groups (Kruskal Wallis, Table 3.8). ASPT varied among ecoregions. In Mpumalanga, number of taxa and ASPT varied among Groups and ASPT varied among bioregions and subregions. Pair-wise examination of classes revealed that differences in Groups, i.e. groups of sites with similar macroinvertebrate assemblages, were primarily the result of differences between Groups 1 and 3 in the Western Cape and Groups 1 and 2, 2 and 3, 2 and 4, and 3 and 5 in Mpumalanga (Kolmogorov Smirnov Test). Median values for each Group are given in Table 3.9.

Table 3.7 **Analysis of Similarity (ANOSIM) for each classification applied to Mpumalanga data. The Global R value is given and significant differences amongst individual classification classes, as determined by pair-wise tests, are indicated with shading ($p < 0.05$). Classes in the same column are not significantly different from one another. Codes: primary: E = Great Escarpment Mountain, H = Central Highlands, L = Lowveld, N = Northern Uplands; secondary: M = mountain stream, C = foothill-cobble bed, G = foothill-gravel bed and R = rejuvenated cascade, and combinations thereof. The number of sites (n) within each classification class are shown.**

Classification	Global R	Class	n	Pair-wise differences							
Ecoregion Level I	0.491	E	26		■						
		H	15			■					
		L	18				■				
Bioregion	0.662	N	47		■						
		L	12			■					
Ecoregion I-subregion	0.520	EM	13		■						
		EC	12		■						
		HM	4			■					
		HC	10			■					
		LC	3				■	■	■		
		LG	11					■			
		LR	3						■		
Bio-subregion	0.425	NM	18		■				■		
		NC	24			■					
		NR	3				■				
		LC	1				■	■	■		
		LG	11					■			
Ecoregion Level II	0.509	E2	4		■		■				
		E3	17		■						
		E4	5		■						
		H11	11			■					
		H12	3			■	■				
		L5	7				■	■			
		L6	7					■			
		L7	4					■			
Ecoregion Level II-subregion	0.532	E3M	9	■	■						
		E4M	4	■							
		E2C	3	■	■					■	
		E3C	8		■						
		H11M	4			■					
		H11C	7			■	■				
		H12C	3				■	■		■	■
		L5C	2			■			■	■	■
		L6G	4						■		
		L7G	6						■		
		L5R	3							■	
Subregion	0.362	M	18		■						
		C	25			■					
		G	11				■				
		R	4					■			

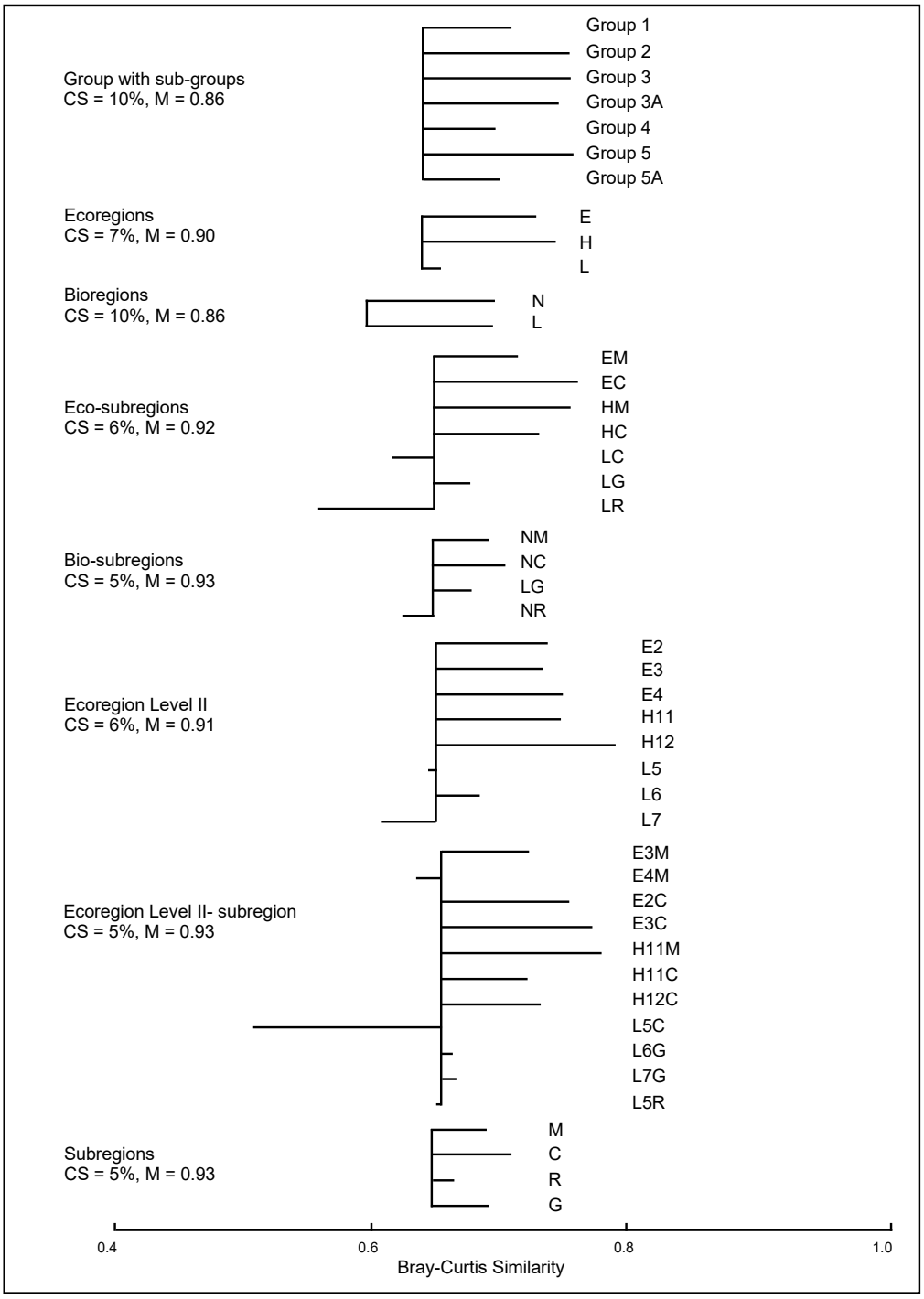


Figure 3.8 **Mean similarity dendrograms of eight alternate classifications for macroinvertebrate assemblages of Mpumalanga. The vertical lines represent the mean between-class similarity (*B*bar) and the horizontal lines terminate at the mean within-class similarity (W_1). M = *B*bar/*W*bar, where *W*bar is the overall weighted mean of all within-class similarities. CS (Classification Strength) = *W*bar-*B*bar. Codes: primary: E = Great Escarpment Mountains, H = Central Highlands, L = Lowveld, N = Northern Uplands; secondary: M = Mountain Stream, C = foothill-cobble bed, R = rejuvenated cascade, G = foothill-gravel bed, and combinations thereof.**

Table 3.8 Results of non-parametric analysis of variance (Kruskal-Wallis test statistic) based on median SASS scores from classes within each classification in the Western Cape (WC) and Mpumalanga (MPU). Significance levels are given, NS = not significant.

Region	Classification	SASS4 Score	Number of Taxa	ASPT
WC	Group	$H = 13.19$; $p < 0.005$	NS	$H = 16.04$; $p < 0.001$
	Ecoregion Level I	NS	NS	$H = 5.53$; $p < 0.05$
	Bioregion	NS	NS	NS
	Eco-subregion	$H = 12.86$; $p < 0.05$	NS	$H = 16.75$; $p < 0.05$
	Bio-subregion	$H = 12.70$ $p < 0.05$	NS	$H = 16.71$; $p < 0.005$
	Subregion	$H = 11.48$; $p < 0.05$	NS	$H = 15.38$; $p < 0.005$
	Proposed	$H = 11.35$; $p < 0.005$	NS	$H = 15.28$; $p < 0.001$
MPU	Group	NS	$H = 14.53$; $p < 0.005$	$H = 19.83$; $p < 0.005$
	Ecoregion Level I	NS	NS	NS
	Bioregion	NS	NS	$H = 5.95$; $p < 0.05$
	Eco-subregion	NS	NS	NS
	Bio-subregion	NS	NS	NS
	Ecoregion Level II	NS	NS	NS
	EcolevII-subregion	NS	NS	NS
	Subregion	NS	NS	$H = 8.56$; $p < 0.05$

Table 3.9 Median values for Groups (i.e. groups of sites with similar macroinvertebrate assemblages) in the Western Cape and Mpumalanga.

	Western Cape			Mpumalanga		
Group	SASS4 Score	No. Taxa	ASPT	SASS4 Score	No. Taxa	ASPT
1	139	16	9.1	148	20	7.4
2	122	15	8.1	174	30	6.5
3	105	16	6.4	180	26	7.1
4	-	-	-	133	20	6.8
5	-	-	-	182	26	6.2

3.5 DISCUSSION

Regional classification of sites, particularly of reference sites, has potential for the management of aquatic resources by providing a framework within which bioassessment is undertaken. This is only true, however, if the regional classification reflects actual spatial differences in the ecosystem component or components being managed. Choice of classification system may, in part, depend on the ease with which new sites can be assigned to classes (Gerritsen *et al.* 2000). Homogeneous regions that are delineated along spatial lines provide for an easier and more logical classification system than non-spatial ones since site membership is determined by the homogeneous region within which a site occurs. The alternative (e.g. classification based on fauna) requires large sets of internally consistent data, obtained from carefully planned and spatially distributed sampling efforts (Van Sickle & Hughes 2000) and site membership is often done by developing predictive models which provide a link between environmental variables and faunal assemblages (e.g. RIVPACS, Wright 1995, AusRivAs, Smith *et al.* 1999).

Studies assessing the ability of spatially-based regional classification systems to partition spatial variability in lotic systems, such that within-class similarity is greater than between-class similarity, differ in their support of the ecological validity of geographic delineaters. Several studies have shown that ecoregions adequately correlate with water chemistry (Ravichandran *et al.* 1996) and macroinvertebrate assemblages (Harding *et al.* 1997, Gerritsen *et al*. 2000, Feminella 2000, Maxted *et al*. 2000, Rabeni & Doisy 2000). By contrast, others have shown that ecoregions cannot adequately explain patterns in water chemistry (Harding *et al.* 1997), macroinvertebrate assemblages (Hawkins & Vinson 2000, Marchant *et al.* 2000) or vegetation (Wright *et al.* 1998). Yet others have observed a degree of congruence between spatial classifications and biotic patterns (Van Sickle & Hughes 2000), streams within an ecoregion being more similar in terms of their vertebrate assemblages than streams in different ecoregions. Van Sickle & Hughes (2000) showed that geographic partitions accounted for a portion of the total variation seen in stream vertebrate assemblages over a large region.

The degree of correspondence between landscape patterns and biota is also in part dependent on the scale of spatial resolution tested (Tate & Heiny 1995, Maxted *et al*. 2000, Johnson 2000). At the broadest scale examined in this study, macroinvertebrate assemblages showed

distinct geographic separation into the Western Cape and Mpumalanga regions as shown by the multivariate analyses, which resulted in separation of sites largely on the basis of geographic region. Exceptions were a few lowland sites in the Western Cape that grouped with Mpumalanga sites. Distinguishing taxa at these sites suggest that this may have been a reflection of taxa often associated with instream and marginal vegetation, such as odonates and hemipterans. In addition, some of these sites support taxa, namely tricorythid mayflies, generally found in more tropical regions. These taxa, together with the shrimps, also characterised several Mpumalanga sites, particularly lowland ones. The distinct differences in distinguishing taxa between the uplands sites of the Western Cape and sites in Mpumalanga appears to be a reflection of biogeographic differences, together with regional variation in the availability of instream and marginal vegetation as habitats for aquatic organisms.

Biogeographically, this regional distinctiveness in macroinvertebrate assemblages is perhaps not unexpected, since the two regions are fairly distinct in many aspects. The Western Cape region has a Mediterranean climate and winter rainfall. The flow regime is described as strongly seasonal, with winter flows peaking in July or August, and low overall predictability (King & Tharme 1994). The distinct sclerophyllous fynbos vegetation and hard, resistant, quartzitic sandstones of the Table Mountain Series in the upper catchments give rivers of this region their characteristic chemical properties. They are dominated by sodium and chloride ions, have a pH less than 7, are poorly buffered, have low conductivity and low concentrations of nutrients, and comparatively high concentrations of humic substances (Dallas *et al.* 1995). Biogeographically, the acid stream fauna of the western and southern Cape comprises the palaeoendemics, referred to as the South Temperate Gondwanian fauna (Harrison 1978), which is largely restricted to this region, together with cold, stenothermal, montane species of the Pan-Ethiopian Afrotropical (sub-Saharan) element (Harrison 1965a, 1965b, 1978). The climatic, botanical and geomorphological characteristics, together with biogeographic features, have contributed to the regional distinctiveness of the macroinvertebrate fauna and to the high degree of endemism within the region (Harrison and Agnew 1962, Wishart & Day in press). This is apparent in the number of endemic families identified as taxa characterising upland sites in the Western Cape.

The north-eastern region, within which Mpumalanga falls, has very different climatic, hydrological, water chemistry and biological characteristics from those of the Western Cape region. Mpumalanga lies in a summer rainfall area, with a flow regime described as

moderate, with mid-summer flows peaking in February, and with a high degree of constancy, high flood predictability and medium to high flood frequency (King & Tharme 1994). Igneous rocks are the main geological formation and rivers in this region are bicarbonate- and calcium-dominated, with near neutral pH, high alkalinity and low conductivity (Dallas *et al.* 1995). The macroinvertebrate fauna is part of the Pan-Ethiopian Afrotropical group (Harrison 1978), and comprises three sub-groups: widespread, hardy species, often associated with marginal vegetation habitats (Harrison 1965b); tropical or warm stenothermal species which has extended southwards from Central Africa into the lowveld of Mpumalanga; and highveld, temperate species characteristic of the elevated "highveld" or central highland regions of Mpumalanga (Harrison 1965b). Regional differences in taxon richness between the Western Cape and Mpumalanga were also apparent, with higher numbers of taxa recorded in the more tropical Mpumalanga region than the temperate Western Cape, a trend also noted in a study comparing species richness from temperate and tropical streams in Australia (Lake *et al.* 1994). This variation in taxon richness between the two geographic regions was reflected in the number of distinguishing taxa, with 17 identified in the Western Cape (Group 1) and 29 in Mpumalanga (Group 5).

Examination of regional classifications within both the Western Cape and Mpumalanga showed that Groups (i.e. groups of sites with similar macroinvertebrate assemblages) were relatively congruent with regional classifications and within-class similarity was consistently higher than between-class similarity. Groups, as measured by mean similarity, however, had greater classification strength than any regional classification. Scarcity of data across eco- and bioregional boundaries in the Western Cape prevented rigorous analysis of these classifications for this region, but in Mpumalanga, bioregions seemed to be better than ecoregions at classifying sites, although all classifications in Mpumalanga were relatively weak. Thus, whilst regional classifications and classifications based on macroinvertebrate assemblages, are capable of partitioning variability in macroinvertebrate assemblages, a considerable amount of variability, not attributable to spatial factors, remains within classification classes.

Several studies have expressed the need for a subregional level (Rabeni & Doisy 2000, Sandin & Johnson 2000) or ecosystem-type (Johnson 2000) classification below that of ecoregions for further reducing spatial variability of faunal assemblages and thereby providing a better understanding of the factors driving biological assemblages. The

subregional level explored in this study, both independently and by combining with ecoregion and bioregion classifications, revealed a high degree of dissimilarity between upper and lower catchment sites. In the Western Cape, incorporating subregions, corresponding to geomorphological zones, into the classification improved the level of within-class similarities. Sites within the two upper catchment subregions, namely mountains stream and foothill-cobble bed, were similar enough to be combined, suggesting that, for the purposes of bioassessment, longitudinal partitioning may be adequately incorporated by separating upland sites from lowland ones. Indeed, studies elsewhere have reported that the clearest differences in biotic assemblages were between montane and non-montane regions (e.g. Ward *et al*. 1994, Tate & Heiny 1995) and ecoregions often partitioned biotic variation best when they differed in topography or climate or both (Hawkins & Vinson 2000). Upper catchments in the Western Cape are known to have a large number of endemic taxa (Harrison 1965a, b), whilst lower reaches are dominated by more widespread, hardy species. Upland sites were clearly dissimilar from lowland sites, but within-class similarity of upland sites was only around 50%, with little distinction between mountain stream and foothill-cobble bed sites. Closer examination of sub-groups within the upland site Group 1 revealed that several taxa, including Teloganodidae, Leptophlebiidae, Elmidae/ Dryopidae, Trichoptera (cased caddis 3 Types), Chironomidae and Simuliidae, were present in all four sub-groups. Several other taxa were characteristic of one sub-group only: the Caenidae, Ecnomidae, Chlorolestidae and Gomphidae of sub-group 1A, the Heptageniidae and Blephariceridae of sub-group 1C, and the Gyrinidae, Limnichnidae and Athericidae of sub-group 1D. Others were not identified as distinguishing taxa from one or other group, including Notonemouridae and Corydalidae (absent from sub-group 1A) and Helodidae (absent from sub-group 1D). Mountain stream channels are chaotically and complexly structured (Grant *et al.* 1990 cited by Hawkins *et al.* 1997) and differences in the availability of habitat or biotopes, and differences in temperature, flow and/or water chemistry, may be contributing to the observed variability in macroinvertebrate assemblages. Hawkins *et al.* (1997) suggest that in mountainous landscapes local processes may be strong enough to mask patterns that would have otherwise emerged in more homogenous landscapes, i.e. they contribute to sampling-scale patchiness. One such local "process", namely availability of SASS-biotopes, is explored in Chapter 4, whilst spatial variability in upland sites is examined further in Chapter 7.

Similar subregional trends were noted for Mpumalanga, with mountain stream and foothill-cobble bed sites showing a high degree of between-class similarity within eco- or bioregional

classes. Upper-catchment sites were approximately 65% similar although differentiation on the basis of longitudinal location was less clear-cut than in Western Cape sites. Differences in SASS scores among classification classes were largely found to be significant only between upper and lower catchment classes. Regional differences in Mpumalanga partially reflected a broad biogeographic pattern of two sub-groups, described by Harrison (1965b) as a highveld, temperate species assemblage and a tropical or warm stenothermal species assemblage of the lowveld. Species belonging to the palaeoendemics, and associated with the escarpment by Harrison (1965b), probably also contributed to the observed separation of macroinvertebrate assemblages from Mpumalanga into Groups 2 and 3. Thirteen distinguishing taxa, including perlid stoneflies, helodid larvae, psychomyiid caddisflies and athericid dipterans, all of which are comparatively sensitive species, were either in Group 2 or 3. This may be a reflection of altitudinal differences, with average altitude at Group 2 sites 200 m higher than at Group 3 sites. Other environmental characteristics of the sites, such as substratum features, may also influence the macroinvertebrate assemblages. The depth of the riffle biotope within the SIC/SOOC biotope-group differed, the average depth at Group 2 sites being 0.17 m, and at Group 3 sites 0.27 m. The relative percentage of bedrock versus cobble substratum differed between groups, with Group 2 sites having relatively more bedrock than Group 3 sites, and the opposite true for cobbled sites. The environmental variables responsible for the observed Groups are examined in more detail in Chapter 6. Transitional regions, i.e. between, for example, ecoregion boundaries, typically have intermediate water qualities and biota, and thus confound the conformity between ecoregions and biotas (Hughes & Larsen cited by Johnson 2000). Group 1 of Mpumalanga may represent such a transitional group and have similarities to at least two other groups. Clearest differentiation of sites was between upland and lowland ones, almost certainly a reflection of differences in altitude together with the associated biogeographic differences.

In summary, cluster and ordination analysis, together with analysis of classification strength of the different regional and faunal classifications, suggest that macroinvertebrate assemblages correspond to regional classifications. Both ecoregions, based on terrestrial geographic delineaters, and bioregions, based on biogeographical and physical features, partitioned spatial variability such that within-class similarity exceeded between-class similarity. Of the two, bioregions had a higher classification strength than ecoregions, although *a posteriori* analysis of macroinvertebrate assemblages suggested the separation of macroinvertebrate assemblages into more groups than were evident from the bioregion

classification. Spatial variability was further partitioned when a second spatial level, that of subregion, was included. The disparity between eco-subregional and bio-subregional classes and the groups derived by *a posteriori* classification of macroinvertebrate assemblages, suggests, that whilst regional classifications partitioned some of the variability, other factors were contributing to the similarities and dissimilarities of sites. These factors may be at the level of river type, and be related to aspects such as stream width, stream depth, substratum composition, biotope availability, hydrological type and canopy cover. The relative influence of geographic patterns, and site-specific physical, chemical and biological characteristics, on the presence and absence of species has been much debated (Gerritsen *et al*. 2000). This is considered further in Chapter 6 by examining the relationship between environmental variables and macroinvertebrate assemblages.

Spatial classifications such as ecoregions, therefore, offer geographic partitions within which to expect somewhat similar conditions but, as Gerritsen *et al*. (2000) concluded, classification of sites should be an iterative process that includes generation of hypotheses, exploratory data analysis, and subsequent evaluation and modification of hypotheses. In this way subjective, *a priori* regional classifications may be modified on the basis of independent, objective *a posteriori* classification of biological assemblages. Regional reference sites selected within the context of the hierarchical spatial framework developed in South Africa are likely to be more representative of specific river types than those selected without using the spatial framework. The within-group variability of macroinvertebrate assemblages is thus likely to be reduced, facilitating more sound comparisons with monitoring sites and thus improving assessment of water quality impairment and reduced river condition. It is apparent, however, that independent and objective *a posteriori* classification of macroinvertebrate assemblages provides information on variables at local levels, such as the importance of substratum type and habitat, which are not necessarily evident from regional classifications.

CHAPTER 4. BIOTOPE AVAILABILITY AND THE UTILISATION OF SASS-BIOTOPES BY MACROINVERTEBRATE TAXA: IMPLICATIONS FOR AQUATIC BIOASSESSMENT

4.1 INTRODUCTION

Spatial heterogeneity is a feature of lotic environments and organisms, such as benthic macroinvertebrates, that inhabit such environments often have a heterogeneous distribution. Understanding this heterogeneity requires examination of factors that may potentially affect the distribution of benthic macroinvertebrates. These factors vary from broad-scale ones at the level of catchment such as geology or climate (Richard *et al*.1997), through to reach-type characteristics such as channel type or riparian canopy cover, down to habitat features such as habitat-type, substratum-type, water depth, and water velocity (Poff & Ward 1990).

Habitat refers to the environment in which an aquatic organism lives and may incorporate aspects such the substrate-type, hydraulic and chemical conditions. The habitat features examined vary amongst studies and include, for example, mesohabitats (Armitage *et al.* 1995), hydraulic biotopes (Padmore 1998), substrate-types (Collier 1995) and SASS-biotopes (South African Scoring System: Chutter 1998, Dallas 1997). SASS-biotopes are specific aquatic macroinvertebrates habitats, which are sampled in the SASS bioassessment protocol (Chutter 1998, Dallas 2000a, b). Habitat availability, or more specifically SASS-biotope availability, may influence bioassessment results. This may simply be a reflection of biotope availability, or it may be preferential utilisation of biotopes resulting from specific substrate or hydraulic requirements of the relevant macroinvertebrate taxa (Poff & Ward 1990).

Although there is limited information available on trends in biotope utilisation by macroinvertebrates in South Africa (Palmer *et al.* 1991, Dallas 1997), studies elsewhere have documented differences, particularly in taxon richness, amongst different biotopes (e.g. Collier 1995, Humphries 1996, Pinder *et al.* 1987, Chessman *et al*. 1997, Kay *et al.* 1999). There is an intuitive acknowledgement that biotope characteristics affect the distribution of macroinvertebrate assemblages in riverine ecosystems and groups of species

have been associated with particular biotopes sufficiently often to permit recognition of biotope-assemblage associations (Palmer *et al.* 1991). Historically, studies have acknowledged this and restricted sampling to identifiable biotopes such as riffles which are likely to be inhabited by distinct species assemblages. In this way spatial variability at the level of habitat is limited, thereby allowing the elucidation of other factors potentially causing differences in macroinvertebrate assemblages between sites.

Biotope types are often differentiated on the basis of hydraulic and substratum characteristics. Biotopes may be erosional, for example riffles, waterfalls, macrophytes in-current; or depositional, including stony backwaters and pools. Three broad biotope types, including stony-bottom biotopes, aquatic macrophytes and sandy biotopes, are commonly sampled. Of these, stony-bottom biotopes and macrophytes generally support a diverse array of macroinvertebrates (e.g. Pridmore & Roper 1985, Wohl *et al.* 1995, Humphries 1996), with more species often recorded from stony-bottom biotopes than from macrophytes (Collier 1995). Sandy biotopes generally support very few invertebrates (Quinn & Hickey 1990, Brewin *et al.* 1995). In addition to substrate differences, local variations in stream flow may be important, with certain taxa better suited to lentic-type habitats, such as backwaters and slow-flowing pools, whilst others are dependent on lotic habitats such as riffles and vegetation-in-current. Several aquatic organisms have morphological adaptations that allow them to occupy a specific hydraulic- and/or substrate-type. Local variation in both of these components may translate into differences in macroinvertebrate assemblages and thus into differences in biotic indices such as SASS scores. Indeed, it has been shown that biotope-related differences in taxon richness affect SASS4 Score, although it has less effect on ASPT (Armitage *et al.* 1983, Chessman *et al*. 1997, Dallas 1997).

Historically, bioassessment was often site-based, i.e. all available biotopes were sampled together, often in proportion to their representation at the site, and biotic index values were calculated for the site as a whole (e.g. Wright 1995, SASS Versions 2 and 3, Chutter 1998). Data interpretation of results from multiple-biotope sampling is often problematic, however, since mixing macroinvertebrates from several biotopes yields samples of unknown heterogeneity (Karr 1999) and sampling a variable number of biotopes may confound the detection of biological impairment because of unequal sampling effort (Parsons & Norris 1996). More recently, major biotopes have been sampled separately

because each biotope has a characteristic macroinvertebrate assemblage and within a given region, differences in assemblages among biotopes are greater than differences in assemblages between sites (Smith *et al*. 1999).

An alternative to sampling all available biotopes is to confine sampling to a particular biotope and thereby to reduce variability resulting from biotope differences (Plafkin *et al.* 1989, Karr 1999) and redundancy associated with multiple-biotope sampling (Parsons & Norris 1996, Hewlett 2000). Problems arise, however, if the specific biotope is not present at all sites assessed or if an anthropogenic disturbance is specific to a particular biotope, and sampling is restricted to a different biotope, then the measurement of human impact may be biased (Kerans *et al.* 1992). For example, Pettigrove (1990, cited by Growns *et al*. 1997) noted that macroinvertebrate assemblages in riffles and pools had differing sensitivities to different environmental disturbances, with nutrient enrichment and removal of riparian vegetation having the greater impact on riffle assemblages, whilst increased turbidity levels had a greater impact on pool assemblages. Given these limitations it seems advantageous to sample all available biotopes, but in a way that allows potential spatial differences resulting from biotope differences to be taken into account.

The aim of this chapter is to examine the influence of the availability of SASS-biotopes at a site on the occurrence of individual taxa, on macroinvertebrate assemblages and on SASS scores. Specifically, it aims to 1) examine the frequency of occurrence of SASS-taxa amongst SASS-biotopes and to compare the results from two geographic regions; 2) ascertain if differences in macroinvertebrate assemblages amongst SASS-biotopes are greater than differences between sites within a region; and 3) examine the effect of SASS-biotope availability on SASS scores. Spatial variability in macroinvertebrate assemblages at the level of SASS-biotopes is discussed in relation to ecological reference conditions and their use in the interpretation of bioassessment data.

4.2 STUDY AREA

Fifty-six sites, situated on 37 rivers, were sampled (Table 4.1). Of these, 14 sites were situated on rivers in the Western Cape region and 42 were on rivers in Mpumalanga. Only minimally-impacted sites, with respect to anthropogenic disturbance, were selected in both regions so that any observed differences in macroinvertebrate assemblages or SASS scores

would not reflect water quality conditions. Sites in the Western Cape were assessed with variable frequency during 1994 and 1995 (total number of assessments = 35, see Appendix A), whilst sites in Mpumalanga were each assessed on three occasions (May, July and September) in 1999 (total number of assessments = 122). Details of the sites assessed in each of the regions are provided in Table 4.1. A subset of sites from Mpumalanga was used in certain analyses as specified in the appropriate section below.

Table 4.1 Sites assessed during this study indicating river and geographic region. The codes for sites on each river are given in parentheses and relate to Table 2.1 and Figures 2.1 and 2.2 in Chapter 2.

Geographic Region	River
Mpumalanga	Alexanderspruit (HC42), Blyde (EC01, EC02), Crocodile (EC06, ER32, HC43, LG60, LG62, LG63, LG64), Dorps (HC36), Elandsfonteinspruit (HM51), Ga-Selati (EM20), Grootfonteinspruit (EM16), Kareekraalspruit (HM52), Kgwete (EM18), Klein-Sabie (EC10), Klip (HC38), Mac-Mac (EC11, LC56), Maritsane (LM71), Nelspruit (EM22, LR72), Ohrigstad (EM19), Sabie (EC12, LC58, LG66, LG68, LR74), Sand (EM29, LG69, LG70), Spekboom (EM13), Sterkspruit (EM14), Tautesloop (HM54), Treu (EC03), Unspecified (EM15, EM24, EM28, EM30), Waterval (HC41) and Wilgekraalspruit (HM55)
Western Cape	Assegaaibosch (CM01), Berg (CM02, CM03, CC01), Eerste (CM04), Elandspad (CM09), Kraalstroom (CM10), Lang (CM05), Molenaars (CC03, CC04), Palmiet (CM07, CF01), Riviersonderend (CM20) and Window (CM06)

4.3 MATERIALS AND METHODS

4.3.1 Benthic macroinvertebrates: SASS4 sampling

Benthic macroinvertebrates were sampled using the qualitative rapid bioassessment method, SASS4 (South African Scoring System). A detailed description of the method is given in Chapter 2. SASS-defined biotopes include stones-in-current (SIC), stones-out-of-current (SOOC), marginal vegetation (MV), aquatic or instream vegetation (AQV), gravel (G), sand (S) and mud (M). In the Western Cape, all available SASS-biotopes were sampled separately with the exception of gravel, sand and mud, which were combined into one group. Biomonitoring practitioners have, however, suggested that such biotope differentiation is

impractical, and they commonly sample only three SASS biotope-groups, namely stones-in-current/stones-out-of-current (SIC/SOOC), aquatic and marginal vegetation (AQV/MV), and gravel/sand/mud (G/S/M). Since data in Mpumalanga were collected in collaboration with these practitioners, sampling in Mpumalanga was undertaken using these biotope-groups.

4.3.2 Data analysis

Frequency data

The relative frequency of occurrence of each SASS-taxon was calculated separately for the Western Cape and Mpumalanga. Since SASS-biotope availability varied amongst sites in the Western Cape, the frequency of occurrence of each SASS-taxon was calculated relative to biotope availability, i.e. relative to the number of times the particular SASS-biotope was sampled. For Mpumalanga data, only sites at which all three SASS biotope-groups were sampled were selected for the analysis (n = 122). In both instances, taxa recorded on less than 5 sampling occasions across the range of sites were omitted from the analysis. The frequency of occurrence of a SASS-taxon within a SASS-biotope is expressed relative to its frequency of occurrence in other SASS-biotopes. For regional comparisons, data from the Western Cape were combined into the three SASS biotope-groups (i.e. SIC/SOOC, AQV/MV and G/S/M), as per the Mpumalanga dataset.

Analysis of macroinvertebrate assemblages

Cluster analysis and non-metric multidimensional scaling (MDS) were used to examine similarities amongst SASS-biotopes and sites based on macroinvertebrate assemblage composition (Clark & Warwick 1994). Analysis of faunal data was undertaken per biotope-group and analysis was done separately for each of two seasons (autumn and spring) and for each geographic region. The following norms have been used for seasonal groupings: spring = September, October and November and autumn = March, April and May. A subset of sites, which were most similar in regional and abiotic characteristics to those of the Western Cape, was selected from the Mpumalanga dataset. Data were transformed using the presence/absence transformation (*PRIMER Version 5*) and the Bray-Curtis coefficient was used on these transformed data. Hierarchical agglomerative clustering, using group-average linking, was used on the data matrix. Ordination of samples by MDS was undertaken, and stress values used to assess the reliability of the MDS ordination. One-way ANOSIM was used to test whether or not there were significant

differences in assemblage structure amongst biotope-groups. The ANOSIM tests were performed on presence/absence transformed data, analysed using the Bray-Curtis measurement of similarity. The distinguishing taxa responsible for the similarity within groups of sites and the dissimilarity amongst groups of sites were established using SIMPER (*PRIMER Version 5*). Those taxa responsible for 90% within-group similarity or dissimilarity were examined.

SASS4 Scores, Number of Taxa and ASPT

SASS scores for each SASS biotope-group were compared with those calculated for the site (i.e. by combining taxa recorded in each separate SASS biotope-group). The sub-set of sites from Mpumalanga was used to calculate median SASS scores for each season. These were compared statistically using the non-parametric Kruskal-Wallis Test. Individual pairs of SASS biotope-groups were compared using the non-parametric Kolmogorov-Smirnov Test. The results of all analyses were considered significant at $p < 0.05$.

4.4 RESULTS

4.4.1 Frequency of occurrence of each SASS-taxon amongst SASS biotope-groups

The frequency of occurrence of each SASS-taxon in each SASS biotope-group has been tabulated for the Western Cape and Mpumalanga (Table 4.2). Certain taxa are more frequently recorded in one biotope-group than in either of the others (relative % > 50%), whilst others occurred across two or three biotope-groups. In the Western Cape, the SIC/SOOC biotope-group supported the highest number of biotope-specific taxa, whilst in Mpumalanga, the SIC/SOOC and AQV/MV biotope-groups supported equal numbers of biotope-specific taxa. Examination of within-biotope-group differences in the frequency of occurrence of SASS-taxa (relative % > 60%), i.e. SIC versus SOOC, AQV versus MV, for the Western Cape (Table 4.3), showed that several taxa were more common in one or the other SASS-biotope.

Comparing patterns observed in the Western Cape with those in Mpumalanga, it seems that several SASS-taxa show similar preferences with respect to SASS biotope-groups. Generally, families within the orders Plecoptera, Ephemeroptera, Coleoptera and Trichoptera, showed a preference for SIC/SOOC, whilst families within the orders

Hemiptera and Odonata, showed a preference for AQV/MV. Families endemic to the Western Cape (Notonemouridae and Teloganodidae) and those restricted to Mpumalanga (Perlidae and Psephenidae) showed a preference for SIC/SOOC.

Table 4.2. Relative frequency of occurrence (expressed as a percentage) of each SASS-taxon in each SASS biotope-group (SIC/SOOC = stones-in-current/stones-out-of-current, AQV/MV = aquatic and marginal vegetation, and GSM = gravel/sand/mud) for the Western Cape and Mpumalanga. Shading indicates frequency of occurrence across biotope-groups (i.e. highest frequency of occurrence in one biotope-group or equal frequency in two or all three biotope-groups). A dash (-) indicates insufficient data, i.e. taxa recorded < 5 times. A blank cell indicates that the taxon does not occur in the geographic region. The number of sampling occasions per biotope-group (n) is given.

Region	**Western Cape**			**Mpumalanga**		
Biotope	**SIC/SOOC**	**AQV/MV**	**GSM**	**SIC/SOOC**	**AQV/MV**	**GSM**
n	*34*	*18*	*7*	*122*	*122*	*122*
Notonemouridae	**74**	26	0			
Perlidae				**86**	6	8
Baetidae 1 Type	20	22	**58**	14	27	**59**
Baetidae 2 Types	**33**	**43**	**24**	**34**	**33**	**33**
Baetidae 3 Types	**65**	35	0	**39**	**39**	**22**
Caenidae	**37**	**23**	**40**	**34**	**30**	**35**
Teloganodidae	**69**	31	0			
Heptageniidae	**100**	0	0	**54**	29	17
Leptophlebiidae	**73**	27	0	**56**	14	30
Tricorythidae	-	-	-	**57**	28	15
Dytiscidae	**10**	**39**	**50**	4	**59**	38
Elmidae/Dryopidae	**55**	45	0	**58**	19	24
Gyrinidae	21	**79**	0	27	**63**	10
Helodidae Larvae	**76**	24	0	23	**63**	13
Hydraenidae	**57**	43	0	0	**50**	**50**
Hydrophilidae	-	-	-	8	**60**	32
Limnichnidae	**68**	32	0	-	-	-
Psephenidae				**76**	8	15
Corydalidae	**100**	0	0			
Ecnomidae	**79**	21	0	-	-	-
Hydropsychidae 1 Type	**51**	**49**	0	**37**	**34**	**29**
Hydropsychidae 2 Types	**100**	0	0	**67**	17	16
Hydropsychidae 3 Types	**100**	0	0	**82**	9	9
Hydroptilidae	-	-	-	32	**56**	12
Philopotamidae	**100**	0	0	**93**	7	0
Psychomyiidae				**80**	4	16
Case Caddis 1 Type	**49**	**33**	**17**	**28**	**39**	**33**
Case Caddis 2 Types	**55**	45	0	18	**54**	28
Case Caddis 3 Types	29	**54**	17	16	**74**	11
Athericidae	**74**	26	0	**60**	17	23
Blephariceridae	**100**	0	0	**93**	0	7
Ceratopogonidae	-	-	-	**31**	**22**	**47**
Chironomidae	**42**	**37**	**21**	**38**	**27**	**35**
Culicidae	-	-	-	5	**86**	10
Dixidae	**44**	**56**	0	0	**96**	4
Muscidae	-	-	-	**75**	0	25

Region	Western Cape			Mpumalanga		
Biotope	**SIC/SOOC**	**AQV/MV**	**GSM**	**SIC/SOOC**	**AQV/MV**	**GSM**
Simuliidae	**47**	**38**	15	**45**	**40**	15
Tabanidae	-	-	-	**76**	5	20
Tipulidae	**73**	0	27	**51**	11	37
Belastomatidae	41	**59**	0	0	**100**	0
Corixidae	**20**	**33**	**48**	**29**	**29**	**43**
Gerridae	35	**65**	0	11	**80**	9
Naucoridae	**24**	**46**	**30**	**25**	**39**	**36**
Nepidae	-	-	-	0	**83**	17
Notonectidae	41	**59**	0	18	**68**	14
Pleidae	-	-	-	6	**88**	6
Veliidae	30	**70**	0	14	**65**	21
Aeshnidae	**58**	42	0	**74**	8	18
Calopterygidae	-	-	-	29	**71**	0
Chlorocyphidae	-	-	-	**39**	**41**	**20**
Chlorolestidae	35	**65**	0	-	-	-
Coenagrionidae	26	**74**	0	3	**78**	18
Corduliidae	-	-	-	**30**	**20**	**50**
Gomphidae	8	15	**77**	37	10	**53**
Libellulidae	**26**	**43**	**31**	**50**	**24**	**26**
Zygoptera Juveniles	-	-	-	14	**67**	19
Oligochaeta	**79**	0	21	**47**	9	**44**
Hydrachnellae	**45**	0	**55**	**51**	33	16
Amphipoda	**70**	30	0			
Brachyura (Crabs)	**68**	32	0	**67**	17	16
Natantia (Shrimps)	-	-	-	20	**65**	15
Planariidae	**100**	0	0	**57**	18	25
Porifera (Sponges)	-	-	-	**60**	20	20
Ancylidae	-	-	-	**53**	29	18
Planorbidae	-	-	-	8	**75**	17
Physidae	**19**	**36**	**46**	-	-	-
Sphaeriidae	-	-	-	26	22	**52**

4.4.2 Analysis of macroinvertebrate assemblages

In the Western Cape, cluster analyses for autumn and spring showed separation by SASS biotope-group, although groupings were less defined in spring (Figure 4.1). MDS ordination (Figure 4.2) supported the results obtained from the cluster analysis for autumn (3D Stress = 0.14) and spring (3D-Stress = 0.12). Autumn faunal samples separated into three groups. A "group" is the term used to describe a group of sites that have similar macroinvertebrate assemblages. The GSM biotope-group was 80% dissimilar from either SIC/SOOC or AQV/MV, whilst SIC/SOOC and AQV/MV were 60% dissimilar. Spring grouping was less distinct, with three of the four GSM samples >85% dissimilar from other samples and three AQV/MV samples were 75% dissimilar from other samples. The remainder were at least 40% similar, and comprised two sub-groups. The first consisted of three AQV/MV and 15 SIC/SOOC samples and at the second was a mixed group

consisting of three AQV/MV and four SIC/SOOC samples. Results of the ANOSIM analysis revealed that the differences between macroinvertebrate assemblages in the three SASS biotope-groups were statistically significant (autumn: Global R = 0.758, $p < 0.01$, spring: Global R = 0.647, $p < 0.01$).

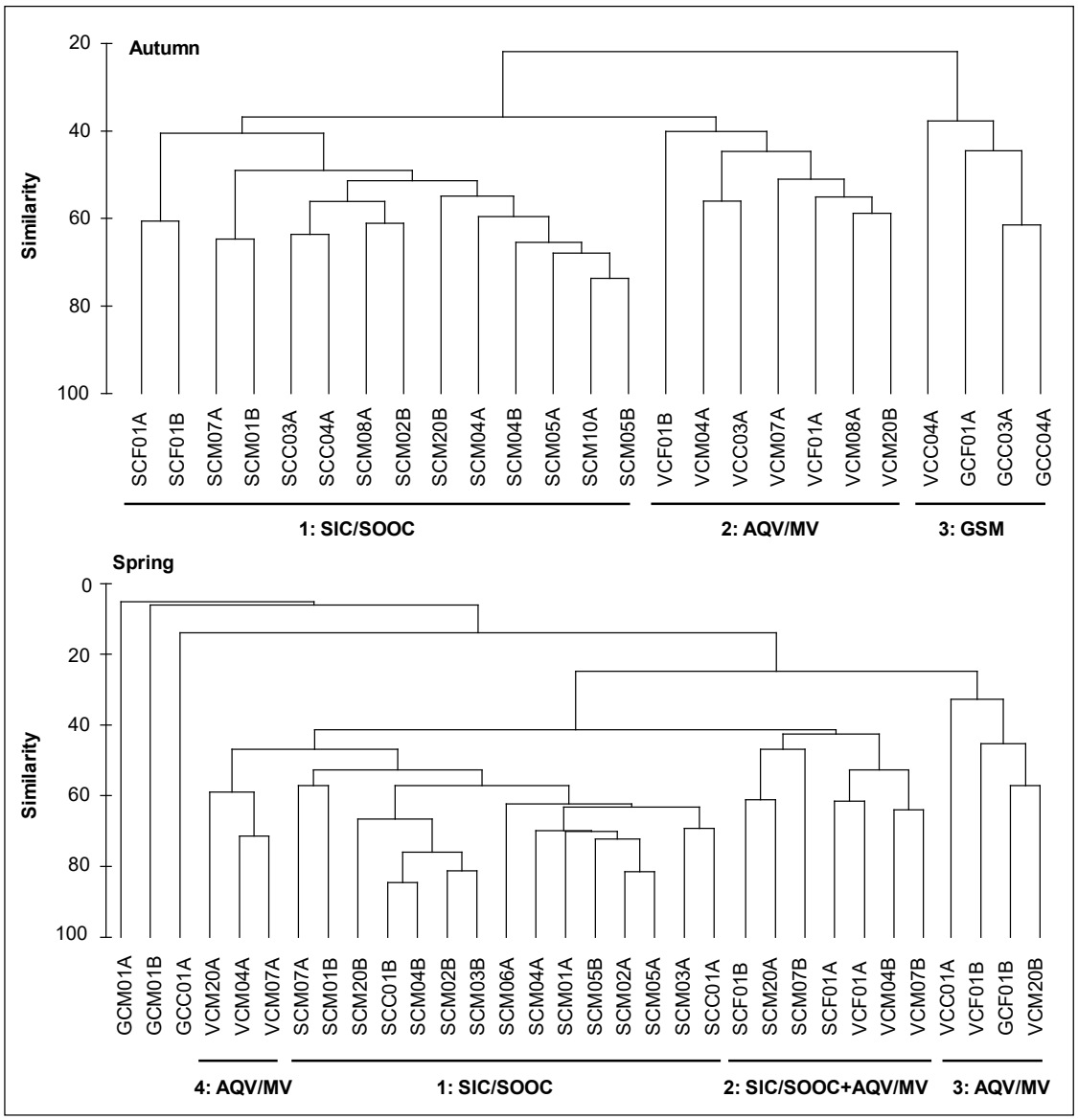


Figure 4.1 Dendrogram showing the classification of sites in the Western Cape based on taxa recorded in each SASS biotope-group on each sampling occasion in autumn (11 sites, 14 sampling occasions) and spring (10 sites, 19 sampling occasions). The site code is prefaced with the biotope-group as follows: S = SIC/SOOC, V = AQV/MV and G = GSM. For autumn sampling, the year follows the site code: A = 1994, B = 1995; whilst for spring, the sampling month follows the site code: A = September, B = November.

Table 4.3. Relative frequency of occurrence (as a percentage) of each SASS-taxon in each SASS-biotope: SIC, SOOC, AQV, MV and GSM for the Western Cape. Shading highlights frequency of occurrence across each pair of biotopes (i.e. >60% frequency of occurrence in one biotope, or equal frequency within both biotopes comprising the biotope-group). The number of sampling occasions per biotope (n) is given.

Biotope	**SIC**	**SOOC**	**AQV**	**MV**	**GSM**
n	*33*	*33*	*8*	*17*	*7*
Notonemouridae	**49**	22	**20**	9	0
Baetidae 1 Type	**16**	**21**	**11**	**15**	37
Baetidae 2 Types	**20**	**22**	**19**	**24**	15
Baetidae 3 Types	**47**	11	15	**28**	0
Caenidae	3	**28**	**29**	7	33
Teloganodidae	**38**	**35**	**14**	**13**	0
Heptageniidae	**57**	**43**	0	0	0
Leptophlebiidae	**40**	**33**	**17**	11	0
Dytiscidae	0	**10**	0	**40**	49
Elmidae/Dryopidae	**34**	11	**29**	**25**	0
Gyrinidae	0	**20**	0	**80**	0
Helodidae Larvae	**33**	**30**	**37**	0	0
Hydraenidae	**40**	9	**24**	**28**	0
Limnichidae	**54**	16	0	**30**	0
Corydalidae	**83**	17	0	0	0
Ecnomidae	9	**54**	**37**	0	0
Hydropsychidae 1 Type	**26**	6	**47**	22	0
Hydropsychidae 2 Types	**42**	**58**	0	0	0
Hydropsychidae 3 Types	**83**	17	0	0	0
Philopotamidae	**100**	0	0	0	0
Case Caddis 1 Type	**30**	**23**	10	**25**	12
Case Caddis 2 Types	**21**	**17**	**53**	8	0
Case Caddis 3 Types	**10**	**12**	**39**	**28**	11
Athericidae	**50**	20	10	**19**	0
Blephariceridae	**85**	15	0	0	0
Chironomidae	**26**	17	**23**	**22**	13
Dixidae	0	**44**	0	**56**	0
Simuliidae	**34**	6	**27**	**23**	10
Tipulidae	**46**	**30**	0	0	24
Belastomatidae	0	**29**	**30**	**42**	0
Corixidae	0	**18**	9	**30**	42
Gerridae	0	**34**	0	**66**	0
Naucoridae	5	**15**	20	**38**	23
Notonectidae	10	**31**	0	**59**	0
Veliidae	**18**	11	0	**71**	0
Aeshnidae	**35**	22	13	**30**	0
Chlorolestidae	8	**25**	**34**	**32**	0
Coenagrionidae	0	**18**	**36**	**46**	0
Gomphidae	4	**8**	0	**15**	73
Libellulidae	**19**	7	**29**	**23**	22
Oligochaeta	**38**	**42**	0	0	20
Hydrachnellae	**31**	**21**	0	0	49
Amphipoda	**34**	**40**	0	**26**	0
Brachyura (Crabs)	**29**	**43**	0	**28**	0
Planariidae	30	**70**	0	0	0
Physidae	**13**	6	**26**	**25**	30

Taxa contributing to within-group similarity varied amongst biotope-groups, particularly in autumn (Table 4.4). Of the twenty-one contributing taxa that were distinct either to SIC/SOOC or AQV/MV in autumn, 12 were important in the SIC/SOOC biotope group and nine in the AQV/MV biotope-group. In spring, only seven taxa in the exclusively SIC/SOOC biotope-group, i.e. Group 1, did not contribute to similarity of macroinvertebrate assemblages at sites in other groups which included AQV/MV biotope-groups. Most notable contributing taxa, exclusive to the SIC/SOOC biotope-group were the Notonemouridae, Heptageniidae, Corydalidae, Philopotamidae, Athericidae, Blepahriceridae and Tipulidae. Taxa contributing to 50% similarity of the GSM biotope-group in spring included Gomphidae and Corixidae, and the number of contributing taxa in total was only 6 compared to 19 in the SIC/SOOC and 16 in the AQV/MV.

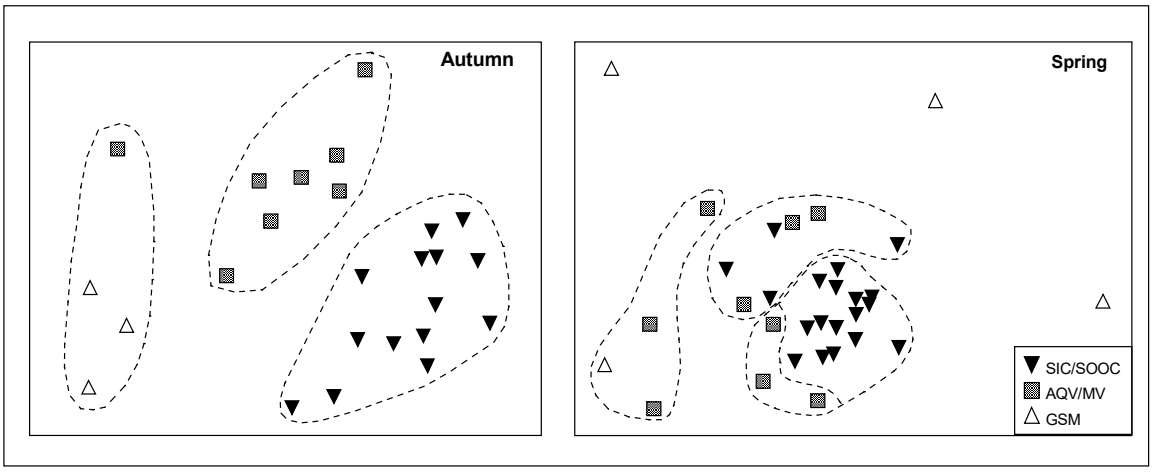


Figure 4.2 Ordination of sites in the Western Cape based on taxa recorded in each SASS biotope-group on each sampling occasion in autumn and spring.

In Mpumalanga, cluster analysis (Figure 4.3) for autumn and spring showed a degree of separation by biotope-group, although groupings were less defined than for the Western Cape. MDS ordination (Figure 4.4) supported the results obtained from the cluster analysis for autumn (3D Stress = 0.17) and spring (3D-Stress = 0.17). Autumn faunal samples essentially separated into five groups. One set of GSM samples (n = 3) were 80% dissimilar from other samples, one set of AQV/MV samples was 70% dissimilar from other samples (n = 4) and another set of GSM samples was 70% dissimilar (n = 2). The remaining samples were all at least 37% similar. The fourth Group comprised AQV/MV samples (n = 13), with three GSM and one SIC/SOOC samples (40% similar). The fifth Group comprised three sub-groups, with 5A mostly SIC/SOOC samples (n = 16) and sub-

Table 4.4 Taxa contributing to within-group similarity of groups identified in the biotope specific analysis in the Western Cape. Results are given separately for autumn and spring. Those taxa contributing to the first 50% of the similarity are indicated by ♦; the remaining taxa contributing to the next 40% (i.e. 90% in total) of the similarity are indicated by □.

Season	**Autumn**			**Spring**			
Group	**1**	**2**	**3**	**1**	**2**	**3**	**4**
Predominant biotope-group	**SIC/SOOC**	**AQV/MV**	**GSM**	**SIC/SOOC**	**SIC/SOOC +AQV/MV**	**AQV/MV**	**AQV/MV**
Average similarity	**48.4%**	**38.4%**	**41.3%**	**60.0%**	**47.7%**	**41.0%**	**63.2%**
Number of distinguishing taxa	19	16	6	15	17	5	6
Notonemouridae	□			□			
Baetidae 1 Type			□				
Baetidae 2 Types	□	□				♦	
Baetidae 3 Types	□			□	□		
Caenidae	□		□		□		
Teloganodidae	□			♦	♦		♦
Heptageniidae	□			□			
Leptophlebiidae	♦	□		♦	♦		
Dytiscidae		□	□				
Elmidae/Dryopidae	♦	♦		□	□		♦
Gyrinidae		♦			□		
Helodidae Larvae	□			□			□
Hydraenidae	□					□	
Limnichnidae	□						
Corydalidae	♦			♦			
Hydropsychidae 1 Type					□		
Hydropsychidae 2 Types				□			
Philopotamidae	♦						
Case Caddis 1 Type	□	□		□			□
Case Caddis 2 Types		□					
Case Caddis 3 Types		□			♦		
Athericidae	♦			□			
Blephariceridae				□			
Chironomidae	□	□		♦	♦		□
Dixidae		□					
Simuliidae	♦	♦		♦	♦	□	♦
Tipulidae				□			
Corixidae			♦		□	□	
Naucoridae		□	□				
Veliidae		♦			□		
Aeshnidae	□	□			□		
Coenagrionidae		♦			♦		
Gomphidae			♦				
Libellulidae		□			□	□	
Oligochaeta					□		
Amphipoda					□		
Planariidae	□						

groups 5B and 5C mostly both GSM. They were at least 40% similar and separated further at 45% into SIC/SOOC and GSM sub-groups. Spring faunal samples separated into three Groups with Group 2 sub-dividing into three sub-groups. The first Group was 75% dissimilar from other samples and comprised ten AQV/MV and one GSM site. The second Group was 65% dissimilar from other samples.

The first sub-group consisted of two GSM samples (40% similar), the second a mix of SIC/SOOC (n = 17), GSM (n = 13), and AQV/MV (n =2) samples and the third sub-group five AQV/MV samples (38% similar). The third Group comprised three GSM samples and was 75% dissimilar from other samples. Results of the one-way ANOSIM analysis revealed that the differences between macroinvertebrate assemblages in the three biotope-groups were statistically significant (autumn: Global $R = 0.465$, $p < 0.01$, spring: Global $R = 0.437$, $p < 0.01$).

Taxa contributing to within-group similarity varied amongst biotope-groups, particularly in autumn (Tables 4.5 and 4.6). In autumn, average similarity was highest in the SIC/SOOC group, and of the 17 taxa that characterised this Group, five were exclusive to the Group, notably Heptageniidae, Psephenidae, Psychomyiidae, Tabanidae and Libellulidae. Taxa contributing to similarity in the AQV/MV groups included a range of taxa from many orders, including Gyrinidae, Gerridae and Veliidae. The number of distinguishing taxa varied considerably amongst the four GSM biotope-groups with only Gomphidae consistently important. In spring, Perlidae and four families of mayfly contributed significantly to within-group similarity of the SIC/SOOC+GSM biotope-group, with Gomphidae again important in this and in the one GSM group. Taxa contributing to AQV/MV similarity were varied and included the following taxa which were not identified as important in other groups: Gyrinidae, Hydropsychidae, Culicidae, Dixidae, Gerridae, Naucoridae, Veliidae, Coenagrionidae and zygopteran juveniles.

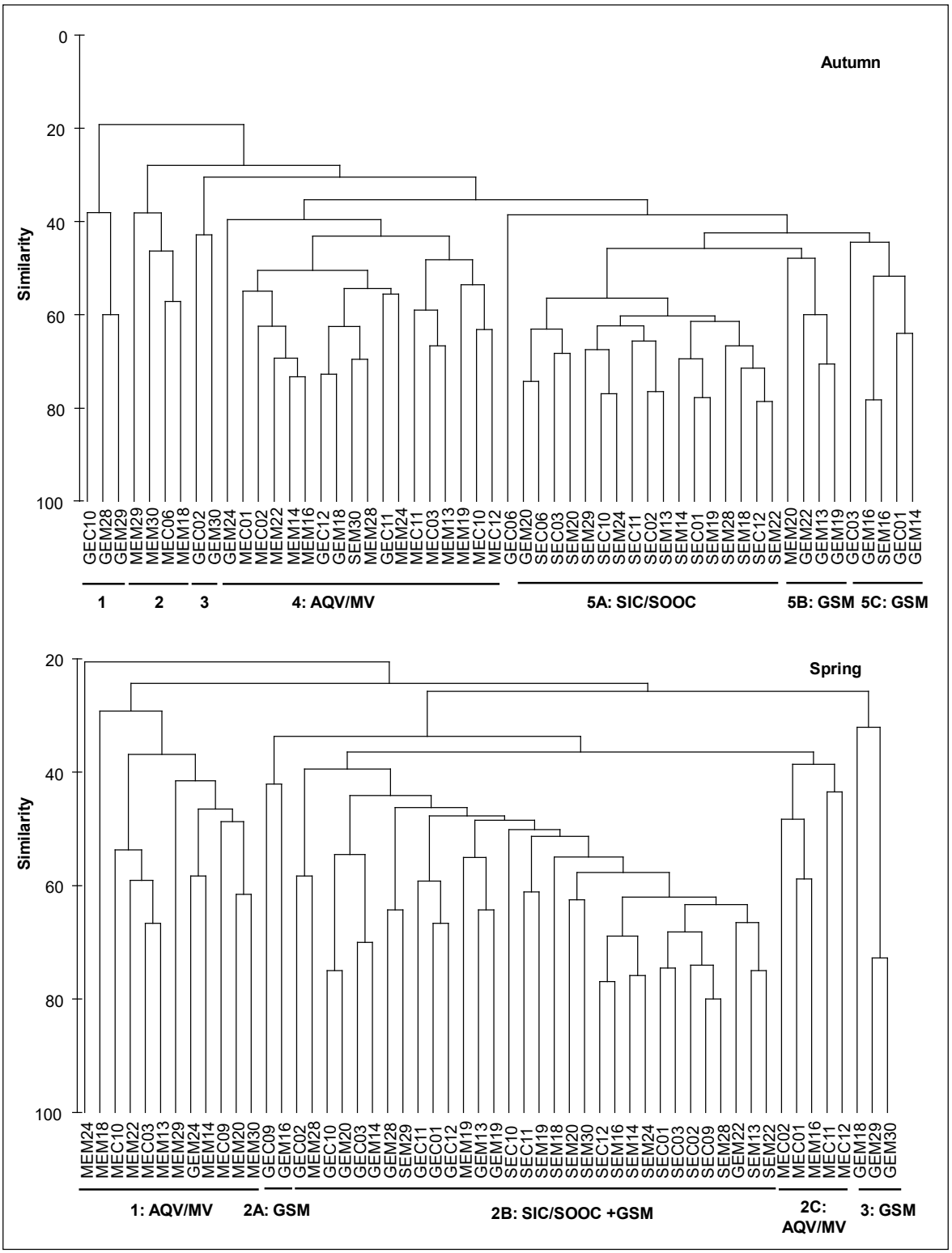


Figure 4.3 Dendrogram showing the classification of 18 sites in Mpumalanga based on taxa recorded in each SASS biotope-group on each sampling occasion in autumn and spring. The site code is prefaced with the biotope-group as follows: S = SIC/SOOC, M = AQV/MV and G = GSM.

Figure 4.4 Ordination of sites in Mpumalanga based on taxa recorded in each SASS biotope-group on each sampling occasion in autumn and spring.

4.4.3 SASS4 Scores, Number of Taxa and ASPT

The availability of SASS-biotopes for sampling may affect SASS4 Scores, number of taxa and ASPT values. This aspect has been examined by calculating 1) the relative percentage contribution of each SASS biotope-group to that calculated for the site; 2) median values of SASS4 Score, number of taxa and ASPT for each SASS biotope-group; and 3) the effect of sampling one, two or three SASS biotope-groups on SASS4 Score, number of taxa and ASPT. Calculations have been done separately for the Western Cape and Mpumalanga, except for 3) which was only performed for Mpumalanga given the limitations of the Western Cape data.

Table 4.5 Taxa contributing to within-group similarity of groups identified in the biotope specific analysis in Mpumalanga in autumn. Those taxa contributing to the first 50% of the similarity are indicated by ♦; the remaining taxa contributing to the next 40% (i.e. 90% in total) of the similarity are indicated by □.

Group	**1**	**2**	**3**	**4**	**5A**	**5B**	**5C**
Predominant biotope-group	**GSM**	**AQV/MV**	**GSM**	**AQV/MV**	**SIC/SOOC**	**GSM**	**GSM**
Average similarity	**45.4**	**44.0%**	**42.9%**	**48.9%**	**60.4%**	**55.7%**	**52.7%**
Number of distinguishing taxa	*4*	*9*	*3*	*14*	*17*	*12*	*11*
Baetidae 1 Type	□						□
Baetidae 2 Types		□					□
Baetidae 3 Types				♦	□	□	
Caenidae				♦		□	□
Heptageniidae					□		
Leptophlebiidae					♦	□	□
Tricorythidae				□	♦		□
Elmidae/Dryopidae			♦	□	□		
Gyrinidae		♦		□			
Psephenidae					♦		
Hydropsychidae 1 Type				□		♦	♦
Hydropsychidae 2 Types				□	□		
Psychomyiidae					♦		
Athericidae					♦	♦	♦
Ceratopogonidae						□	
Chironomidae		□		□	□	□	
Simuliidae		□		♦	□		
Tabanidae					□		
Tipulidae		□		□	□	♦	♦
Corixidae	♦						
Gerridae		♦					
Veliidae		♦		♦		♦	
Aeshnidae			□		□		□
Chlorocyphidae		□					
Coenagrionidae				□		□	
Gomphidae	♦		♦			♦	♦
Libellulidae					□		
Oligochaeta					□		♦
Brachyura (Crabs)	□			□	♦	□	
Planariidae				□			

Table 4.6 Taxa contributing to within-group similarity of groups identified in the biotope specific analysis in Mpumalanga in spring. Those taxa contributing to the first 50% of the similarity are indicated by ♦; the remaining taxa contributing to the next 40% (i.e. 90% in total) of the similarity are indicated by □.

Group	**1**	**2A**	**2B**	**2C**	**3**
Predominant biotope-group	AQV/MV	GSM	SIC/SOOC +GSM	AQV/MV	GSM
Average similarity	**36.5%**	**42.1%**	**49.6%**	**43.1%**	**45.6%**
Number of distinguishing taxa	13	4	17	10	4
Perlidae			□		
Baetidae 2 Types	□				□
Baetidae 3 Types			♦	□	
Caenidae	□	□	♦	♦	
Leptophlebiidae		□	♦		
Tricorythidae			♦	□	
Elmidae/Dryopidae			□		
Gyrinidae	□				
Psephenidae			□		
Hydropsychidae 1 Type	□				
Hydropsychidae 2 Types			□		
Case Caddis 1 Type	♦	♦			
Athericidae			□		
Chironomidae	□		♦	♦	♦
Culicidae	□				
Dixidae	□				
Simuliidae	♦		♦	♦	
Tabanidae					
Tipulidae		♦	□		
Corixidae					□
Gerridae	□				
Naucoridae				□	
Veliidae	♦				
Aeshnidae			□		
Coenagrionidae	♦			□	
Gomphidae			♦		♦
Zygoptera Juveniles				□	
Oligochaeta			□		
Hydrachnellae	□				
Brachyura (Crabs)			□		
Planariidae			□		
Ancylidae				□	
Planorbidae				□	

Relative percentage contribution

The mean (plus standard deviation) percentage contribution of taxa within each SASS biotope-group to SASS4 Score, number of taxa and ASPT to those of the site has been calculated (Figure 4.5). Because certain taxa are found in more than one biotope the summed percentages from the biotopes do not equal 100%. Instead the percentage given for each biotope-group is that percentage relative to the total calculated for the site (i.e. biotope-groups

combined). Thus if the SASS4 Score in the SIC/SOOC was 145 compared to 175 for the site, then the percentage contribution of taxa in the SIC/SOOC biotope-group to the site would be 83%. Similarly, if ASPT in SIC/SOOC was 9.3 compared to 8.9 for the site, the percentage contribution would be 104%. Because ASPT is calculated by dividing SASS4 Score by number of taxa, subsequent calculation of the percentage contribution of ASPT sometimes resulted in an ASPT greater than 100%. Sites in the Western Cape at which the SIC/SOOC biotope-group was exclusively present were omitted from the analysis.

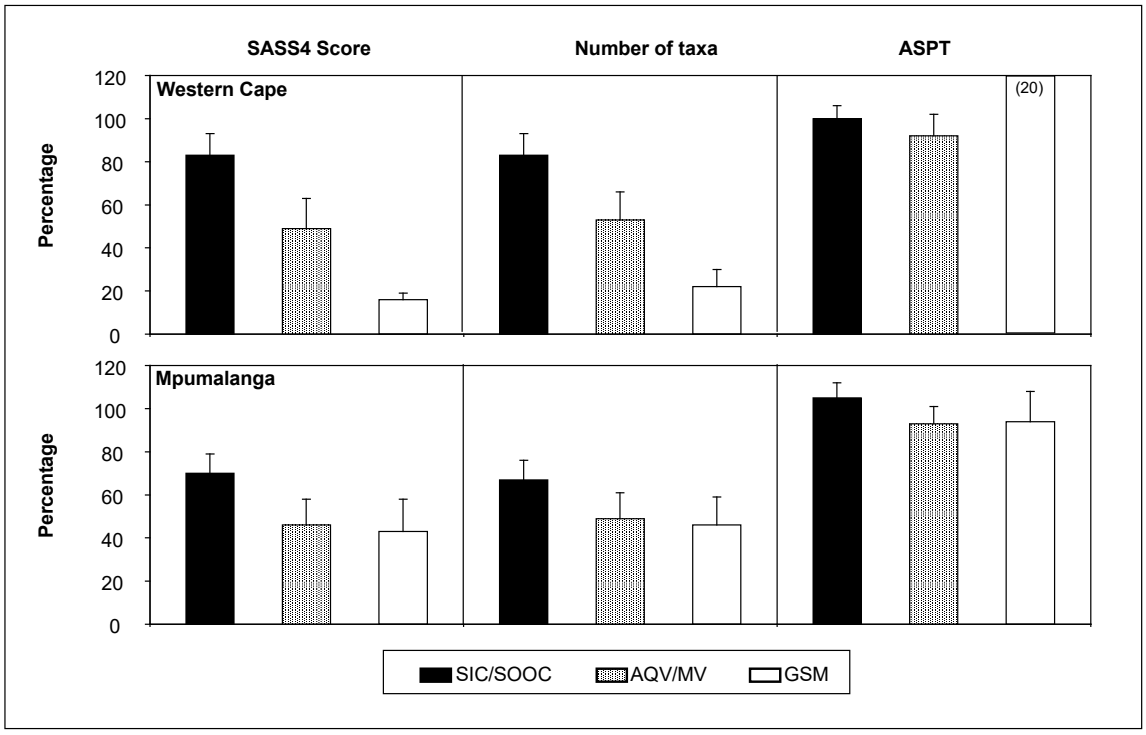


Figure 4.5 **Mean (+ SD) of percentage contribution of SASS4 Scores, number of taxa and ASPT for SASS samples collected in three separate biotope-groups to SASS4 Scores, number of taxa and ASPT calculated for the site. Mean values have been calculated for the Western Cape and Mpumalanga. Biotope-groups are: SIC/SOOC = stones-in-current/ stones-out-of-current, AQV/MV = aquatic/marginal vegetation and GSM = gravel, sand and mud. The percentage ASPT in the GSM biotope-group has been truncated and the ASPT for the site contributing to the high ASPT is given in parenthesis.**

Based on these data, taxa present in the SIC/SOOC biotope-group constituted 83% of the SASS4 Score and number of taxa, and 100% of the ASPT in the Western Cape (n = 18), and 70%, 67% and 105% of the SASS4 Score, number of taxa and ASPT in Mpumalanga (n = 53). Taxa present in the AQV/MV biotope-group constituted 49%, 53% and 92% of the SASS4 Score, number of taxa and ASPT in the Western Cape (n = 17), and 46%, 49% and

93% of the SASS4 Score, number of taxa and ASPT in Mpumalanga (n = 53). Taxa present in the GSM biotope-group constituted 16%, 22% and 88% of the SASS4 Score, number of taxa and ASPT in the Western Cape (n = 6), and 43%, 46% and 94% of the SASS4 Score, number of taxa and ASPT in Mpumalanga (n = 53). In both regions, the SIC/SOOC biotope-group had the highest percentage contribution to SASS4 Score and number of taxa. The GSM biotope-group generally supported fewer taxa in the Western Cape compared to Mpumalanga, where differences in number of taxa and SASS4 Scores from AQV/MV and GSM were less pronounced.

One Western Cape site had a single high-scoring taxon present, namely "Trichoptera (cased caddis 3 Types)" in the GSM biotope. This "taxon" has a sensitivity/tolerance score of 20 and resulted in a very high ASPT (20) for this site (truncated in Figures 4.5 and 4.6). Variation in ASPT between all three SASS biotope-groups was less pronounced, particularly between AQV/MV and GSM. The percentage contribution of ASPT was greater than or equal to 100% in the SIC/SOOC biotope-group, suggesting that more of the sensitive and high scoring taxa are present in this biotope-group.

Median values

SASS4 Score, number of taxa and ASPT values were significantly different amongst SASS biotope-groups in the Western Cape ($p < 0.01$; SASS4 Score: Kruskal-Wallis test statistic $H = 35.85$; number of taxa: $H = 29.30$ and ASPT: $H = 14.21$; Figure 4.6). SASS4 Score, number of taxa and ASPT values were also significantly different amongst SASS biotope-groups in Mpumalanga ($p < 0.01$; SASS4 Score: Kruskal-Wallis test statistic $H = 60.18$; number of taxa: $H = 50.82$ and ASPT: $H = 27.94$; Figure 4.6). Applying the Kolmogorov-Smirnov test, however, revealed that in Mpumalanga these differences were the result of differences between SIC/SOOC and AQV/MV and SIC/SOOC and GSM biotope-groups. SASS4 Score, number of taxa and ASPT values were not significantly different between the AQV/MV and GSM biotope-groups. The SIC/SOOC biotope-group had significantly higher median values than either the AQV/MV or GSM biotope-groups.

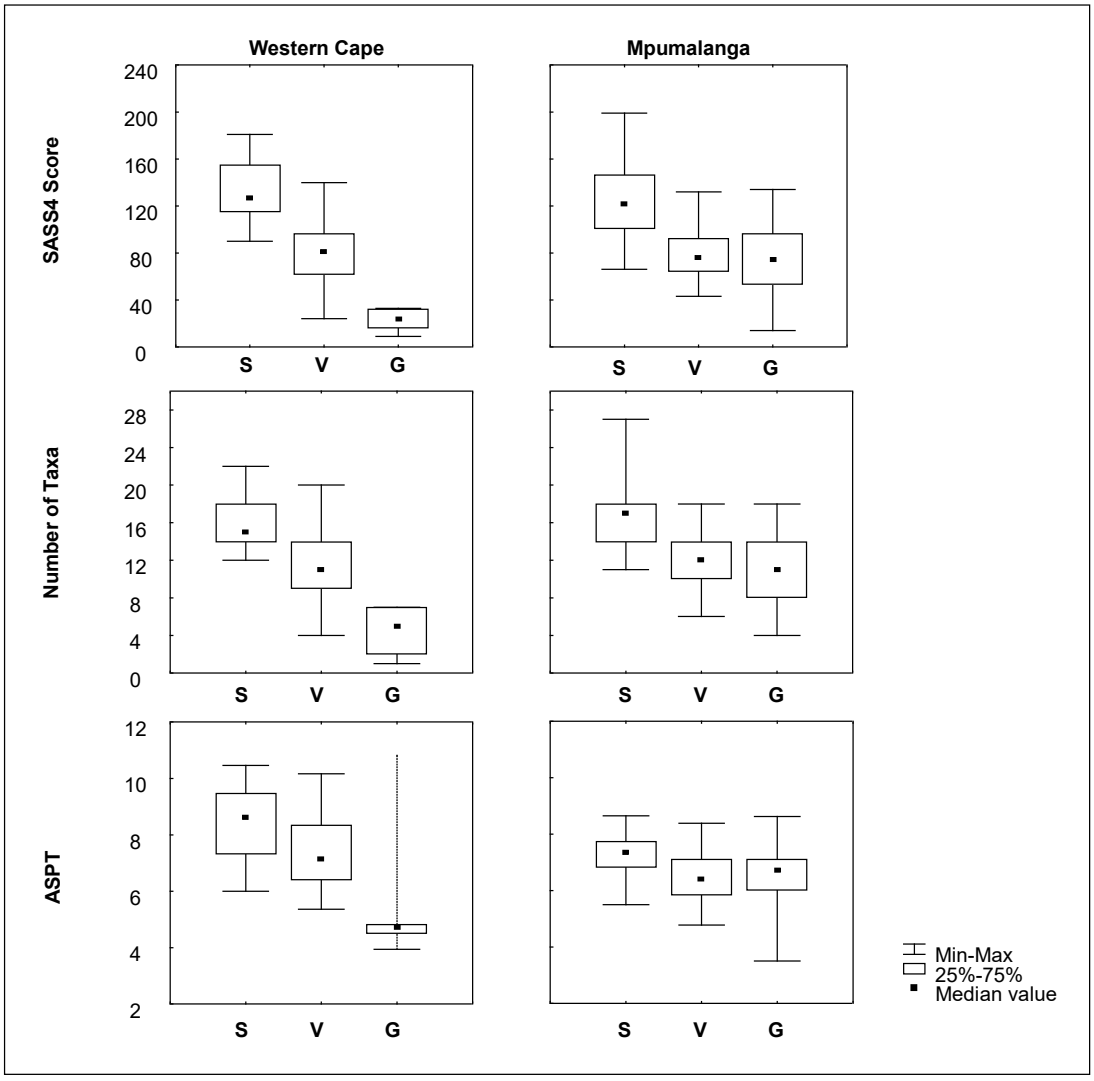


Figure 4.6. Median values for each biotope-group in the Western Cape and Mpumalanga. Biotope-groups are: SIC/SOOC = stones-in-current/ stones-out-of-current, AQV/MV = aquatic/marginal vegetation and GSM = gravel, sand and mud.

Effect of sampling one, two or three biotope-groups on SASS scores

The number of additional taxa recorded per biotope-group was assessed by comparing the number of taxa (mean ± standard deviation) recorded in a single SASS biotope-group with the number of additional taxa recorded if a second SASS biotope-group was sampled, followed by a third SASS biotope-group. Data from a subset of Mpumalanga sites at which all three biotope-groups were sampled were used for calculations on a per-sampling-occasion basis (n = 53). Analysis was run twice, first with the SIC/SOOC biotope-group assessed first and then with AQV/MV assessed first (Figure 4.7). Results showed that if the SIC/SOOC biotope-group was assessed first, then the mean number of taxa recorded in

the SIC/SOOC biotope-group was 16.7 (SD ± 3.5). This represents approximately 67% of the total number of taxa recorded in all three SASS biotope-groups. Adding the AQV/MV biotope-group resulted in an additional 5.9 (SD ± 2.1) taxa (totalling 91% of the total number of taxa) and adding the GSM biotope-group an additional 2.0 (SD ± 1.8) taxa. If AQV/MV biotope-group was assessed first, then the mean number of taxa recorded in the AQV/MV biotope-group was 12.1 (SD ± 3.0). This represents approximately 49% of the total number of taxa recorded in all three biotope-groups Adding the SIC/SOOC biotope-group resulted in an additional 10.4 (SD ± 3.2) taxa.

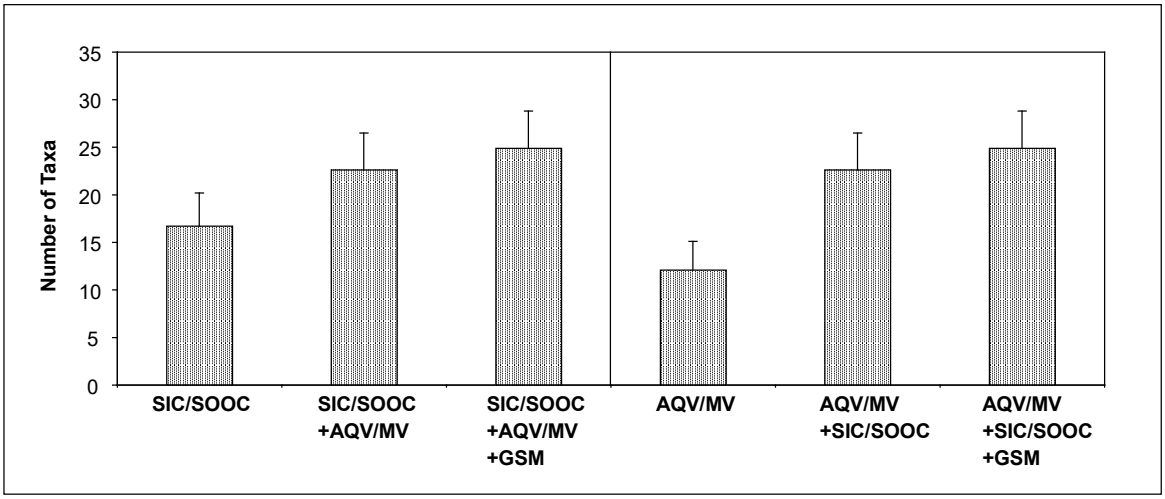


Figure 4.7 Number of taxa (mean ± standard deviation) recorded in a single biotope-group, showing the number of additional taxa recorded when a second and third biotope-group are included. A: SIC/SOOC, (SIC/SOOC+AQV/MV), (SIC/SOOC+AQV/MV+ GSM); B: AQV/MV, (AQV/MV+SIC/SOOC), (AQV/MV+SIC/SOOC+GSM).

The hypothesis that SASS4 Score and number of taxa increase, whilst ASPT decreases as a function of the number of biotopes or biotope-groups sampled, was tested. For Western Cape data up to seven separate SASS-biotopes were sampled, and data are plotted as SASS scores for the site against total number of biotopes sampled. In Mpumalanga all three biotope-groups were sampled at each site and SASS scores were calculated for each SASS biotope-group or combination of SASS biotope-groups (Figure 4.8).

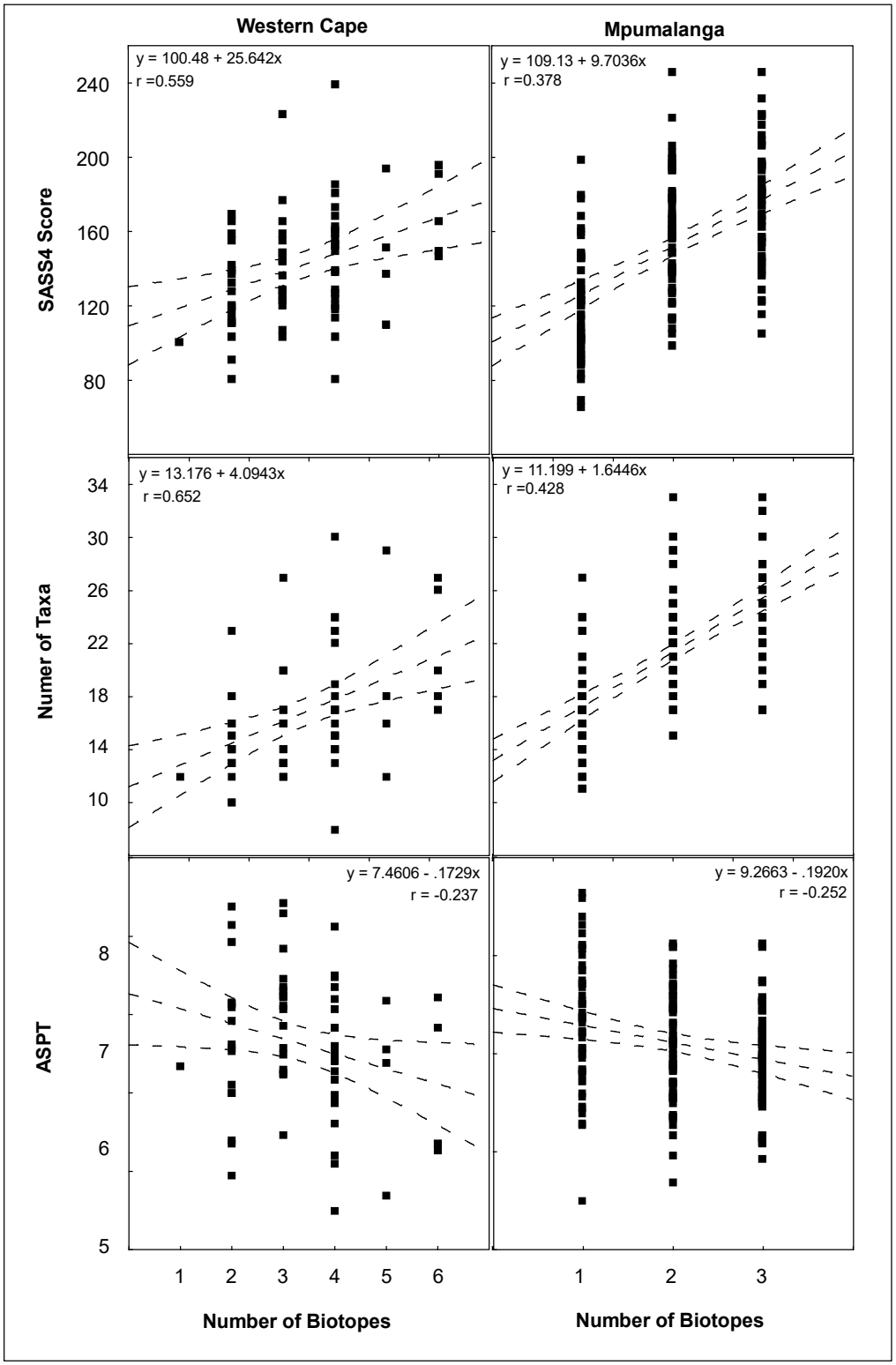


Figure 4.8 Regression analysis of SASS4 Score, number of taxa and ASPT plotted as a function of the number of biotopes sampled for the Western Cape (n = 67 sampling occasions at 32 sites,) and for Mpumalanga (n = 159 on 53 sampling occasions at 19 sites). The dotted lines represent the 95% confidence intervals.

The SIC/SOOC biotope-group represented the first biotope-group, followed by AQV/MV and then by GSM. SASS4 Score and number of taxa were significantly positively correlated with number of biotopes in both regions, whilst ASPT was significantly negatively correlated with number of biotopes ($p < 0.05$). When Mpumalanga data were re-analysed with the AQV/MV biotope-group representing the first biotope-group, both SASS4 Score ($r = 0.77$) and number of taxa ($r = 0.78$) were significantly positively correlated with number of biotope-groups, although ASPT ($r = 0.27$) was no longer negatively correlated but was significantly positively correlated with number of biotopes ($P < 0.05$).

Differences in SASS scores amongst different combinations of SASS biotope-groups in Mpumalanga (Figure 4.9) were examined and found to be significant. SASS4 Score, number of taxa and ASPT values were significantly different amongst different combinations of biotope-groups. For SIC/SOOC, SIC/SOOC+AQV/MV and SIC/SOOC+ AQV/MV+GSM, all three metrics differed significantly from one another ($p < 0.01$ for SASS4 Score: Kruskal-Wallis Test Statistic $H = 53.13$ and number of taxa: $H = 73.45$; and $p < 0.05$ for ASPT: $H = 8.45$). Similarly, for AQV/MV, SIC/SOOC+AQV/MV and SIC/SOOC+AQV/MV+GSM ($p < 0.01$ for SASS4 Score: $H = 103.23$, number of taxa: $H = 106.12$; ASPT: $H = 16.70$). Applying the Kolmogorov-Smirnov test however, revealed SIC/SOOC was significantly different from SIC/SOOC+AQV/MV ($p < 0.01$) for SASS4 Score and number of taxa, but not for ASPT. AQV/MV was significantly different for all three metrics ($p < 0.01$).

4.5 DISCUSSION

This study indicates that there were differences in the frequency of occurrence of SASS-taxa amongst SASS-biotopes, that differences in macroinvertebrate assemblages were greater amongst SASS-biotopes than between sites within a region, that each biotope-group had a characteristic macroinvertebrate assemblage associated with it, and that SASS scores differed amongst the three SASS biotope-groups.

Several taxa demonstrated a degree of biotope specificity and it is likely that these preferences reflect substrate, hydraulic and/or thermal requirements of individual taxa, particularly in the physically harsh environment of Western Cape mountain streams, and/or

food specialisation of individual taxa. Morphological and behavioural adaptations allow organisms to inhabit the habitat to which they are morphologically best suited.

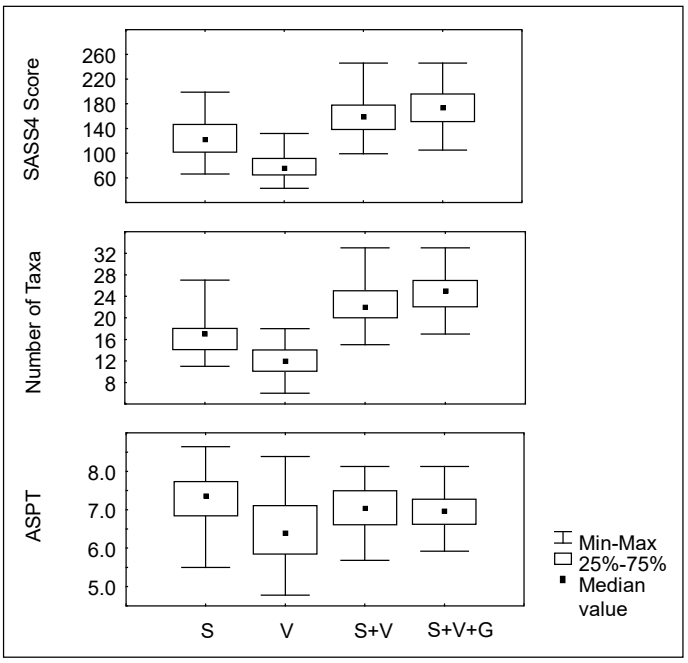


Figure 4.9 Median values for the SIC/SOOC and AQV/MV biotope-groups in Mpumalanga, together with those when taxa from these two biotope-groups are combined (S+V) and when taxa from all three biotope-groups are combined (S+V+G). (S: stones-in-current/stones-out-of-current, V: aquatic/marginal vegetation.

For example, heptageniid and leptophlebiid mayflies occur in stony habitats, often with flowing water. Their flattened body forms enable these aquatic nymphs to remain within the boundary layers of rocks where current drops near to zero (Davies & Day 1998). Gill shape is another example of morphological adaptation, with leptophlebiids having long filamentous gills more suitable to flowing water. In contrast, baetids have a generalised gill form and may be found in most biotopes, whilst caenids have gills protected by a gill cover. This enables caenids to survive in backwaters that are often blanketed in fine sediment, and indeed caenids were one of the distinguishing taxa for the GSM biotope-group in the Western Cape in spring. The larval and pupal stages of the dipteran Blephariceridae, which were recorded in the SIC/SOOC biotope-group, adhere to rock surfaces in fast-flowing water, and even to vertical cascades (Scholtz & Holm 1985).

Feeding adaptations also reflect the habitat preferences of aquatic organisms. For example, members of the genus *Tricorythus* are filter feeders and normally occur on the underside of stones in swift currents. Hydropsychids such as *Cheumatopsyche afra* and *C. thomassetti*

are predominantly found in riffles, runs and cascades and require swiftly-flowing water to support their silken food-collecting nets. They have also been noted in aquatic vegetation in fast-flowing water. In such cases it is likely that the hydraulic environment is more important than substrate type. Amphipods are stony-bottom dwellers and occur in a variety of flow types including fast-riffles, medium-run and slow-flowing backwaters. Riffles and backwaters are most efficient at trapping detritus upon which amphipods feed (Snaddon *et al.* 1991) and backwaters appear to be important refuges during high-flow events and important areas for food trapping.

The observed biotope specificity of individual taxa was reflected in the analysis of macroinvertebrate assemblages, with differentiation of sites occurring on the basis of macroinvertebrates associated with particular SASS-biotopes rather than at site level. Within- and between-biotope similarity varied with season and geographic region. Seasonal differences in biotope specificity, with macroinvertebrate assemblages being more biotope-specific in autumn versus spring in the Western Cape, is probably related to seasonal differences in discharge. In early autumn, assemblages have not yet been exposed to elevated discharges and resemble those of the low-flow summer conditions, and thus the distinctiveness of assemblages is still apparent. In the United Kingdom, Armitage *et al.* (1995) also found that mesohabitat distinctiveness varied with season, with boundaries between mesohabitats most distinct under low flow conditions (summer), remaining distinct under intermediate flows (autumn) and becoming least distinct at high flow. Water levels affect not only the areal extent and availability of lotic habitat, but also the degree of biotope isolation, and therefore the availability of refugia for species during vulnerable stages (Power *et al*. 1988).

The nature of the biotopes change with discharge and within the SIC/SOOC biotope group, riffles are probably more common under low-flow conditions, becoming runs as discharge increases (Padmore 1998). Such seasonal differences highlight the importance of hydraulic conditions, with several taxa which were only associated with the SIC/SOOC biotope-group in autumn contributing to group similarity of mixed SIC/SOOC + AQV/MV and AQV/MV biotope-groups in spring. Marginal vegetation in flowing water provides a very different environment to aquatic organisms than marginal vegetation in standing water. The transformation of a lentic environment to a lotic one, under high-flow conditions, restricts the presence of several hemipteran families, such as Gerridae and

Vellidae which, since they are surface swimmers, are dependent on smooth surface of the water, whilst encouraging the colonisation by taxa requiring a flowing water environment. In addition to these physical factors, biotic factors may come into play, with the distribution of lotic organisms being mediated by interactions with other organisms. Cooper (1984) noted that gerrids remained near the stream margins in the presence of trout, an important predator, but foraged in central areas of the channel when trout were absent. This observation emphasises the interactive nature of the organisms inhabiting lotic systems and the dependence on, and interdependence of, biotic and abiotic factors.

On the basis of observed temporal differences in biotope specificity, macroinvertebrate assemblages might be expected to exhibit greater specificity in spring in Mpumalanga (i.e. spring is hydrologically equivalent to autumn in the Western Cape), since this is a summer-rainfall region, with lowest flows in winter. However, this was not observed and differentiation into biotope-groups was generally less clear with no seasonal difference. Certain taxa, including Perlidae, Heptageniidae, Psephenidae and Psychomyiidae within the SIC/SOOC biotope-group, several species of Hemiptera and Zygoptera in AQV/MV and gomphid dragonflies in GSM, were however, fairly biotope-specific

Of the three SASS biotope-groups examined in this study, SIC/SOOC proved to be most consistent in terms of the macroinvertebrate assemblages associated with it. This was true for both geographic regions. Several of the more sensitive, and thus high-scoring taxa in terms of the SASS sensitivity/tolerance scores, occurred more frequently in this biotope-group than in others. Many taxa recorded were in the orders Ephemeroptera, Plecoptera and Trichoptera. These orders include several taxa considered to be obligate erosional species since they have clinging and scraping behaviours most suited to substrates such as stones and boulders (Richards *et al*. 1997). Taxa recorded in the SIC/SOOC constituted the highest relative percentage contribution (> 67%) to each of the SASS metrics and the SIC/SOOC biotope-group had significantly higher median values for each of these metrics than other biotopes. Sampling the SIC/SOOC on its own would ensure collection of approximately 67% of the taxa recorded if AQV/MV and GSM were also sampled. Problems may arise, however, when SIC are absent, or where the substratum is predominantly bedrock or boulder. In both instances, fewer taxa are likely to be recorded than if SIC were present or if substratum was dominated by cobbles. This supports the results of Quinn & Hickey (1990).

The AQV/MV biotope-group supported a greater number of SASS-taxa in Mpumalanga (20) compared to the Western Cape (eight), but in both instances, several of the more tolerant and low-scoring taxa were represented, often within the order Hemiptera. The adults of many of these families are air-breathers and hence not dependent on water as a medium, thus being less sensitive to a reduction in water quality, particularly reduced levels of dissolved oxygen, than families that are dependent on water during part of their life cycle. The AQV/MV biotope-group is variable with respect to the quality and quantity of habitat available for sampling, and both type (Humphries 1996) and biomass (Collier *et al.* 1999) of aquatic macrophytes have been shown to affect macroinvertebrate assemblages and abundances. Because macrophytes are living plants, their biomass changes over time, particularly when compared to mineral substrates such as stones and boulders, which are more stable over time (Beisel *et al.* 1998). As already mentioned, movement of water around the vegetation may affect the suite of invertebrates recorded in this biotope-group, with vegetation in fast-flowing water providing habitat suitable for taxa normally restricted to stones-in-current biotopes, whilst vegetation in slow-flowing water is more likely to harbour those taxa that utilise backwater and slackwater areas of flow. All of these factors may have contributed to the variability observed in macroinvertebrate assemblages associated with the AQV/MV biotope-group.

Harrison (2000) suggests that aquatic invertebrates that inhabit marginal vegetation may be grouped on the basis of how they use this biotope. Certain taxa are almost always associated with marginal vegetation. These include damselflies, true bugs and certain simuliid and caddis species. Other taxa utilise this biotope in addition to other biotopes, particularly if situated in flowing water. Examples include elmid beetles and baetid mayflies. Some taxa use margins as a temporary biotope and spend only the first parts of their lives in this habitat following adult oviposition, when marginal vegetation acts as a conduit between the aquatic and terrestrial environments, or as temporary refugia during spates. The fourth group is found in greater abundance on marginal vegetation but is common in other biotopes, i.e. taxa that have no biotope preference, and the fifth group include terrestrial or semi-aquatic taxa that spend a large proportion of their lives at the water's edge and thus marginal vegetation provides a co-dominant biotope. Harrison (2000) also considers marginal vegetation to be vital for the reproduction of aquatic insects, including both terrestrial insects, such as beetles, and aquatic insects and non-insects, such as snails, leeches and mites, and observed that many adult invertebrates laid

eggs in the margins. The adults may also use marginal vegetation to emerge from the water or to enter it in order to lay eggs in other biotopes. Harrison (2000) concludes that streams and rivers that lack marginal vegetation may have depressed levels of invertebrate recruitment, as a result. Research into the importance of the AQV/MV biotope-group in South Africa is scarce but it probably provides an important habitat for aquatic organisms as shown by the fact that sampling this biotope-group on its own would ensure collection of approximately 49% of the taxa recorded if SIC/SOOC and GSM were also sampled. Sampling the AQV/MV biotope-group is particularly important for bioassessment and management of lowland systems (Collier *et al.* 1999), since the SIC/SOOC biotope-group is often absent from these rivers (e.g. Collier *et al.* 1998).

The GSM biotope-group generally had the lowest number of taxa associated with it and only three taxa were more frequently recorded in this biotope. The unstable nature of gravel, sand and mud, whose fine sediments typically move at much lower velocities than do those in larger particles (Richards *et al*. 1997) leads to this biotope supporting lower densities of macroinvertebrates than larger particled substrates do (Quinn & Hickey 1990, Starke 1993, Brewin *et al*.1995, Cogerino *et al.* 1995, Johnson & Vaughn 1995). This biotope-group was also the least consistent in terms of the macroinvertebrate assemblages associated with it, and groups were sometimes clustered with AQV/MV in the Western Cape and AQV/MV and SIC/SOOC in Mpumalanga. It is likely that the type of substrate sampled, i.e. mud, sand or gravel, and the flow condition, i.e. flowing or stagnant, will influence which taxa are present. Pardo & Armitage (1997) observed that under low-flow conditions, depositional habitats were dominated by burrowing collector gatherers, comprising on average, lower-scoring taxa (Starke 1993). There were however instances in Mpumalanga when GSM supported taxa similar to those of the SIC/SOOC biotope-group. Examination of the characteristics of these sites showed a substantially higher percentage of gravel at these sites relative to other sites. When gravel is situated in flowing water, at a discharge below that needed to cause mobilisation of particles, it provides habitat resembling SIC biotope and taxa normally associated with SIC are likely to be recorded.

Given the observed biotope specificity of certain macroinvertebrate taxa and the variability in the availability of biotopes from site to site, it is clear that consideration needs to be given to the effect of biotope availability on bioassessment and the incorporation of the

observed biotope-related differences into a bioassessment programme represents a challenge. In practise it is not uncommon for at least one SASS biotope-group to be absent, particularly in lowland rivers that often have no SIC/SOOC biotope-group present. Options for taking biotope differences into account include:

(1) ignoring biotope differences and interpreting data on a site basis;
(2) restricting sampling to a single biotope (Parsons & Norris 1996, Hewlett 2000); or
(3) comparing data from sites separately for each SASS biotope-group (e.g. Chessman 1995, Kay *et al.* 1999).

Option 1 is likely, in the absence of any one biotope-group at a site, to lead to erroneous conclusions regarding the condition of a site and is thus to be discouraged. Option 2 is appropriate if the same biotope-group is likely to be present at all future monitoring and reference sites. This is unlikely to be the case in South African rivers, which are diverse in their physical characteristics and where not all SASS biotopes are necessarily present. Consistency and comparability are critical for the successful implementation of any national biomonitoring programme, and limiting bioassessment to a single biotope-group would severely limit the national utility of such a programme. Option 3 seems to be the most appropriate and scientifically-defensible option which will enable differences in biotope availability between monitoring sites and between monitoring and reference sites to be taken into account.

Results from the Western Cape, however, suggest that differences in the relative frequencies of occurrence of taxa within SASS biotope-groups exist, most probably as a reflection of flow conditions. This was particularly apparent when SIC assemblages were compared with SOOC assemblages. Generally SIC assemblages included taxa recorded in the SOOC biotope, whilst several SIC taxa were absent from SOOC assemblages. Results from a bioassessment where only the SOOC biotope was sampled, as part of the SIC/SOOC biotope-group, would thus reflect differences in biotope availability rather than water quality. A recent study compared two bioassessment procedures, the first aimed at assessing lotic and lentic habitats within a stream, where stones-in-current are grouped with vegetation-in-current (lotic) and stones-out-of-current and are grouped with vegetation-out-of-current (lentic); and the second using biotope groups (i.e. SIC/SOOC and AQV/MV) (N. Bonado & H.F. Dallas, unpublished data). Results showed that the "lotic" and SIC/SOOC assemblages were very similar, whilst the "lentic" and AQV/MV

assemblages were very different. In that study, SIC were always available and AQV was often represented by the aquatic sedge, *Isolepis*, known to be an important habitat for aquatic organisms in the Western Cape (pers. obs). This highlights the importance of factoring in the hydraulic features in relation to each biotope, and viewing the hydraulic-biotope condition in combination.

A fourth approach, which also has general application, i.e. is suitable for all river types, and which aims to reduce spatial variability further, whilst still being efficient in terms of sampling duration, may also be worth investigating. It is suggested that sampling be limited to two key biotopes, namely riffles, i.e. swift sections with broken water, within the SIC biotope, and vegetation. Riffles have been shown to support several sensitive taxa and in a study comparing taxa from riffles and runs, i.e. swift sections with unbroken water, sampling riffles ensured collection of all taxa recorded in runs (Pridmore & Roper 1985). Differences in SIGNAL values (i.e. the Australian equivalent of ASPT), between reference and monitoring sites were more pronounced in riffles than pool edges (Growns *et al*.1997). Most sites assessed will have riffles or marginal vegetation or both of these biotopes, with upland sites having a greater probability of the availability of riffles, and lowland sites vegetation. If both are present they should be assessed separately. Each of these biotopes represents a more specific biotope than the biotope-groups within which they are currently grouped, i.e. SIC/SOOC and AQV/MV. The third biotope-group, namely gravel, sand and mud (G/S/M), has been shown to contribute very little in terms of number of taxa or SASS scores, and hence could confidently be omitted from a new bioassessment procedure. By limiting bioassessment to very specific biotopes, intrinsic spatial variability will be further reduced and should result in more robust classifications and reference conditions for use within bioassessment programmes. The suitability of the proposed sampling strategy will need to be tested by examining macroinvertebrate assemblages associated with each specific biotope and comparing SASS scores calculated for reference sites across a broad geographic range. In this way the consequences of spatially restricted sampling can be determined and the validity of this sampling strategy evaluated.

CHAPTER 5. TEMPORAL VARIABILITY OF MACROINVERTEBRATES ASSEMBLAGES AND IMPLICATIONS FOR AQUATIC BIOASSESSMENT

5.1 INTRODUCTION

In regions with seasonal climates such as South Africa, lotic systems often exhibit daily, seasonal and annual periodicity. Since many aquatic organisms are known to have specific hydraulic requirements, seasonal variation in factors such as stream hydrology (McEravy *et al.* 1989), temperature (Hawkins *et al*. 1997) and biotope availability (Armitage & Pardo 1995, Armitage *et al.* 1995) may lead to variation in the distribution and abundance of benthic macroinvertebrates. Seasonal variation in discharge often translates into differences in wetted perimeter, hydraulic conditions and biotope availability. For example, stony bottom biotopes such as runs become riffles under low flow conditions, whilst marginal vegetation may change from being lotic to lentic. Temperature is thought to influence macroinvertebrate assemblage structure by influencing developmental rates of individual taxa and by excluding taxa unable to tolerate certain temperature ranges (Hawkins *et al*. 1997). Many aquatic organisms have life history stages such as emergence, feeding and growth that are cued into intrinsic seasonal changes and seasonal differences may therefore occur at the assemblage level.

Temporal variability in taxon richness (McElravy *et al.* 1989, Linke *et al*. 1999) is reflected in biotic indices that are based on the macroinvertebrate assemblages, and thus when macroinvertebrate assemblages are used for bioassessment, temporal variation of individual taxa may influence judgement as to whether or not a site is disturbed. Indeed, the seasonal dependence of biotic indices is a common criticism of such indices (Zamora-Munoz *et al*. 1995). Resh and Jackson (1993) assessed the effect of season on the accuracy of biotic indices and found that there were seasonal differences in almost all of the measures. Linke *et al*. (1999) noted consistent differences in number of taxa and the biotic index between summer and winter samples. Bioassessment based on these results would indicate better water quality in the same streams in winter relative to summer even though the relative degree of disturbance had remained constant. Kay *et al.* (1999), however, in a study of nine sites in north-western Australia, and Ruse (1996), in a study of 16 sites in the

River Mole catchment, United Kingdom, found very little temporal variation in faunal assemblages. The effect of season of sampling is therefore not clear-cut, with some studies showing definite seasonal effects and others showing few. Of consequence is the extent to which macroinvertebrate assemblages and SASS scores at reference sites vary temporally, and whether this variability is significant enough to impede the detection of disturbance when a monitoring site is compared with the reference site or reference condition.

In bioassessment programmes, homogeneous regions are delineated, either based on *a priori* regional classification of sites (e.g. Gerritsen *et al*. 2000, Van Sickle & Hughes 2000), or *a posteriori* analysis of biological data (e.g. Wright 1995, Smith *et al*. 1999). Classification, in both cases, attempts to group sites with similar biota together, such that macroinvertebrate assemblages within a group of sites are less variable than observed in the absence of site classification. Approaches for incorporating the effects of temporal variability in bioassessment vary from limiting a suite of assessments to a short time period, to incorporating bioassessment data from two or three seasons so that a seasonally-composite assemblage of macroinvertebrate taxa at a site is obtained (e.g. Furse *et al.* 1984, Turak *et al*. 1999). At sites exhibiting seasonal differences, a more accurate inventory of the expected macroinvertebrate taxa at a site would be obtained by combining data from two or three seasons, and taxa that exhibit seasonal dependence will be recorded in the list of expected taxa. Problems arise, however, when a single assessment of the macroinvertebrate assemblage at a monitoring site is undertaken and then compared to a seasonally-composite reference condition or a reference condition defined for a different season without taking cognisance of seasonal variability. Is the observed effect a reflection of disturbance at the site or merely an artefact of seasonal variability? Since one of the key objectives of bioassessment is to establish the degree to which a monitoring site has been disturbed relative to a reference condition, it is important to understand, reduce or eliminate the potential influence of temporal or seasonal variability.

The aim of this chapter is to evaluate the influence of season on bioassessment by focusing on seasonal variability at three levels, namely 1) individual taxa; 2) macroinvertebrate assemblages; and 3) SASS scores. Results are discussed in relation to ecological reference conditions and the interpretation of bioassessment data.

5.2 STUDY AREA

Sixty sites, situated on 40 rivers, were sampled (Table 5.1). Of these, eight sites were situated on rivers in the Western Cape region and 52 were on rivers in Mpumalanga. Only minimally-impacted sites, with respect to anthropogenic disturbance, were selected in both regions such that any observed differences did not reflect water quality conditions. Sites in the Western Cape were assessed with variable frequency during 1994 and 1995 (total number of assessments = 44, see Appendix A), whilst sites in Mpumalanga were each assessed on three occasions (May, July and September) in 1999 (total number of assessments = 122). Details of the sites assessed in each region are provided in Table 5.1. A subset of 16 sites from Mpumalanga was used in certain analyses as specified in the appropriate section.

Table 5.1 Sites assessed during this study indicating river and geographic region. The codes for sites on each river are given in parenthesis and relate to Table 2.1 and Figures 2.1 and 2.2 in Chapter 2.

Geographic Region	River
Mpumalanga	Alexanderspruit (HC42), Blyde (EC01, EC02), Blystaanspruit (EC04), Crocodile (ER32, HC43, LG60, LG62, LG63, LG64), Dorps (HC36), Elands (EC07, EC08) Elandsfonteinspruit (HC44, HM51), Ga-Selati (EM20), Grootfonteinspruit (EM16), Houtbosloop (EC09), Kareekraalspruit (HM52), Kgwete (EM18), Klein-Sabie (EC10), Klip (HC38), Lunsklip (HC45), Mac-Mac (EC11, LR73), Marite (LC57), Maritsane (LM71), Mohlombe (EM26), Nelspruit (EM22, LR72), Ohrigstad (EM19), Sabie (EC12, LC58, LG66, LG68, LR74), Sand (EM29, LG69, LG70), Spekboom (EM13, HC39), Sterkspruit (EM14), Tautesloop (HM54), Treu (EC03), Unspecified (EM15, EM24, EM28, EM30), Waterval (HC40, HC41), Wilge (HG33) and Wilgekraalspruit (HM55)
Western Cape	Assegaaibosch (CM01), Berg (CM02, CC01), Eerste (CM04) Lang (CM05), Palmiet (CM07, CF01) and Riviersonderend (CM20).

5.3 MATERIALS AND METHODS

5.3.1 Benthic macroinvertebrates: SASS4 sampling

Benthic macroinvertebrates were sampled using the qualitative rapid bioassessment method, SASS4 (South African Scoring System). The following norms have been used for seasonal groupings: spring = September, October and November; summer = December, January and February; autumn = March, April and May; and winter = June, July and August. A detailed description of the SASS method is given in Chapter 2.

5.3.2 Data analysis

Frequency data

The relative frequency of occurrence of each SASS-taxon was calculated separately for the Western Cape and Mpumalanga. Since sampling frequency varied amongst sites in the Western Cape, the frequency of occurrence of each SASS-taxon was calculated relative to the number of sampling occasions per season. For Mpumalanga data, only sites assessed in all three seasons were included (n = 52). In both instances, taxa recorded on fewer than 5 sampling occasions across the range of sites were omitted from the analysis. The frequency of occurrence of a taxon within a season is expressed relative to its frequency of occurrence in other seasons. Given the observed differences in the relative frequency of occurrence of SASS-taxa amongst SASS-biotopes (Chapter 4), seasonal patterns in the frequency of occurrence of SASS-taxa was also assessed for a single SASS biotope-group, namely the SIC/SOOC biotope-group (stones-in-current and stones-out-of-current) for the Western Cape.

Analysis of macroinvertebrate assemblages

Cluster analysis and non-metric multidimensional scaling (MDS) were used to examine similarities amongst seasons and sites based on macroinvertebrate assemblage composition (Clark & Warwick 1994). Three separate analyses were undertaken on Western Cape data. The first included all data from all seasons, the second included autumn and spring data from upper-catchment sites (mountain stream and foothill-cobble bed sites) only, and the third included autumn and spring data at sites where the SIC/SOOC biotope-group (stones-in-current and stones-out-of-current) had been assessed separately. A subset of sites (n = 16), which were most similar in regional and abiotic characteristics to those of the Western

Cape, was selected from the Mpumalanga dataset. Three separate analyses were conducted, the first on all data from all sites, the second on autumn and spring data for the SIC/SOOC biotope-group only and the third for autumn and spring data for the AQV/MV biotope-group (aquatic and marginal vegetation) only. Data were transformed using the presence/absence transformation (*PRIMER Version 5*) and the Bray-Curtis coefficient was used on these transformed data. Hierarchical agglomerative clustering, using group-average linking, was used on the data matrix. Ordination of samples by MDS was undertaken, and stress values used to assess the reliability of the MDS ordination. One-way ANOSIM was used to test whether or not there were significant differences in assemblage structure amongst seasons. The ANOSIM tests were performed on presence/absence transformed data, analysed using the Bray-Curtis measurement of similarity. The distinguishing taxa responsible for the similarity within groups of sites and the dissimilarity amongst groups of sites were established using SIMPER (*PRIMER Version 5*). Those taxa responsible for 90% within-group similarity were identified.

SASS4 Scores, Number of Taxa and ASPT

SASS scores for each season were compared with those generated by combining three seasons into a multiple-season site assessment (i.e. taxa recorded in autumn, winter and spring were combined). The sub-set of sites from Mpumalanga was used to calculate median SASS scores for each season. These were compared statistically using the non-parametric Kruskal-Wallis Test. Individual pairs of biotope-groups were compared using the non-parametric Kolmogorov-Smirnov Test. The results of all analyses were considered significant at $p < 0.05$.

5.4 RESULTS

5.4.1 Frequency of occurrence of each SASS-taxon amongst seasons

The frequency of occurrence of each SASS-taxon in each season has been tabulated for the Western Cape and Mpumalanga (Table 5.2). Most taxa were distributed equally amongst all the seasons examined and seasonal differences were minimal, with only eight taxa exhibiting a seasonal pattern in the Western Cape and fifteen in Mpumalanga. Those taxa that were more frequently recorded in a particular season, such as the twelve taxa recorded more frequently in spring in Mpumalanga, were often also present in the other seasons.

Restricting analysis to a single biotope-group (SIC/SOOC) in the Western Cape revealed differences in the relative frequency of occurrence of each SASS-taxon within each of two seasons, namely autumn and spring (Table 5.3).

Table 5.2 Relative frequency of occurrence (expressed as a percentage) of each SASS-taxon in each season (AU = autumn, WI = winter, SP = spring and SU = summer). Shading indicates frequency of occurrence across season (i.e. highest frequency of occurrence in one season). Percentages are given separately for Western Cape and Mpumalanga data. A dash (-) indicates insufficient data, i.e. taxa recorded < 5 times. Blank indicates the taxon does not occur in the geographic region. The number of sampling occasions per season (n) is given.

	Western Cape				Mpumalanga		
Taxon	**AU**	**WI**	**SP**	**SU**	**AU**	**WI**	**SP**
n	*13*	*8*	*18*	*5*	*52*	*52*	*52*
Notonemouridae	21	30	23	27			
Perlidae					31	31	38
Baetidae 2 Types	29	32	14	25	43	37	20
Baetidae 3 Types	25	14	39	22	29	32	39
Caenidae	48	13	17	21	35	28	37
Teloganodidae	16	36	39	8			
Heptageniidae	22	14	29	35	34	35	30
Leptophlebiidae	24	28	26	22	34	31	35
Oligoneuridae					0	**100**	0
Prosopistomatidae					30	30	40
Tricorythidae	-	-	-	-	34	35	31
Dytiscidae	15	0	45	40	33	29	38
Elmidae/Dryopidae	31	5	27	37	27	36	36
Gyrinidae	28	18	24	29	33	36	31
Helodidae Larvae	14	34	33	18	39	7	**54**
Hydraenidae	33	8	34	25	0	**67**	33
Hydrophilidae	-	-	-	-	33	33	33
Limnichnidae	36	39	26	0			
Psephenidae					32	37	31
Corydalidae	32	18	29	21			
Ecnomidae	33	32	19	17	14	43	43
Hydropsychidae 1 Type	40	40	7	13	24	31	44
Hydropsychidae 2 Types	13	0	37	50	39	36	25
Hydropsychidae 3 Types	-	-	-	-	39	28	33
Hydroptilidae	-	-	-	-	9	48	43
Philopotamidae	36	20	13	31	46	24	30
Psychomyiidae					38	35	27
Case Caddis 1 Type	36	12	15	37	37	31	32
Case Caddis 2 Types	19	30	27	24	34	31	34
Case Caddis 3 Types	14	23	38	25	25	19	**56**
Athericidae	33	30	18	19	33	30	37
Blephariceridae	23	25	33	20	44	33	22
Ceratopogonidae	**73**	0	27	0	33	20	47
Chironomidae	28	21	23	28	33	31	36
Culicidae	-	-	-	-	29	17	**54**
Dixidae	-	-	-	-	35	23	42

Muscidae	-	-	-	-	23	15	**62**
Psychodidae	-	-	-	-	9	45	45
Simuliidae	26	23	26	26	34	30	36
Tabanidae	-	-	-	-	38	31	31
Tipulidae	15	25	33	27	35	33	32
Belastomatidae	-	-	-	-	25	25	**50**
Corixidae	27	0	26	47	35	32	33
Gerridae	-	-	-	-	27	42	31
Naucoridae	25	0	9	**66**	23	38	39
Nepidae	-	-	-	-	45	18	36
Notonectidae	25	0	9	**66**	35	38	27
Pleidae	-	-	-	-	33	17	**50**
Veliidae	62	23	15	0	29	40	30
Pyraustidae	19	0	7	**74**	38	13	**50**
Aeshnidae	39	7	9	45	36	33	31
Calopterygidae	-	-	-	-	21	29	**50**
Chlorocyphidae	-	-	-	-	38	34	28
Chlorolestidae	**54**	18	0	28	-	-	-
Coenagrionidae	27	22	15	36	32	34	35
Corduliidae	-	-	-	-	33	26	41
Gomphidae	21	17	8	54	33	33	34
Libellulidae	22	0	21	57	30	36	34
Zygoptera Juveniles	-	-	-	-	35	35	30
Oligochaeta	29	19	37	15	31	35	34
Hydrachnellae					20	26	**54**
Amphipoda	28	22	**50**	0			
Brachyura (Crabs)	35	14	6	45	33	33	34
Natantia (Shrimps)					15	46	38
Planariidae	**70**	0	0	30	32	27	41
Porifera (Sponges)					33	17	**50**
Ancylidae					34	28	37
Physidae	**100**	0	0	0	43	0	**57**
Planorbidae					27	18	**55**
Sphaeridae					35	4	**62**

5.4.2 Analysis of macroinvertebrate assemblages

In the Western Cape, macroinvertebrate assemblages did not group by season when assemblages from all seasons were considered (Figure 5.1, MDS: 3D-stress = 0.18). One site (CF01), situated in the lower reaches of the Palmiet River, separated from most other sites although a few other upper-catchment sites grouped with it. This, together with a scarcity of data for summer and winter, prompted analysis of upper-catchment sites in autumn and spring only (Figure 5.2). This resulted in two groups of sites that were 46% dissimilar (MDS: 3D-stress = 0.17). Eight of the eleven autumn samples grouped together, whilst the spring and remaining three autumn samples grouped together. There was however, considerable within-group variability. Analysis of assemblage data for the

SIC/SOOC biotope-group revealed similar broad autumn and spring Groups (Figure 5.3, MDS: 3D-stress = 0.15). ANOSIM analysis revealed that there were significant seasonal differences in macroinvertebrate assemblages (Global R = 0.245, $p < 0.01$) with only autumn and summer assemblages not significantly different in the pair-wise analysis. Taxa contributing to within-group similarity of spring assemblages but not to autumn ones included Heptageniidae, Helodidae, Tipulidae and Oligochaeta, whilst nine taxa contributed to autumn assemblages, including families of Trichoptera, Hemiptera and Odonata (Table 5.4). Spring sub-groups had contributing taxa common to all sub-groups but also a few exclusive to a specific sub-group.

Table 5.3 Relative frequency of occurrence (expressed as a percentage) of each SASS-taxon in the SIC/SOOC biotope-group for autumn (AU) and spring (SP) in the Western Cape. SASS-taxa with a relative % > 60% are shaded. The number of sampling occasions per season and the number of times each taxon was recorded are given.

Taxon	AU	SP	n
n	*10*	*16*	
Notonemouridae	47	53	17
Baetidae 2 Types	**62**	38	10
Baetidae 3 Types	26	**74**	11
Caenidae	**80**	20	7
Teloganodidae	24	**76**	18
Heptageniidae	38	**63**	11
Leptophlebiidae	46	54	23
Elmidae/Dryopidae	**64**	36	17
Helodidae Larvae	39	**61**	14
Hydraenidae	57	43	11
Corydalidae	51	49	23
Case Caddis 1 Type	**62**	38	12
Case Caddis 2 Types	29	**71**	5
Case Caddis 3 Types	24	**76**	6
Ecnomidae	**76**	24	6
Hydropsychidae 1 Type	**76**	24	9
Hydropsychidae 2 Types	31	**69**	9
Philopotamidae	**73**	27	8
Athericidae	55	45	14
Blephariceridae	35	**65**	8
Chironomidae	53	47	22
Simuliidae	49	51	24
Tipulidae	26	**74**	11
Corixidae	52	48	5
Veliidae	**89**	11	6
Aeshnidae	**76**	24	9
Libellulidae	55	45	7
Oligochaeta	55	45	14
Amphipoda	39	**61**	7
Planariidae	**100**	0	7

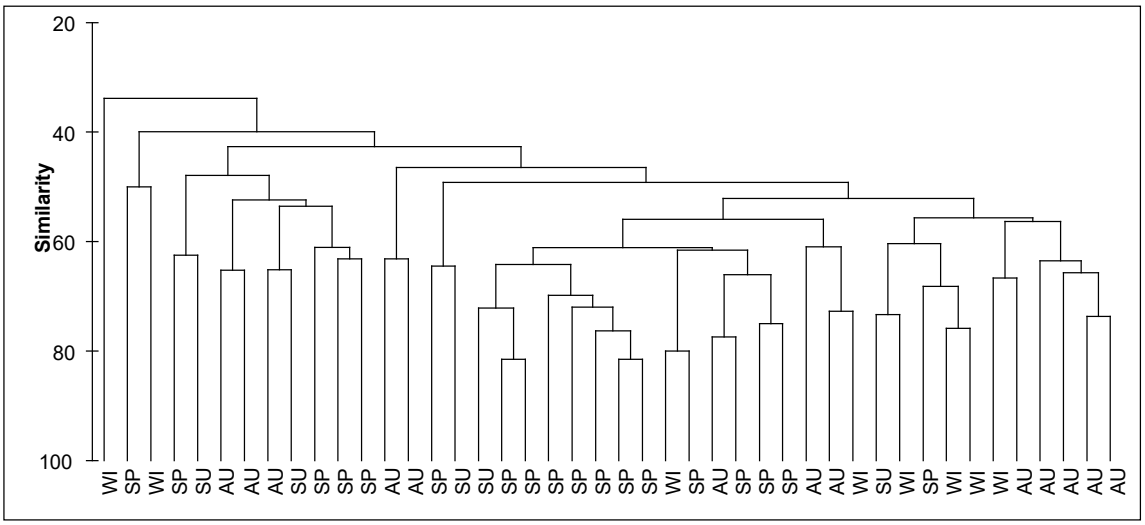


Figure 5.1 Dendrogram showing the classification of sites in the Western Cape based on taxa recorded in each season (AU = autumn, WI = winter, SP = spring and SU = summer).

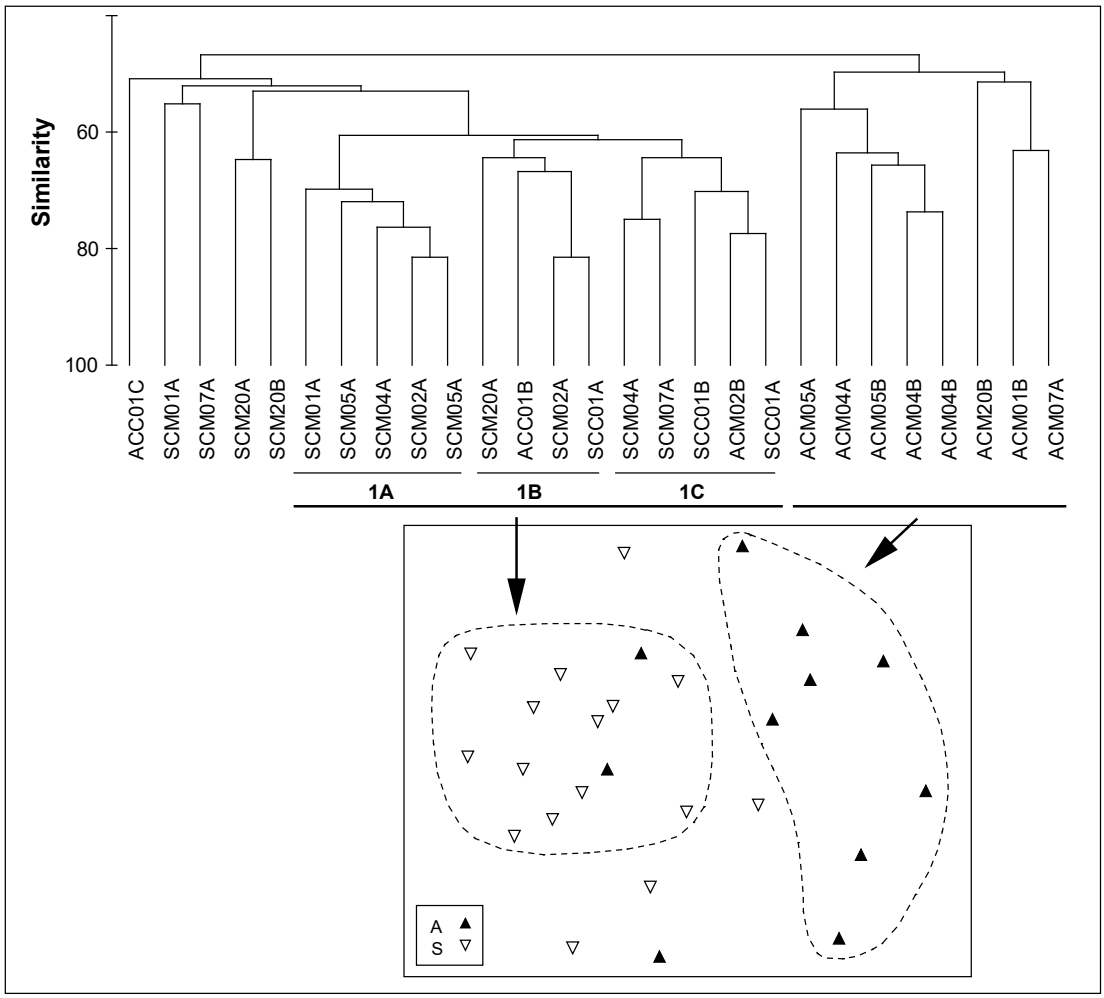


Figure 5.2 Dendrogram and MDS ordination showing the classification of upper-catchment sites in the Western Cape based on taxa recorded in autumn (A) and spring (S). The sampling year follows the site code: A = 1994, B = 1995, C = 1996.

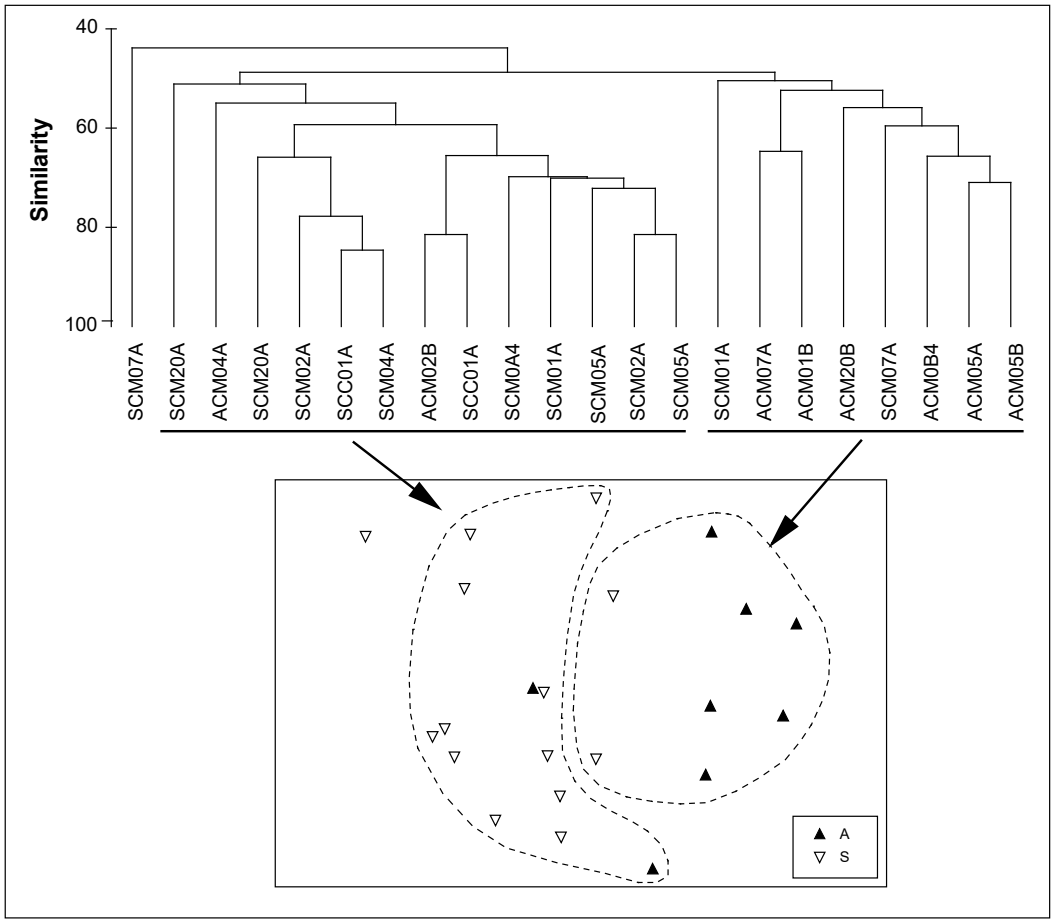


Figure 5.3 Dendrogram and MDS ordination showing the classification of upper-catchment sites in the Western Cape based on taxa recorded in the SIC/SOOC biotope-group in autumn (A) and spring (S). The year of assessment and site code follows the season.

In Mpumalanga, macroinvertebrate assemblages did not group by season (Figure 5.4, MDS: 3D-stress = 0.19). Analyses per SASS biotope-group, showed no seasonal grouping and most faunal samples were at least 55% similar for the SIC/SOOC biotope-group (Figure 5.5, MDS: 3D-stress = 0.16) and 35% similar for the AQV/MV biotope-group (Figure 5.6, MDS: 3D-stress = 0.17). ANOSIM analysis revealed that macroinvertebrate assemblages at sites were not significantly different amongst seasons (Global R = 0.021). Restricting ANOSIM analysis to a single biotope-group revealed there were significant seasonal differences in macroinvertebrate assemblages from the SIC/SOOC biotope-group (Global R = 0.049, $p < 0.05$) but that these differences were largely because of differences between autumn and spring.

Table 5.4 Taxa contributing to within-group similarity of groups identified in the seasonal analysis in the Western Cape. Results are given separately for autumn and spring. Those taxa contributing to the first 50% of the similarity are indicated by ♦; the remaining taxa contributing to the next 40% (i.e. 90% in total) of the similarity are indicated by □.

Predominant Season	**Spring**	**Autumn**	**Spring Sub-groups**		
Group	**1**	**2**	**1A**	**1B**	**1C**
Average similarity	**63.4%**	**54.7%**	**72.9%**	**68.1%**	**67.9%**
Number of distinguishing taxa	*13*	*18*	*12*	*11*	*13*
Notonemouridae	□	□	□	□	♦
Baetidae 2 Types		□			
Baetidae 3 Types	♦		♦	□	□
Teloganodidae	♦	□	♦		♦
Heptageniidae	□			♦	♦
Leptophlebiidae	♦	♦	♦	♦	♦
Dytiscidae					□
Elmidae/Dryopidae	□	♦	□	□	□
Helodidae Larvae	□		♦		♦
Hydraenidae	□	□		♦	□
Corydalidae	♦	♦	♦	□	□
Ecnomidae					□
Hydropsychidae 1 Type		□			
Hydropsychidae 2 Types				□	
Philopotamidae		□	□		
Case Caddis 1 Type		□			
Athericidae		□			
Blephariceridae			□		
Chironomidae	♦	♦	□	□	□
Simuliidae	♦	♦	♦	♦	♦
Tipulidae	□			♦	
Veliidae		♦			
Aeshnidae		□			
Chlorolestidae		□			
Oligochaeta	□		□		
Brachyura (crabs)		□			
Planariidae		□			

5.4.3 SASS4 Scores, Number of Taxa and ASPT

The season in which sampling is conducted may affect SASS4 Score, number of taxa and ASPT values. This aspect has been examined by calculating 1) the relative percentage contribution of a single season to SASS4 Score, number of taxa and ASPT calculated for multiple-season site assessments (i.e. combining data from each season); and 2) median values of SASS4 Score, number of taxa and ASPT for each season. Trends in SASS scores at four representative sites, two in each region, have also been examined.

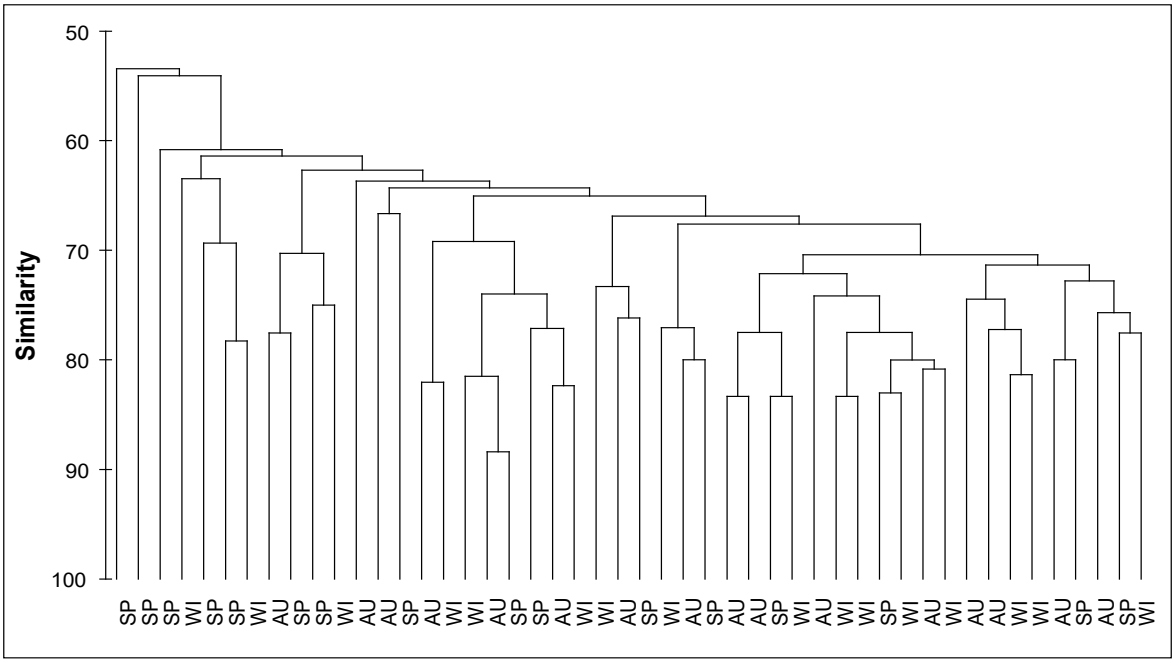


Figure 5.4 Dendrogram showing the classification of sites in Mpumalanga based on taxa recorded in three seasons (AU = autumn, WI = winter, SP = spring).

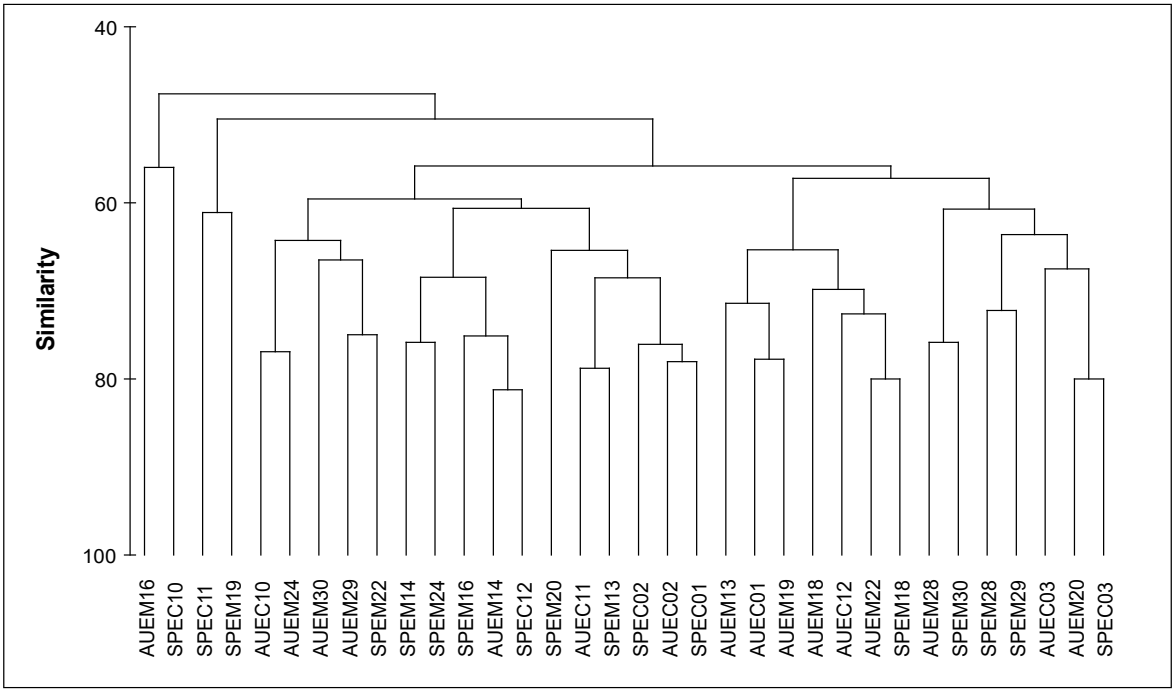


Figure 5.5 Dendrogram showing the classification of sites in Mpumalanga based on taxa recorded in the SIC/SOOC biotope-group in autumn (AU) and spring (SP). The site code follows the season.

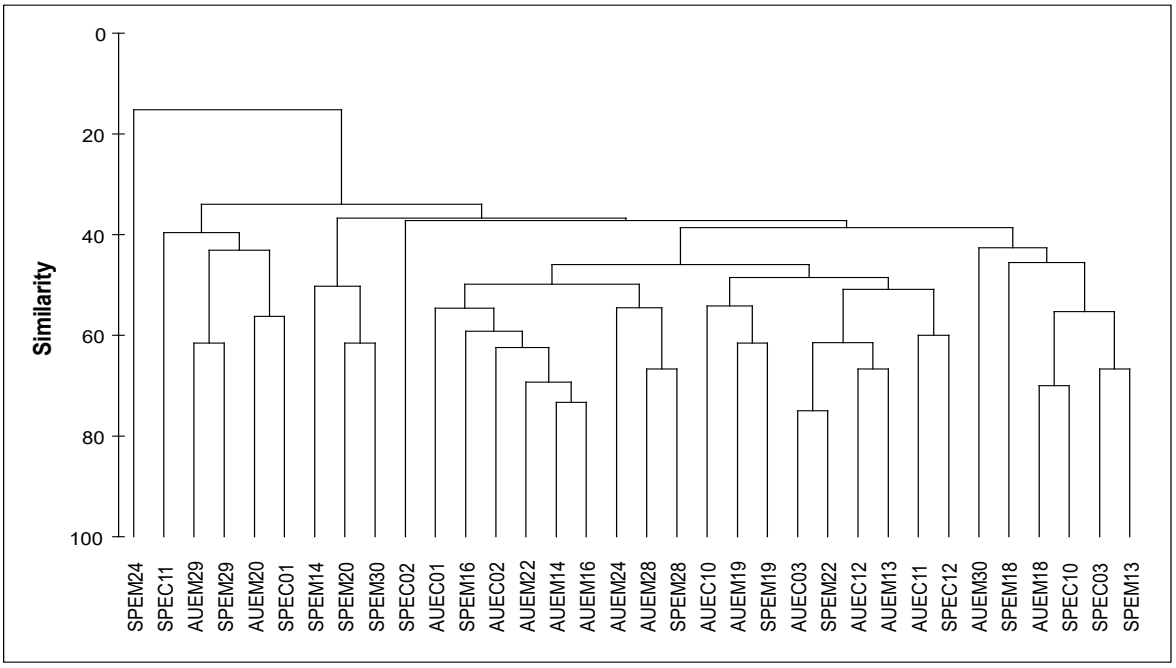


Figure 5.6 Dendrogram showing the classification of sites in Mpumalanga based on taxa recorded in the AQV/MV biotope-group in autumn (AU) and spring (SP). The site code follows the season.

Relative percentage contribution

The mean (and standard deviation, given as an error bar) percentage contribution of taxa within each of three seasons (autumn, winter and spring), to SASS4 Score, number of taxa and ASPT of the multiple-season site assessments (i.e. data from three seasons combined) have been calculated (Figure 5.7). Thus, if the SASS4 Score in spring was 145 compared to 175 for the multiple-season assessment, then the percentage contribution of taxa in spring to the multiple-season assessment would be 83%. Similarly, if ASPT in spring was 9.3 compared to 8.9 for the multiple-season assessment, the percentage contribution would be 104%. Because ASPT is calculated by dividing SASS4 Score by number of taxa, subsequent calculation of the percentage contribution of ASPT sometimes resulted in an ASPT greater than 100%. Because certain taxa are found in more than one season the summed percentages from the seasons do not equal 100%. Instead the percentage given for each season is that percentage relative to the total calculated for the multiple-season site assessments. Because ASPT is calculated by dividing SASS4 Score by number of taxa, subsequent calculation of the percentage contribution of ASPT sometimes resulted in an ASPT greater than 100%. Sites in the Western Cape sampled in all three seasons, namely autumn, winter and spring, were limited and results are based on data from six sites only.

Based on these data, taxa present in autumn respectively constituted 62%, 71% and 87% of the SASS4 Score, number of taxa and ASPT in the Western Cape (n = 6), and 71%, 72% and 99% of the SASS4 Score, number of taxa and ASPT in Mpumalanga (n = 16). Taxa present in winter constituted 58%, 54% and 111% of the SASS4 Score, number of taxa in the Western Cape, and 79%, 77% and 103% of the SASS4 Score, number of taxa and ASPT in Mpumalanga. Taxa present in spring constituted 70%, 66% and 106% of the SASS4 Score, number of taxa and ASPT in the Western Cape, and 72%, 74% and 97% of the SASS4 Score, number of taxa and ASPT in Mpumalanga. There were differences in the relative percentage contribution between regions, with Western Cape sites often having lower contributions to SASS4 Scores and number of taxa, but higher contributions to ASPT, particularly in winter, than Mpumalanga sites. The Western Cape is a winter-rainfall area as opposed to Mpumalanga, which is a summer-rainfall area, so this result is not unexpected. In the Western Cape, percentage contribution of number of taxa was highest in autumn, whilst in Mpumalanga it was highest in winter. Variation in ASPT between all three seasons was less pronounced in Mpumalanga.

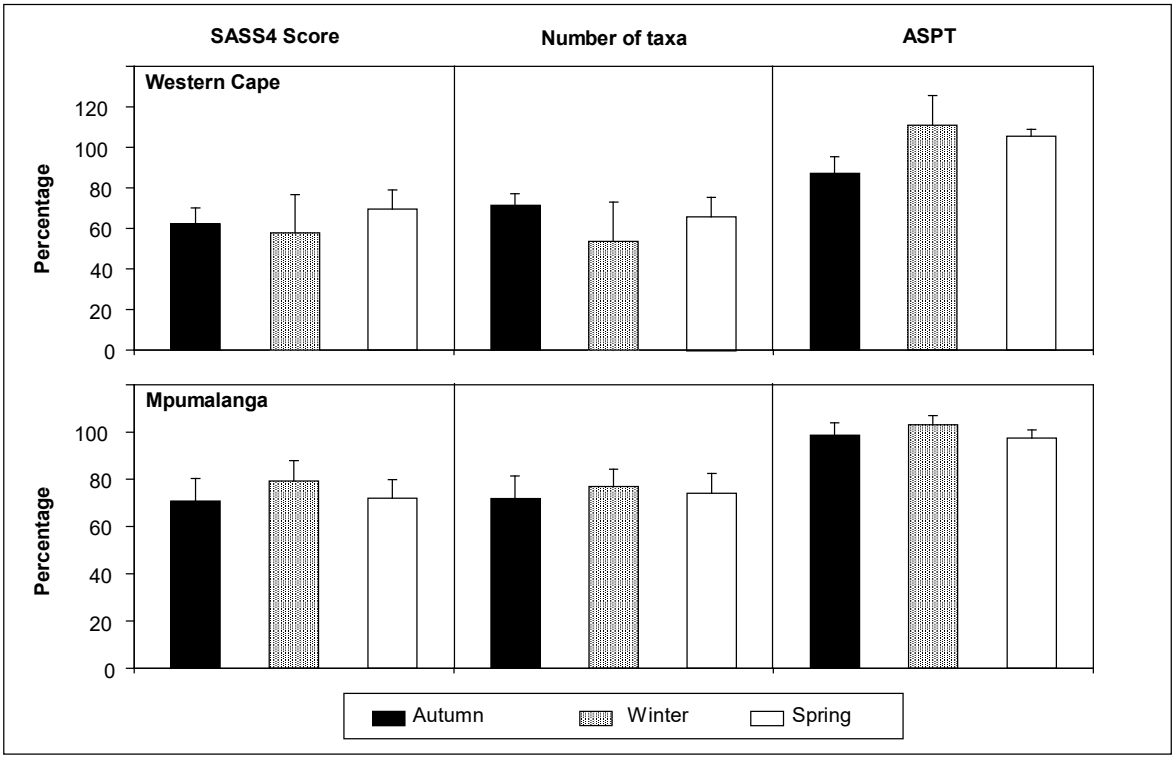


Figure 5.7 Mean (+ SD) of percentage contribution of SASS4 Scores, number of taxa and ASPT for SASS samples collected in three separate seasons (autumn, winter and spring) to SASS4 Scores, number of taxa and ASPT calculated for the multiple-season site assessment. Mean values have been calculated for the Western Cape and Mpumalanga.

Median values

Number of taxa and ASPT values were significantly different amongst seasons in the Western Cape (number of taxa: Kruskal-Wallis test statistic H = 10.35, $p < 0.05$; and ASPT: H = 15.26, $p < 0.01$, Figure 5.8). Applying the Kolmogorov-Smirnov test between pairs of seasons, however, revealed that differences were the result of differences between winter and summer for number of taxa ($p < 0.05$) and between autumn and winter, autumn and spring, and winter and summer for ASPT ($p < 0.05$). Significantly fewer taxa were recorded in winter compared to summer, and significantly higher ASPT values were recorded in winter and spring in comparison to summer and autumn. SASS4 Score, number of taxa and ASPT values were not significantly different amongst seasons in Mpumalanga. Applying the Kolmogorov-Smirnov test between pairs of seasons revealed that in Mpumalanga winter and spring were significantly different with respect to ASPT ($p < 0.05$).

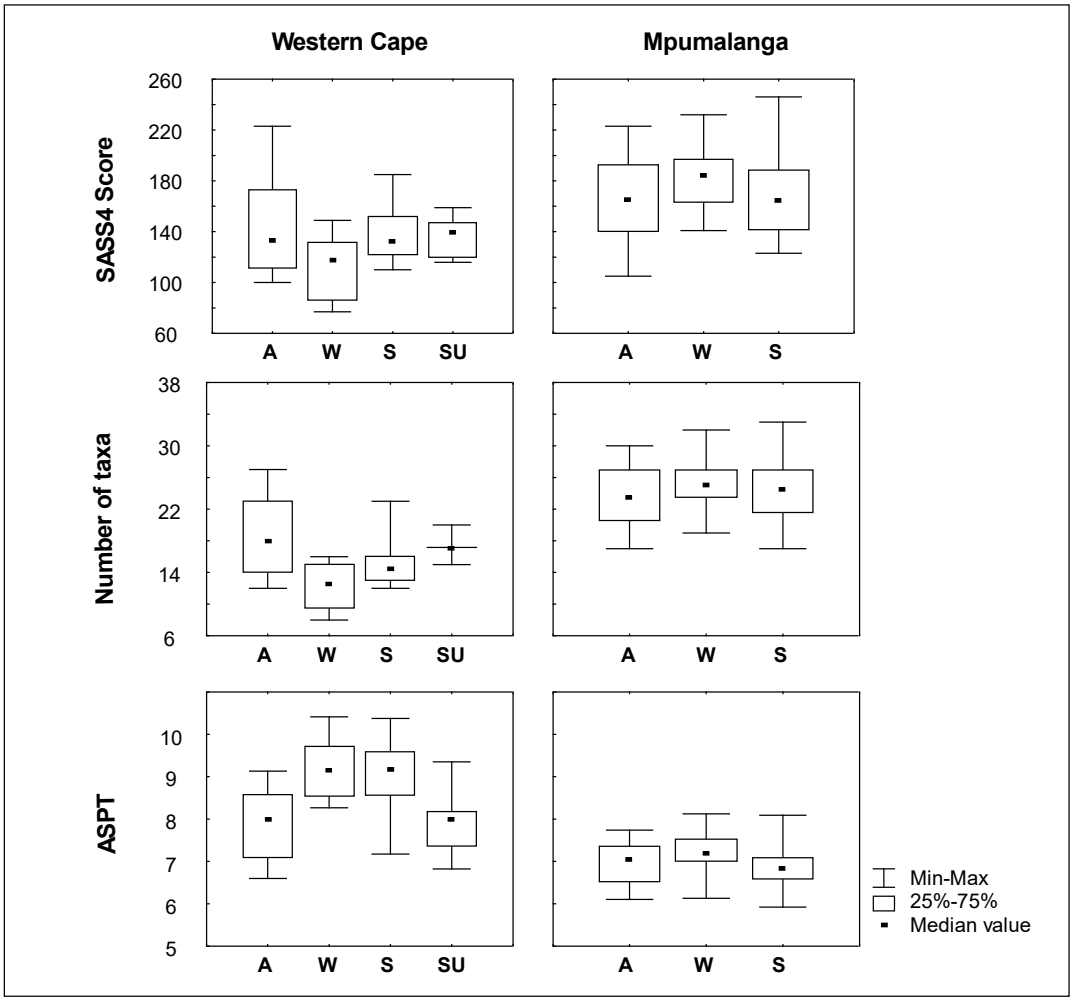


Figure 5.8. Median values for each season in the Western Cape and Mpumalanga (A = autumn, W = winter, S = spring and SU = summer).

The temporal variation in SASS scores at representative sites has been examined for a minimally-impacted mountain stream and a minimally-impacted foothill-cobble bed site in the Western Cape (Figures 5.9 and 5.10) and in Mpumalanga (Figure 5.11 and 5.12). In each case SASS4 Score, number of taxa and ASPT are given for each sampling occasion. Cluster analysis and MDS ordination were performed on macroinvertebrate assemblage data for each site and dendrograms showing temporal grouping at each site are given. SASS4 Score and number of taxa were variable and were often highest in spring in the western cape and winter in Mpumalanga. ASPT varied the least amongst sampling occasions. Faunal samples within sites were between 50 and 60% similar and clustering on the basis of season was not evident.

5.5 DISCUSSION

This study indicates that seasonal differences in the frequency of occurrence of individual taxa were limited, with most differences evident from the analysis of the SIC/SOOC biotope-group in the Western Cape. Several sensitive and high-scoring taxa, such as Teloganodidae, Heptageniidae, Helodidae, Blephariceridae and Amphipoda, were more common in spring compared to summer, whilst Elmidae/Dryopidae, Ecnomidae and Philopotamidae were more common in autumn. These observations were reflected in the macroinvertebrate assemblages with seasonal clustering into predominantly autumn- versus spring-groups. This was especially evident when macroinvertebrates associated with the SIC/SOOC biotope-group were examined, possibly because this biotope is more sensitive to changes in flow than other biotopes. Similar results were obtained by Chessman *et al*. (1997) who found negligible seasonal variation in values of SIGNAL (similar to ASPT), with only riffles within the SIC biotope showing a difference between spring and autumn. Whilst more taxa were recorded in autumn than in spring in the Western Cape, a higher proportion of sensitive and high-scoring taxa were recorded in spring than in autumn.

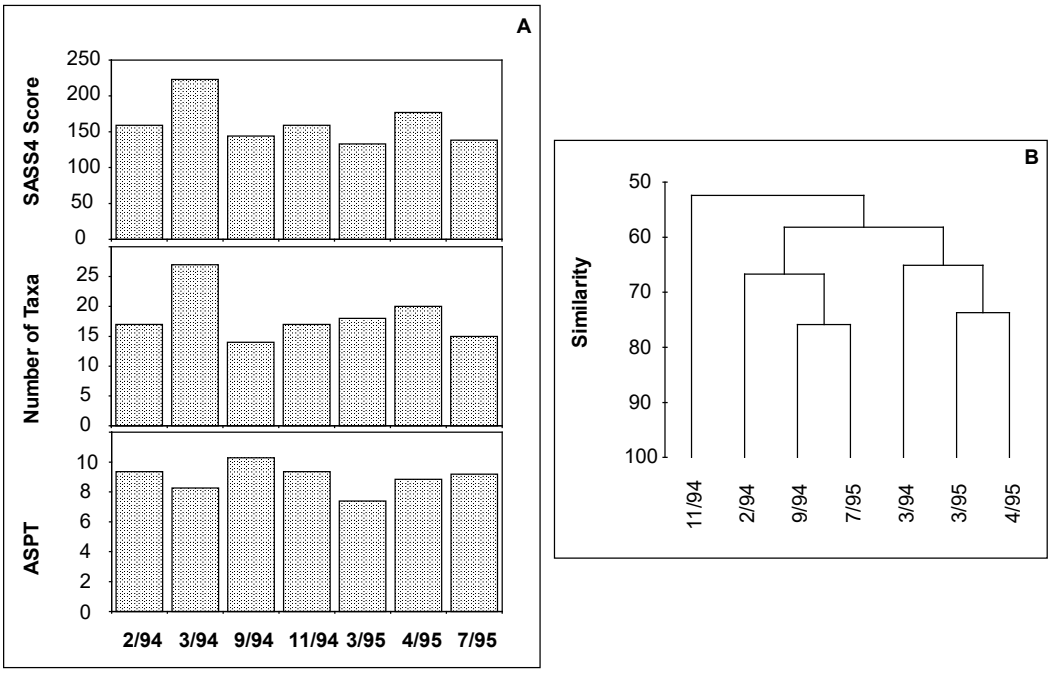


Figure 5.9 **A: SASS4 Score, number of taxa and ASPT per sampling occasion for a minimally-impacted site (CM04) on the upper Eerste River in the Western Cape. B: Dendrogram showing classification of faunal samples.**

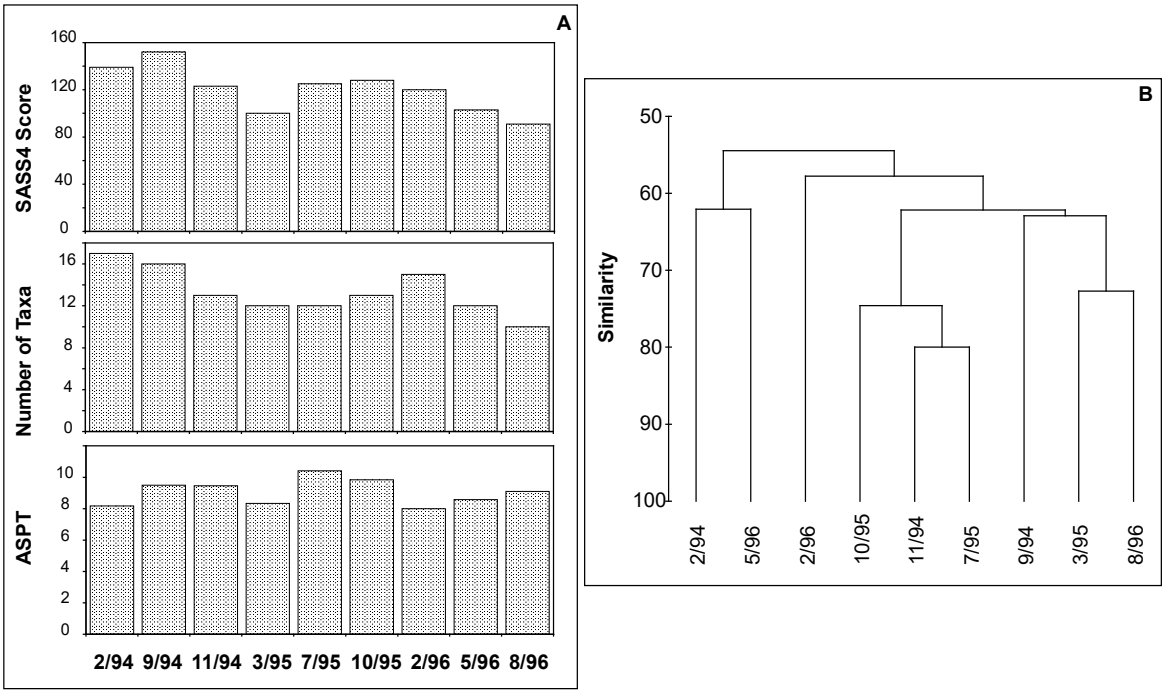


Figure 5.10 **A: SASS4 Score, number of taxa and ASPT per sampling occasion for a minimally-impacted site (CC01) on the upper Berg River in the Western Cape. B: Dendrogram showing classification of faunal samples.**

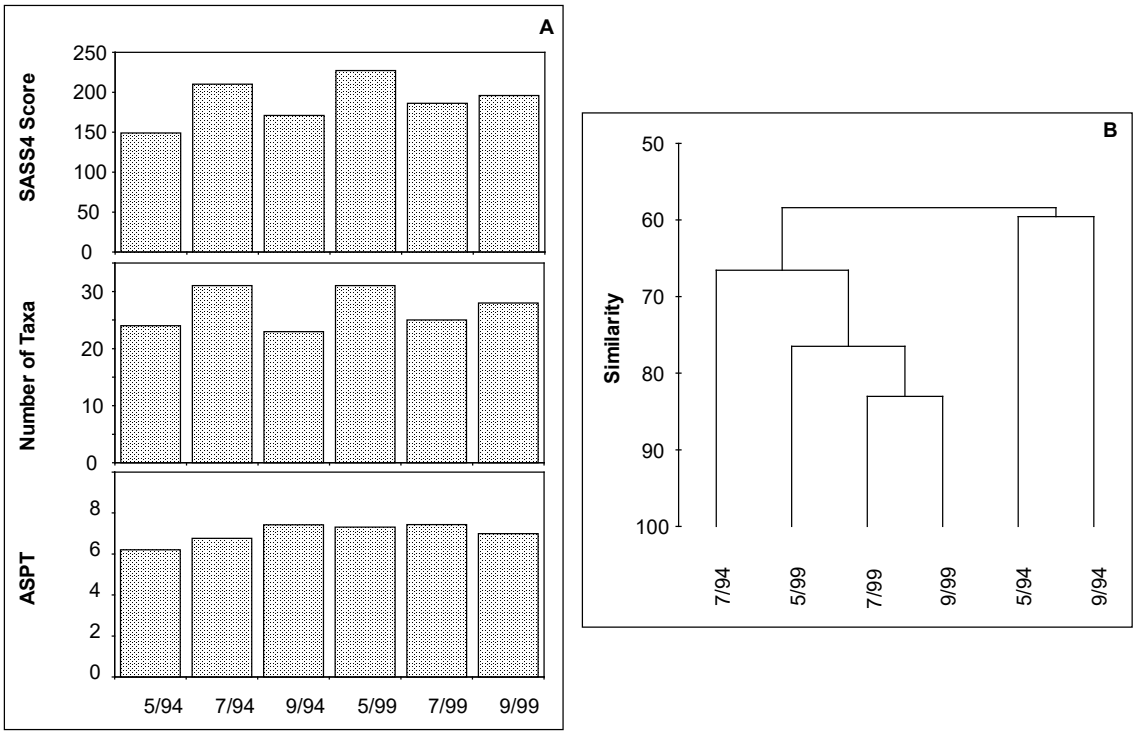


Figure 5.11 A: SASS4 Score, number of taxa and ASPT per sampling occasion for a minimally-impacted site (EM19) on the Ohrigstad River in Mpumalanga. B: Dendrogram showing classification of faunal samples.

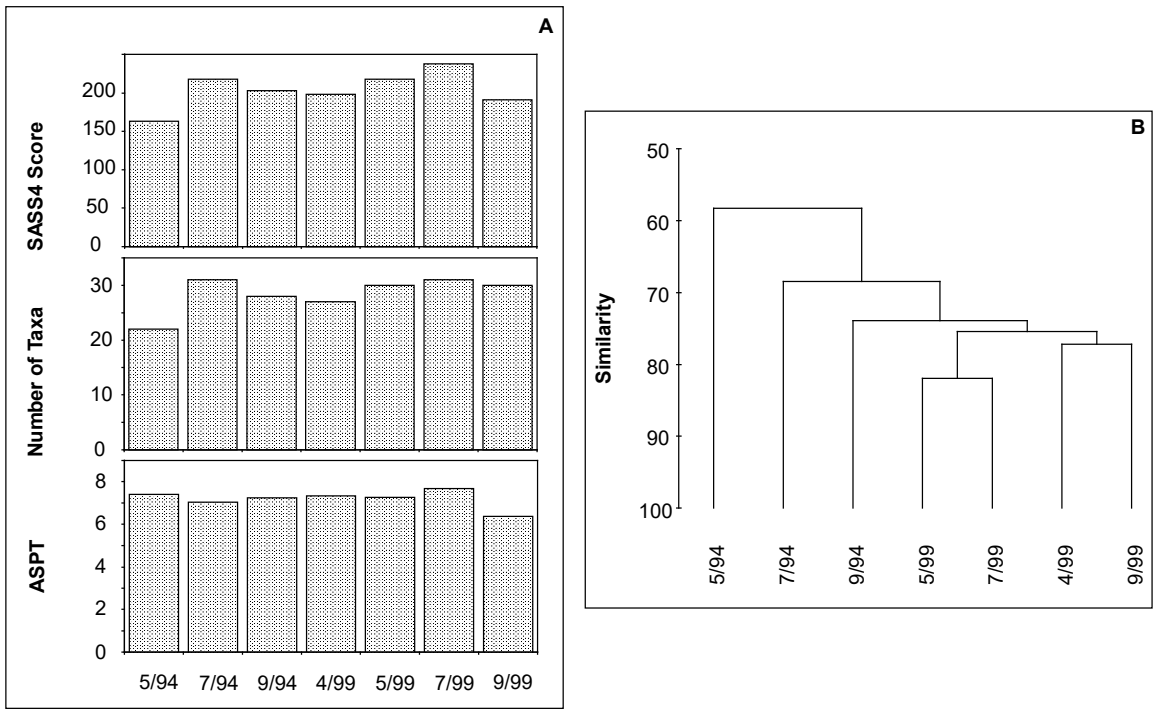


Figure 5.12 A: SASS4 Score, number of taxa and ASPT per sampling occasion for a minimally-impacted site (EC01) on the Blyde River in Mpumalanga. B: Dendrogram showing classification of faunal samples.

Seasonal patterns in the distribution and abundance of macroinvertebrates reflect life history characteristics of individual taxa. Temporal differences in taxonomic makeup of macroinvertebrate assemblages within streams may be due to the differences among insect life cycles (e.g. Yanoviak & McCafferty 1996). 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 & Day 1998). King (1981) and King *et al*. (1988) recorded no major temporal changes in the composition of macroinvertebrate assemblages in two mountain streams of the Western Cape, although densities were highest in late spring (King *et al*. 1988, Britton 1991). King *et al*. (1988) attributed this to the slow growth of species in the highly oligotrophic waters of upland reaches of this region. In the present study, the sampling method, SASS, utilises a collecting apparatus with comparatively large mesh-size (1 mm), thus smaller instars of aquatic insects such as heptageniid and teloganodid mayflies, may not be collected and observed seasonal differences in upper catchments may be heightened when SASS sampling is undertaken. For example, the heptageniid*, Afronurus harrisoni,* which King (1981) considers to be a summer species, was more frequently recorded in spring than in autumn. It is possible that by autumn, emergence had taken place and individuals of the next generation were too small to be collected. Many stream insects emerge sequentially over the period of early to late summer (Sweeney 1984, Newbold *et al.* 1994) and spring is considered to be a period when river discharge is starting to decrease, and the number of over-wintering invertebrates on the streambed surface increases, but emergence has not yet begun (King *et al.* 1988).

Seasonal patterns in the distribution and abundance of macroinvertebrates reflect temperature regimes and may also reflect the availability of food resources. 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 elevated water temperatures. Britton (1991) further explored the postulated synchronization between abundance of shredders and the annual pulse of leaf-fall. The key shredder in Swartboskloof, namely the amphipod, *Paramelita nigroculus*, as well as Plecoptera, which are thought to exploit coarse leaf particles (King *et al*. 1988), were most abundant in spring, after which their numbers dropped remarkably, despite litter-fall from riparian trees occurring in this summer period. Bunn (1986a, b), in a study of northern jarrah forest streams of western Australia, which are also within a mediterranean ecosystem, observed a similar lack of

synchronization in shredder life cycles and summer leaf-fall. Failure of the shredders to take advantage of summer leaf-fall has been attributed to poor quality of senescent leaves, high summer water temperatures, and elevated concentrations of polyphenols in stream water (Bunn 1986b, 1988, Britton 1991). Britton (1991) postulates that microbial processing of leaf-litter during autumn and winter, aided by physical abrasion of leaf material at high discharges, results in high-quality course particulate organic matter (CPOM) becoming available to shredders in spring. It is postulated that they are most abundant in spring and have life cycles synchronized to exploit this food resource once it becomes nutritionally available.

In the summer-rainfall region of Mpumalanga, most taxa were recorded in winter, whilst autumn exhibited the most within-season variability, and spring had the highest frequency of occurrence of 12 taxa. These differences were not reflected in the cluster and ordination analysis, however, suggesting that, at the assemblage-level, seasonal differences in macroinvertebrate assemblages in Mpumalanga are negligible. In the Western Cape, a winter-rainfall region, periods of lowest baseflow are coupled with high temperatures, whilst in Mpumalanga periods of lowest baseflow occur in winter, and are thus not coupled with high temperatures. The lotic environment in the Western Cape may therefore be thought of as a more stressful environment than that of Mpumalanga and seasonal patterns of macroinvertebrate assemblages at upper-catchment sites of this region may reflect adaptations of aquatic organisms to these harsh conditions over evolutionary time.

An understanding of the natural temporal variability of individual taxa, macroinvertebrate assemblages and SASS scores at a site is necessary for detection of SASS scores that are outside the expected intrinsic range of variability. Using preliminary guidelines for establishing if a site in the Western Cape is disturbed or not (Table 5.5, modified from Dallas *et al*. 1998), individual site assessments at CM04 (Figures 5.9) would always class as minimally-disturbed and those at CC01 (Figure 5.10) as minimally-disturbed with the exception of SASS4 Score on three occasions, although ASPT on these occasions always exceeded 7.5. Upper-catchment sites in the Western Cape are known for their relatively low SASS4 Score and associated high ASPTs. This phenomenon has been attributed to the low biotope diversity of such sites, with frequent absence or scarcity of instream and marginal vegetation. This results in fewer taxa being recorded, hence low SASS4 Score, although many of these are sensitive and high-scoring ones, hence high ASPT. In contrast,

upper-catchment sites in Mpumalanga often have a diverse array of biotopes, including vegetation, and sites in this region tend to have much higher SASS4 Scores, whilst ASPTs are often lower than those recorded in the Western Cape. SASS4 Scores at sites in Mpumalanga (Figures 5.11 and 5.12) far exceeded the guidelines of Chutter (1998), as did ASPT values. Chutter's guidelines (1998) do not differentiate between subregions. Using median values and Observed/Expected ratios generated for SASS4 Score and ASPT at upper-catchment sites in Mpumalanga on a seasonal basis (Dallas 2000b), all site assessments would indicate that both site EM19 and site EC01 were always within biological band A, i.e. reference (see Table 1.2, Chapter 1). It seems then, that even in instances where seasonal differences are present at the level of macroinvertebrate assemblage, when translated into biotic indices, SASS scores are such that sites remain in the class designated as minimally-disturbed or reference.

Table 5.5 Preliminary SASS4 Scores and ASPT values for identifying impacted sites (Western Cape: modified from Dallas *et al*. 1998, Mpumalanga: Chutter 1998).

Geographic region	Subregion	SASS4 Score	ASPT
Western Cape	Mountain Stream	140	7.5
	Foothill-cobble bed	120	7.5
Mpumalanga	Subregions not separated	100	6.0

In summary, if data interpretation is based on SASS scores alone, seasonal differences may not be evident. However, at both the individual taxon and assemblage levels, differences amongst season are evident in the Western Cape. For this reason, if a monitoring site is assessed in autumn only, it should preferably be compared to the reference condition for autumn, if available, or to the general reference condition, taking into account those taxa that are reported to occur more frequently in spring. The absence of one of these "spring" taxa, may merely reflect a seasonal pattern, as opposed to one related to water quality impairment or reduced river health. Of the three biotope-groups in the Western Cape, macroinvertebrate assemblages from the SIC/SOOC biotope-group exhibit distinct seasonal variability. Sampling season and comparison with appropriate reference conditions defined for this biotope-group is thus particularly crucial.

Given the observed seasonal variability of macroinvertebrate assemblages in the Western

Cape, it would be advantageous to assess the variability of reference assemblages and SASS scores on an annual basis. Although Barbour *et al.* (1996) found minimal annual differences in reference sites in Florida, United States, over three sampling years, in a climatically variable country such as South Africa, annual assessment would be useful in understanding intrinsic variability in reference assemblages and SASS scores. As noted, the extent of the variability is likely to vary geographically. During the implementation of biomonitoring programmes, annual bioassessment of the reference sites therefore needs to take precedence. In this way the extent of the annual and seasonal variability of reference assemblages and SASS scores may be quantified. Understanding this variability is important since, as variability among reference sites increases, so do the differences necessary to discriminate monitoring from reference sites (Hawkins *et al.* 1997).

CHAPTER 6. THE EFFECT OF BIOTOPE-SPECIFIC SAMPLING ON REFERENCE SITE CLASSIFICATION AND IDENTIFICATION OF ENVIRONMENTAL PREDICTORS

6.1 INTRODUCTION

Riverine ecosystems are extremely complex systems, driven and affected by a multitude of abiotic and biotic factors that may interact to generate biotic patterns. As a result of these interactions, biotic assemblages within river systems exhibit a high degree of spatial heterogeneity (Poff & Ward 1990, Cooper *et al.* 1997, Palmer & Poff 1997, Townsend *et al.* 1997). Understanding which environmental variables are responsible for this heterogeneity has been the focus of several studies (e.g. Statzner & Higler 1986, Allan *et al*. 1997, Hawkins *et al.* 1997, Wiley *et al*. 1997). In the context of bioassessment and the establishment of ecological reference conditions, the identification of environmental variables that best explain the observed spatial distribution of biotic assemblages is important, particularly if predictive models are to be developed. Such models are increasingly being applied within bioassessment programs (e.g. Wright 1995, Smith *et al.* 1999) and the prediction system is heavily dependent on the strength of the relationship between the biological and environmental attributes of the reference sites. Spatial heterogeneity and variability appear to be scale-dependent (Wiley *et al*. 1997) with predictability partially dependent on the hierarchical level at which the study is undertaken (Frissel *et al.* 1986, Rowntree *et al.* 1998). An understanding of the factors determining the distribution and abundance of stream organisms requires research at many scales (e.g. Cooper *et al.* 1998). Establishing relationships between environmental variables and biological variables is also dependent on the choice and measurement of the environmental variables as well as the efficacy of the biological classification (Carter *et al.* 1996). Ultimately, however, understanding the significance of different scales and the potential influence of environmental variables on the spatial distribution biotic assemblages is important, particularly if these assemblages are to form the basis of bioassessment.

Broad-scale environmental variables occur at the level of catchment and their potential effect on riverine ecosystems has been aptly described by Hynes's (1975) statement that: "In every respect, the valley rules the stream". Altitude (Wright 1995, Carter *et al.* 1996,

Marchant *et al.* 1997, Bailey *et al.* 1998, Smith *et al.* 1999, Turak *et al.* 1999, Monaghan *et al*. 2000), longitude/latitude (Wright 1995, Marchant *et al.* 1997, Reynoldson *et al*. 1997, Smith *et al.* 1999, Turak *et al.* 1999), distance from source (Wright 1995, Marchant *et al.* 1997, Bailey *et al.* 1998, Linke et al. 1999, Smith *et al.* 1999, Turak *et al.* 1999), upstream catchment area (Bailey *et al.* 1998, Linke et al. 1999) and channel slope (Collier 1995, Tate & Heiny 1995) have all been shown to be catchment-scale environmental variables that explain biotic distribution patterns, and, in particular, contribute towards the discrimination between identified macroinvertebrate groups. Thus, broad-scale environmental variables set the total "potential" community, whilst its actual structure and composition are determined by site and habitat-scale variables.

At the scale of site, factors such as stream width (Wright 1995, Reynoldson *et al*. 1997, Beisel *et al.* 1998, Linke et al. 1999), stream depth (Wright 1995, Reynoldson *et al*. 1997, Collier *et al.* 1998, Smith *et al.* 1999), flow pattern (Wright 1995, Smith *et al.* 1999) and current velocity (Beisel *et al.* 1998) have been strongly associated with macroinvertebrate assemblage structure. In particular, the depth of shallow-water biotopes such as riffles has been shown to correlate with taxonomic richness (Collier 1995). The characteristics of the riparian vegetation at a site, specifically the extent of the canopy cover and thus the shade ratio, i.e. the ratio of mean bank height plus riparian vegetation height over channel width, has also been shown to contribute to the richness of macroinvertebrate assemblages (Collier 1995).

At the scale of habitat, variables such as the nature of the substratum, including substrate diversity (Marchant *et al.* 1997), type (Wohl *et al.* 1995, Collier *et al.* 1998), size (Collier 1995, Beisel *et al.* 1998, Lammert & Allan 1999) and texture (Downes *et al.* 1998), and the extent of particular substrate types such as percentage bedrock or cobble or silt (Reynoldson *et al*. 1997), are considered to exert a strong influence on biotic community structure (Minshall 1984, Collier *et al.* 1998, Linke et al. 1999). Beisel *et al.*'s (1998) findings were consistent with the hypothesis that benthic macroinvertebrate abundance increases with substrate size up to cobble size and decreases as the substrate becomes boulder and bedrock (e.g. Quinn & Hickey 1990). Downes *et al.* (1998) provided experimental evidence that local processes such as substrate texture may regulate stream communities. In their study, habitat structure altered faunal diversity and abundances, with the majority of common species reaching highest abundances on creviced or rough

surfaces. Wohl *et al.* (1995) examined invertebrate assemblages in three habitat-types, depositional (sand), cobble-riffle and bedrock outcrop, and found that each had a distinctive macroinvertebrate community structure, fundamentally determined by the local geomorphology and related to physical parameters. The amount of submerged wood was an important correlate with the number of taxa and the percentage of the dominant taxon for lowland streams assessed in Waikato, New Zealand (Collier *et al.* 1998), whilst the amount of fines (e.g. mud, silt or clay), was an important factor in influencing assemblage structure in the Vaal catchment, South Africa (Chutter 1970) and in the Fraser River, British Columbia (Reynoldson *et al*. 1997). Richards *et al.* (1997) found that the percentage fines (less than 2 mm in diameter) was highly predictive of species traits such as life history, functional group, mode of existence, habitat specificity and mobility.

Stream water chemistry, which is influenced by, for example, geology (Day & King 1995), may strongly influence macroinvertebrate assemblages (Poff & Allan 1995, Richard *et al.* 1997). Stream temperature (Collier 1995, Tate & Heiny 1995, Hawkins *et al.* 1997, Webster & Meyers 1997, Turak *et al.* 1999), air temperature (Wright 1995), conductivity (Collier 1995, Tate & Heiny 1995, Marchant *et al.* 1997), alkalinity (Wright 1995, Reynoldson *et al*. 1997) and dissolved oxygen (Dallas & Day 1993) are water chemistry variables known to influence biotic assemblages. The concentration of nutrients such as organic nitrogen + ammonia and total phosphorus, have also been implicated in contributing to observed differences in macroinvertebrate assemblages between upland and lowland areas (Tate & Heiny 1995). In such instances, however, the observed effect may reflect trends in water quality, since lowland systems are often impacted to some extent by anthropogenic activities.

Clearly, a multitude of environmental variables may potentially affect the spatial distribution of macroinvertebrate assemblages. It is the aim of this chapter to investigate the relationship between environmental variables and macroinvertebrate assemblages, with the goal of identifying the importance of variables at different scale in discriminating between identified groups of sites with similar macroinvertebrate assemblages (i.e. Groups). In addition, the influence of sampling different biotope-groups is examined by comparing reference site classifications based on each separate SASS biotope-group. Environmental variables that best discriminate between the identified Groups within each biotope-group classification are identified and general implications with respect to

bioassessment and the establishment of ecological reference conditions are discussed.

6.2 STUDY AREA

Fifty-nine sites on 34 rivers, used in the composite classification of reference sites in Mpumalanga (Chapter 3), were incorporated in this chapter (Table 6.1). Sites were assessed on three occasions (May, July and September) in 1999.

Table 6.1 Sites in Mpumalanga assessed during this study indicating the river name and site code (in parenthesis). These relate to Table 2.1 and Figures 2.1 and 2.2 in Chapter 2.

River (site codes)
Alexanderspruit (HC42), Blyde (EC01, EC02), Blystaanspruit (EC04), Crocodile (EC05, EC06, ER32, HC43, LG60, LG61, LG62, LG63, LG64), Dorps (HC36), Elands (EC07, EC08) Elandsfonteinspruit (HC44, HM51), Ga-Selati (EM20), Grootfonteinspruit (EM16), Heddle (EM17), Houtbosloop (EC09), Kareekraalspruit (HM52), Kgwete (EM18), Klein-Sabie (EC10), Klip (HC38), Lone Creek (EM25), Lunsklip (HC45), Mac-Mac (EC11, LC56, LR73), Marite (LC57), Maritsane (LM71), Mohlombe (EM26), Nelspruit (EM22, LR72), Ohrigstad (EM19), Sabie (EC12, EM27, LC58, LF59, LG66, LG67, LG68, LR74), Sand (EM29, LG69, LG70), Selon (HC35), Spekboom (EM13, HC39), Sterkspruit (EM14), Tautesloop (HM54), Treu (EC03), Unspecified (EM15, EM24, EM28, EM30, EM31, HM48), Waterval (HC40, HC41), Wilge (HG33) and Wilgekraalspruit (HM55)

6.3 MATERIALS AND METHODS

6.3.1 Benthic macroinvertebrates: SASS4 sampling

Benthic macroinvertebrates were sampled using the qualitative rapid bioassessment method, SASS4. A detailed description of the method is given in Chapter 2. Three SASS-defined biotope-groups, namely stones-in-current/stones-out-of-current (SIC/SOOC), aquatic and marginal vegetation (AQV/MV) and gravel/sand/mud (G/S/M), were sampled separately. The "composite samples" for each site were derived by combining the lists of taxa recorded in each SASS biotope-group at each site.

6.3.2 Environmental variables

The environmental variables measured at each site are listed in Table 6.2 together with their abbreviations, the transformation procedures used, and details of the measurement and categorisation procedures applied. Variables were divided into four types, namely catchment variables such as longitude, latitude, altitude, distance from source and stream order; site variables such as channel pattern, hydrological type, stream width, habitat depths, geological and vegetation types and canopy cover; habitat variables such as substratum richness, composition and dominance, the percentage of each substratum type, percentage embeddedness of the stones, the number and combination of biotopes, the percentage of each biotope present, and the percentage cover of algae and macrophytes; and water chemistry variables including pH, temperature, conductivity, turbidity, dissolved oxygen, alkalinity and nutrients (total phosphorus, Kjeldahl nitrogen, nitrate + nitrite, ammonium and silica). Details pertaining to the analytical procedures of the chemical variables are given in Chapter 2. The mean of the three sampling occasions was calculated in instances where the value was a dimension (e.g. stream width, shallow-water habitat depth), concentration (e.g. temperature, conductivity) or percentage (e.g. percentage bedrock, percentage SIC/SOOC).

6.3.3 Data analysis

Analysis of macroinvertebrate assemblages

Cluster analysis and non-metric multidimensional scaling (MDS) were used to examine similarities amongst sites based on macroinvertebrate assemblage composition (Clark & Warwick 1994). Analyses were based on data generated from macroinvertebrates collected in three seasons (autumn, winter and spring) for each biotope-group separately. Classification of sites based on two or three seasons rather than one season is often recommended as it is considered a more robust means of classifying sites, since temporal variation is reduced (Turak *et al.* 1999). Four separate classifications were undertaken, the first a composite classification in which macroinvertebrate assemblages recorded in all three biotope-groups were combined, the second a classification of assemblages from the SIC/SOOC biotope-group, the third based on macroinvertebrate assemblages from the AQV/MV biotope-group and the fourth based on assemblages from the GSM biotope-group.

Table 6.2. Environmental variables measured at sites in the study area with details of measurement and categorisation procedures. Variables prefixed with a L were $log_{10}(x)$ transformed and variables commonly affected by anthropogenic activities are indicated with an asterisk (*).

Variable Type	Variable	Code	Categories	Measurement and categorisation details
Catchment variables	Longitude	LONG		GIS co-ordinates
	Latitude	LAT		GIS co-ordinates
	Altitude	LALT		Obtained from 1: 250 000 maps, in metres
	Distance from source	LDIS		Obtained from 1: 250 000 maps, in kilometres
	Stream order	ORD	1, 2, 3, 4	Obtained from 1: 250 000 maps
Site variables	Channel pattern	CHP	S, SS, MA	Based on descriptions by Rowntree and Wadeson (1999). S: single thread: low sinuosity: single channel, laterally inactive; SS: single thread: high sinuosity - stable-sinuous: single channel, moderately, laterally inactive; MA: multiple thread: anatomosing/anabranching: multi-thread channels separated by vegetated or otherwise stable alluvial islands or bedrock
	Hydrological-type	HYDRO	P	All sites assessed were perennial (P)
	Stream width	LW		Mean width of water, in metres
	Shallow-water habitat depth	LSAVG		Mean depth, in metres
	Shallow-water habitat-type	SHType	1, 2, 3	1: bedrock rapid, 2: bedrock rapid/cobble riffle mix, 3: cobble riffle
	Deep-water habitat depth	LDAVG		Mean depth, in metres
	Geological/ lithostratigraphic type	GEOL	Jj, Jl, Vgwb, Vm, VMlw, Vp, Vro, Vru, Z, Zba	Based on Vegter's (1995) simplified lithostratigraphic units. Jj: Rhyolite, granophyre, syenite, tuff, breccia, minor sedimentary rocks; Jl: Basalt; north-south trending dolerite dykes along Lebombo range; Vgwb: Lava, tuff, quartzite, shale, conglomerate; Vm: Dolomite, chert, subordinate quartzite, conglomerate, shale; diabase and syenite dykes and sills; VMlw: Pyroclastics, lava, quartzite, conglomerate, sandstone siltstone; grit, shale, diabase sills; Vp: Quartzite, shale, conglomerate, iron formation, breccia, diamicitite, limestone, dolomite; Vro: Rhyolite, pyroclastics; Vru: Bronzitite, harzitite, harzhurgite, norite, pyroxenite, anorthotise, gabbro, diorite; Z: Granite, granodiorite, tonalite, gneiss, migmatite; Zba: Sandstone, shale, conglomerate, greywacke, lava, pyroclastic rocks

Variable Type	Variable	Code	Categories	Measurement and categorisation details
	Vegetation type	VEG	AF, MSHG, NEMG, RHG, MOB, MB, SOLB, MLB, SWLB, LAMB	Based on Low and Rebelo's (1996) potential natural vegetation of South Africa, Lesotho and Swaziland. AF: Afromontane Forest; MSHG: Moist Sandy Highveld Grassland; NEMG: North-Eastern Mountain Grassland; RHG: Rocky Highveld Grassland; MOB: Mopane Bushveld; MB: Mixed Bushveld; SOLB: Sour Lowveld Bushveld; MLB: Mixed Lowveld Bushveld; SWLB: Sweet Lowveld Bushveld; LAMB: Lebombo Arid Mountain Bushveld
	Canopy cover	CC	1, 2, 3	1: open, 2: partially open, 3: closed
Habitat variables	Substratum richness	SUBRICH	1, 2, 3 4	The percentage area of each substratum type in the sample area was estimated by eye. Size classes for each substratum type have been modified from the Wentworth grade scale (units are in mm) as follows: bedrock, boulder - x > 256, cobble - 100 < x < 256, pebble - 16 < x < 100, gravel - 2 < x < 16 (fine pebble or small gravel of Wentworth), sand - 0.06 < x < 2, mud/silt/clay - x < 0.06. In analyses, cobble and pebble were combined, and a group consisting of gravel, sand and mud/silt/clay was used. Richness: number of substratum types, including BR: bedrock, B: boulder, CP: cobble/pebble, G: gravel/sand/mud
	Substratum composition	SUBCOMP	1, 2, 3, 4	1: BR/B/CP/G, 2: BR/CP/G, 3: B/CP/G and 4: CP/G
	Substratum dominance	SUBDOM	BR, BR/B, BR/CP, BR/G, B/CP, B/G, CP, CP/G, G	If any one substratum-type was > 60%, then single dominant type; otherwise two dominant substrata are given
	% Bedrock	BR		An estimate of the mean percentage bedrock in the sample area
	% Boulder	B		An estimate of the mean percentage boulder in the sample area
	% Cobble/pebble	CP		An estimate of the mean percentage cobble/pebble in the sample area
	% Gravel/sand/mud	GSM		A calculated total mean percentage of gravel, sand and mud/silt/clay in the sample area
	% Gravel	G		An estimate of the mean percentage gravel in the sample area
	% Sand	S		An estimate of the mean percentage sand in the sample area

Variable Type	Variable	Code	Categories	Measurement and categorisation details
	% Mud	M		An estimate of the mean percentage mud/silt/clay in the sample area
	% Embeddedness	EMB	1, 2, 3, 4	An estimate of the extent to which boulder/cobble/gravel particles are embedded in the surrounding fine sediments such as small gravel, sand, silt and/or mud. 1: 0-25%, 2: 26-50%, 3: 51-75%, 4: 76-100%
	Biotope number	BIOTNO	1, 2, 3	Number of biotope-groups sampled including stones-in-current/stones-out-of-current (SIC/SOOC), aquatic and marginal vegetation (AQV/MV) and gravel/sand and mud (GSM)
	Biotope combination	BIOTCOMB	1, 2, 3, 4, 5	1: all three biotopes, 2: SIC/SOOC + AQV/MV, 3: SIC/SOOC + G/S/M, 4: AQV/MV + G/S/M and 5: SIC/SOOC only
	% SIC/SOOC	SIC/SOOC		An estimate of the mean percentage SIC/SOOC in the sample area
	% AQV/MV	AQV/MV		An estimate of the mean percentage AQV/MV in the sample area
	% G/S/M	G/S/M		An estimate of the mean percentage G/S/M in the sample area
	% Algae*	ALGAE		An estimate of the mean percentage of sample area covered by algae
	% Macrophytes*	MACRO		An estimate of the mean percentage of sample area covered by macrophytes
Water chemistry variables	pH*	pH		Mean
	Temperature	LTEMP		Mean, in ^{o}C
	Conductivity *	LCOND		Mean, in mS m^{-1}
	Turbidity *	LTURB		Mean, in NTU
	Dissolved oxygen *	LDO		Mean, in mg l^{-1}
	Alkalinity *	LCACO3		Mean, in meq l^{-1}
	Total Phosphorus *	LTP		Mean, in mg P l^{-1}
	Kjeldahl nitrogen *	LKN		Mean, in mg N l^{-1}
	Nitrate+Nitrite *	LNO3+NO2-N		Mean, in mg N l^{-1}
	Ammonium *	LNH4-N		Mean, in mg N l^{-1}
	Silica *	LSI		Mean, in mg l^{-1}

Sites that did not group with other sites in the composite classification were considered to be outliers and were excluded from subsequent SASS biotope-group classifications. Since not all biotope-groups were sampled at each site, the number of sites within each classification varied as follows: composite: 59, SIC/SOOC: 55, AQV/MV: 56, GSM: 54. Taxa present at less than 5% of sites were considered to be rare and were excluded from the classifications. All data were presence/absence transformed (*PRIMER Version 5*) and the Bray-Curtis coefficient was used on these transformed data. Hierarchical agglomerative clustering, using group-average linking, was used on the data matrix.

Ordination of samples by MDS was undertaken, and stress values used to assess the reliability of the MDS ordination.

Classification strength

The classification strength of each classification was assessed by comparing the mean of all between-class similarities (*Bbar*) with the overall weighted mean of within-class similarities (*Wbar*) using *MEANSIM6* (Van Sickle 1997, Van Sickle & Hughes 2000).

Environmental variables

Variables were tested for normality and, where necessary, data were log-transformed to approximate normality prior to analyses. Correlation analyses was undertaken to provide insight into the degree of association amongst the variables. The environmental variables distinguishing each Group were identified using stepwise Discriminant Function Analyses (DFA, *Statistica Version 5.5 for Windows*). A " Group" is the term used to describe a group of sites that have similar macroinvertebrate assemblages.

Variables commonly affected by anthropogenic activity were omitted from the initial DFA (see Table 6.2 for details), but were subsequently included to assess differences in the relative importance of environmental variables. Some of these variables, such as pH and dissolved oxygen, whilst often affected by anthropogenic activities, may also be important variables influencing macroinvertebrate distributions under natural or undisturbed conditions. Prior to DFA, variables were analysed using a non-parametric analysis of variance (Kruskal-Wallis: KW) using Group membership as the factor variable. In general, environmental variables that showed significant differences ($p < 0.05$) among groups were chosen for further analyses. DFA was run on Groups from the composite classification and on groups from each separate biotope-group classification. A stepwise approach is recommended for finding the minimum subset of environmental variables that provides adequate prediction of group membership (Parsons & Norris 1996). Several combinations of environmental variables were tested in the stepwise DFA and the combination which produced the lowest error in predicting Group membership of a site in the DFA was selected as the subset of environmental variables which best discriminated between groups. The number of predictor variables was limited to one per 10 sites. This follows that used by Smith *et al*. (1999) although they limited this number to one per 20

sites, which is unrealistic in the present study, since fewer reference sites were included in the final classifications (59).

6.4 RESULTS

6.4.1 Macroinvertebrate assemblage analysis

Cluster and ordination analysis of composite macroinvertebrate data resulted in separation into five groups (Figure 6.1, MDS 3-D Stress = 0.16). Sites identified as outliers, and thus excluded from the final classification and from subsequent biotope-group classifications, included sites EM14, EM16, EM17, EM31 and HM48. Groups 1, 2 and 3 comprised mostly upland sites within the mountain stream and foothill-cobble bed subregions. Group 4 consisted of sites from several subregions. Group 5 comprised mostly lowland sites in the foothill-gravel bed subregion. Two sub-groups, 3A and 5A, were apparent within Groups 3 and 5 respectively.

Cluster and ordination of the SIC/SOOC macroinvertebrate data resulted in separation into three Groups (Figure 6.2, MDS 3-D Stress = 0.16). Group numbers used in the composite classification (i.e. Figure 6.1) have been used where the majority of sites show within-faunal-group consistency. Groups 2 and 3 consisted of upland sites and were approximately 67% dissimilar. Group 2 comprised mostly sites within the Central Highlands ecoregions, whilst Group 3 comprised mostly sites within the Great Escarpment Mountain ecoregion. Group 5 consisted of lowland sites of the Lowveld ecoregion and sites in this Group were 59% dissimilar from sites in Groups 2 and 3. Four sites did not cluster within the identified Groups and all sites were at least 58% similar to one another.

Cluster and ordination of the AQV/MV macroinvertebrate data resulted in separation into three Groups (Figure 6.3, MDS 3-D Stress = 0.21). The ordination was not particularly strong as indicated by the high stress value. Groups 2 and 3 included mostly upland sites and Groups were approximately 48% dissimilar. Group 5 comprised many of the lowland sites, together with four upland sites. This Group was 45% dissimilar from Groups 2 and 3. Four sites did not cluster within the identified Groups and the remaining sites were at least 46% similar to one another.

Cluster and ordination of the GSM macroinvertebrate data, resulted in separation into three Groups (Figure 6.4, MDS 3-D Stress = 0.19). Group 2 consisted of a mix of sites, Group 3 of Great Escarpment Mountain sites and Group 5 of the lowland sites together with several upland sites. Groups 2 and 3 were 50% dissimilar and Group 5 was 42% dissimilar from

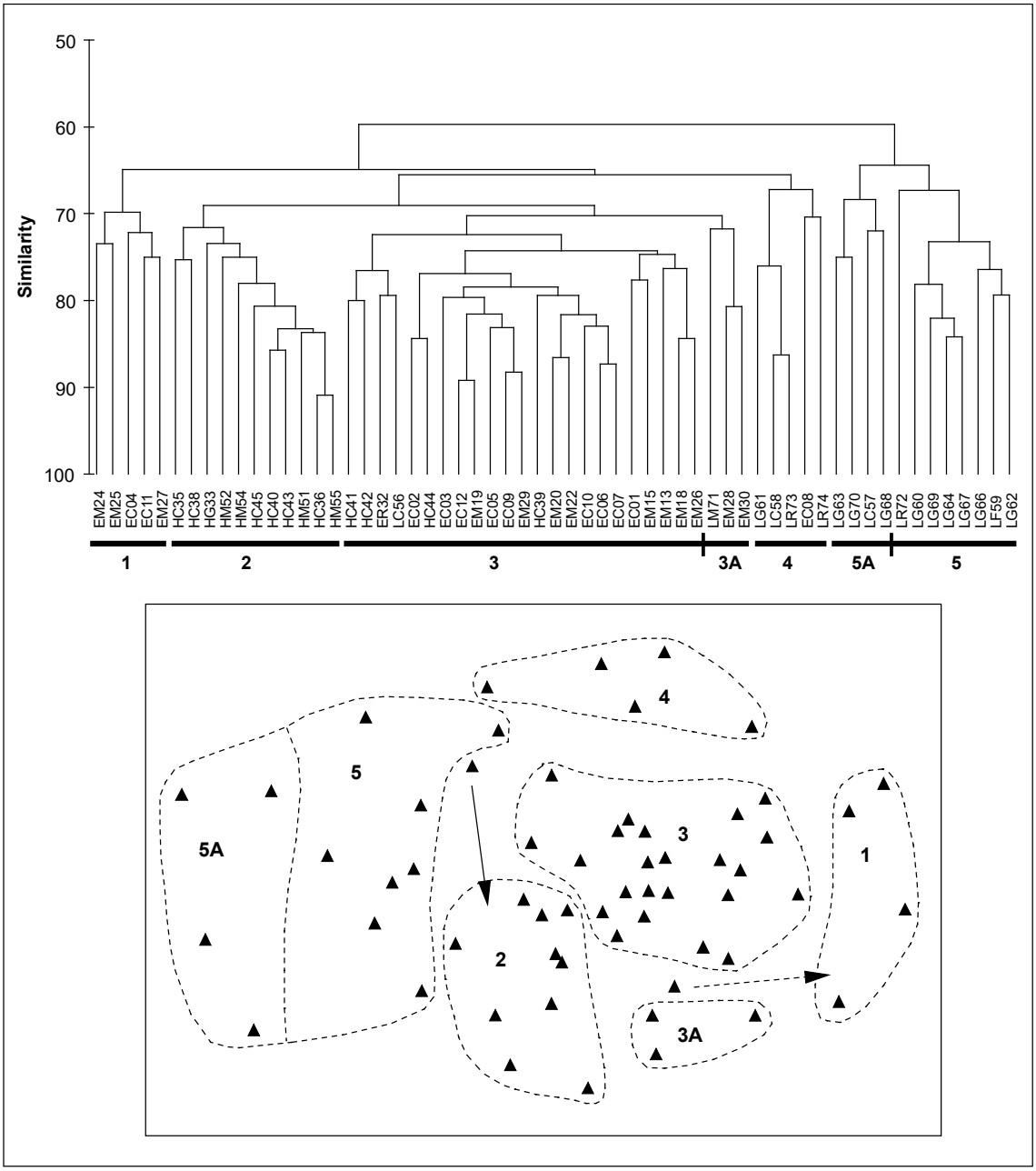


Figure 6.1 Dendrogram and MDS ordination showing the classification of sites in Mpumalanga based on macroinvertebrate taxa recorded in three seasons (autumn, winter and spring) from all three biotope-groups. Codes: primary: E = Great Escarpment Mountain, H = Central Highland, L = Lowveld ecoregion; secondary: M = mountain stream, C = foothill-cobble bed, G = foothill-gravel bed, R = rejuvenated cascade and F = rejuvenated foothill.

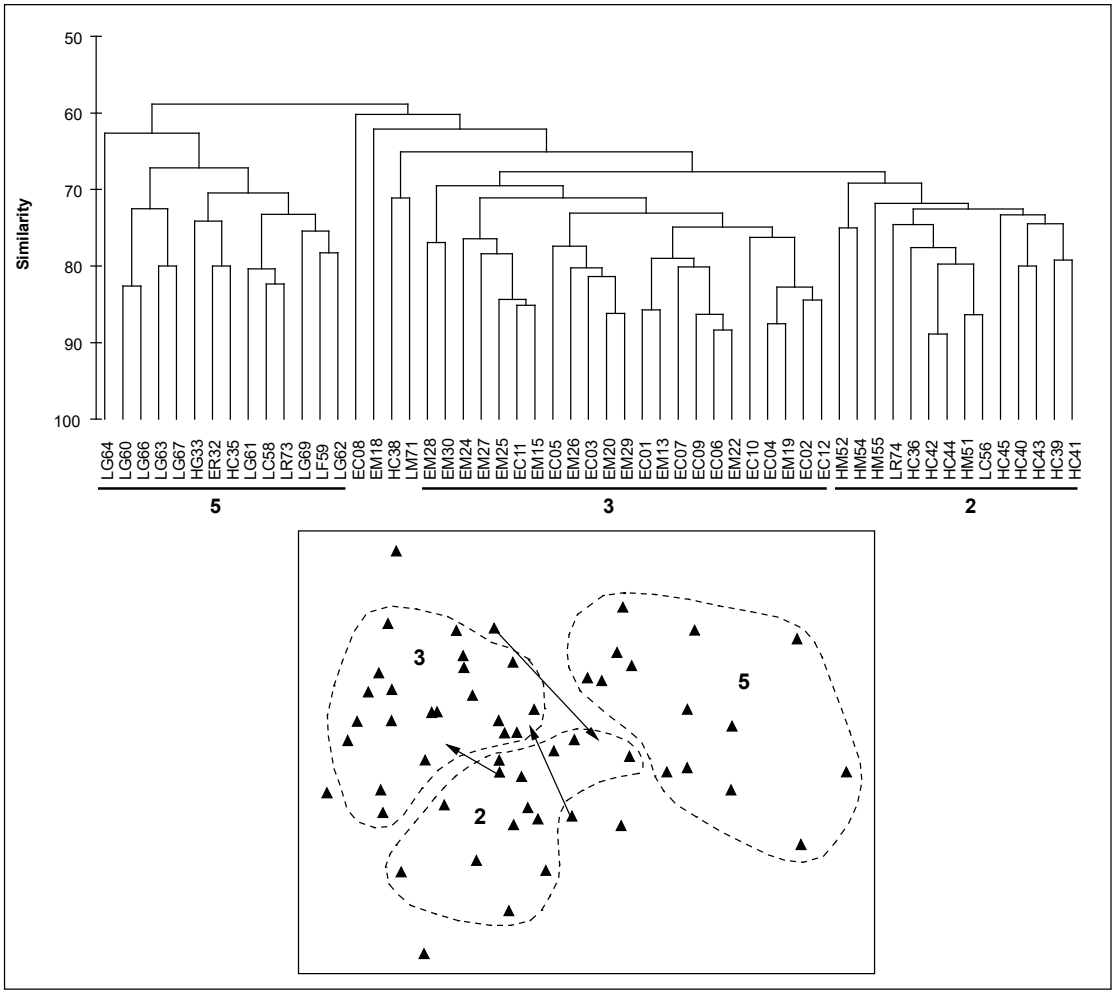


Figure 6.2 Dendrogram and MDS ordination showing the classification of sites in Mpumalanga based on macroinvertebrate taxa recorded in three seasons (autumn, winter and spring) in the SIC/SOOC biotope-group. Codes: primary: E = Great Escarpment Mountain, H = Central Highland, L = Lowveld ecoregion; secondary: M = mountain stream, C = foothill-cobble bed, G = foothill-gravel bed, R = rejuvenated cascade and F = rejuvenated foothill.

Groups 2 and 3. Three sites did not cluster within the identified Groups and the remaining sites were at least 42% similar to one another.

The number of sites that grouped with the same sites and in the same "Group" across the classifications varied depending on the Group. Using the composite Groups as the basis for subsequent Group numbering, sites in Group 1 formed part of Group 3 in the separate-biotope classifications. This suggests that macroinvertebrate assemblages associated with each separate biotope were similar to assemblages characterising Group 3 sites. Twelve of the 26 Group 3 sites also classified as Group 3 in all separate biotope-group classifications.

In other words, the macroinvertebrate assemblages of each separate-biotope classification contributed to the within-group similarity of the composite classification of these sites. Group membership of the remaining 14 sites varied and different sites grouped with Groups 2, 3 and 5, suggesting that macroinvertebrate assemblages at these sites were more variable and group membership depended on the biotope sampled. Group 2 of the composite classification was the least consistent and, with the exception of strong agreement between the composite and SIC/SOOC classifications, sites were classed as Groups 3, 4 and 5. Group 5 was the most consistent, with eight of the 11 sites classed as Group 5 in all the classifications, including both the composite and separate-biotope classifications.

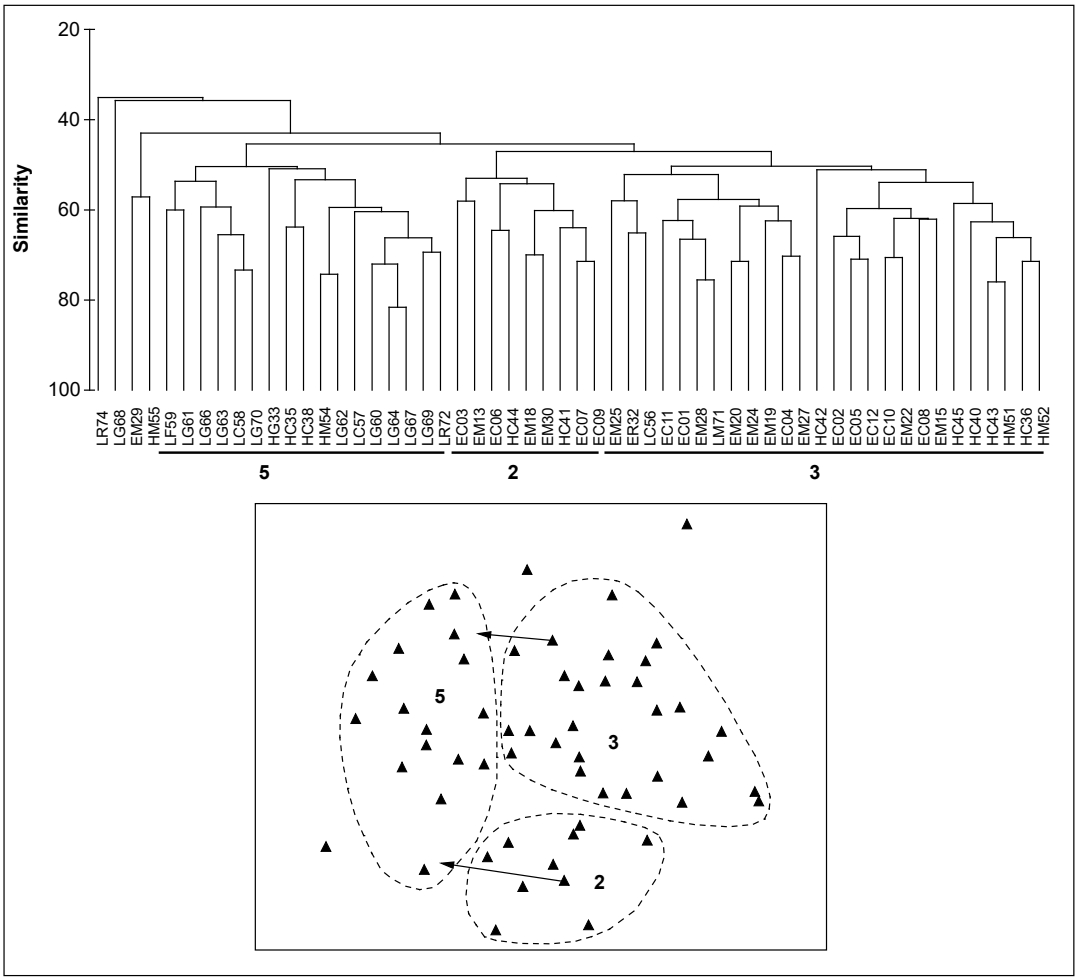


Figure 6.3 Dendrogram and MDS ordination showing the classification of sites in Mpumalanga based on macroinvertebrate taxa recorded in three seasons (autumn, winter and spring) in the AQV/MV biotope-group. Codes: primary: E = Great Escarpment Mountain, H = Central Highland, L = Lowveld ecoregion; secondary: M = mountain stream, C = foothill-cobble bed, G = foothill-gravel bed, R = rejuvenated cascade and F = rejuvenated foothill.

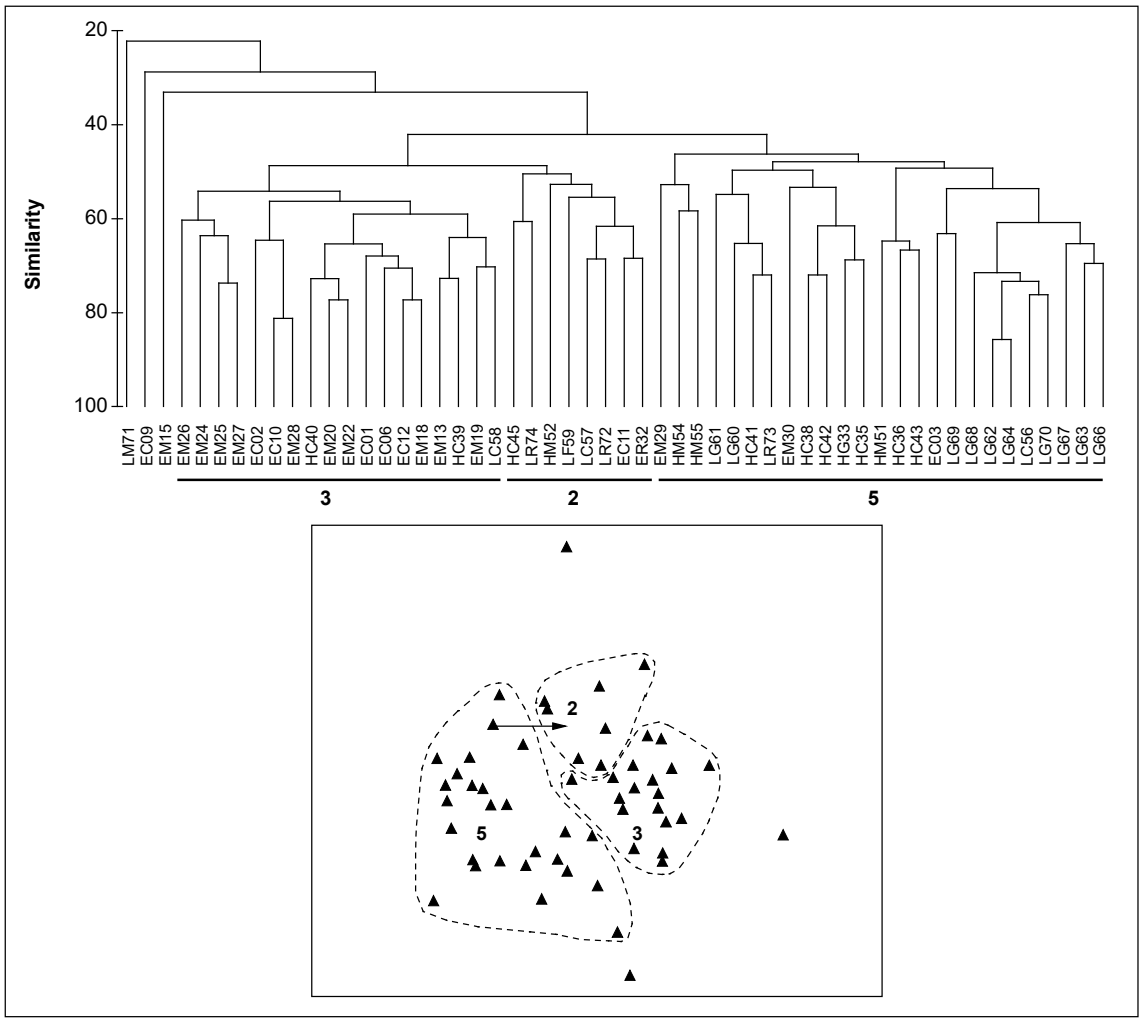


Figure 6.4 Dendrogram and MDS ordination showing the classification of sites in Mpumalanga based on macroinvertebrate taxa recorded in three seasons (autumn, winter and spring) in the GSM biotope-group. Codes: primary: E = Great Escarpment Mountain, H = Central Highland, L = Lowveld ecoregion; secondary: M = mountain stream, C = foothill-cobble bed, G = foothill-gravel bed, R = rejuvenated cascade and F = rejuvenated foothill.

Group 4 sites were either classed as Group 2, 3 or 5 in the biotope specific classifications. Results suggest that there is considerable variation between classifications with respect to group membership. In general, Group 1 could be considered part of Group 3, and only groups 3 and 5 showed a degree of Group consistency. Of the biotope-group classifications, the SIC/SOOC was the most similar to the composite classification, and the GSM classification was the least similar.

6.4.2 Relative classification strength

The results of the classification strength analysis suggest that macroinvertebrate assemblages within Groups are more similar than macroinvertebrate assemblages between Groups. In all classifications the hypothesis that there is no class structure was rejected (10 000 permutations, $p < 0.0001$) and macroinvertebrate assemblages were therefore considered more homogeneous within than between Groups (Figure 6.5). Of the five classifications tested, the composite classification wherein sub-groups were considered separately had the highest CS (10%) followed by the composite classification without sub-groups and the AQV/MV classification. Between-class similarity (*Bbar*) was lowest in the GSM classification, followed by AQV/MV and SIC/SOOC classifications, showing that between-class similarity in the GSM and AQV/MV classification classes was lower than in the SIC/SOOC classification classes.

Comparing these classifications with other studies (Van Sickle & Hughes 2000) on the basis of the M-ratio, however, suggests that all classifications are fairly weak. Classification strength increases progressively as M-ratio decreases from 1.0 to 0. The M-ratios in these analyses were ≥ 0.85. In some instances, the overall weighted mean of within-class similarities (*Wbar*) was less than the between-class similarities (*Bbar*) suggesting that macroinvertebrates assemblages from sites within the particular class are exceedingly variable. The Group 2 of the GSM classification is such an example.

6.4.3 Environmental variables

A subset of environmental variables that produced the lowest error in predicting group membership of a site in the DFA was identified for each classification (Table 6.3). The environmental variables within the subset have been ranked such that the variable with the greatest predictive potential (PP) is ranked 1, the one with the second highest PP is ranked 2, the third 3, etc., up until the maximum number of variables identified as predictors for each classification.

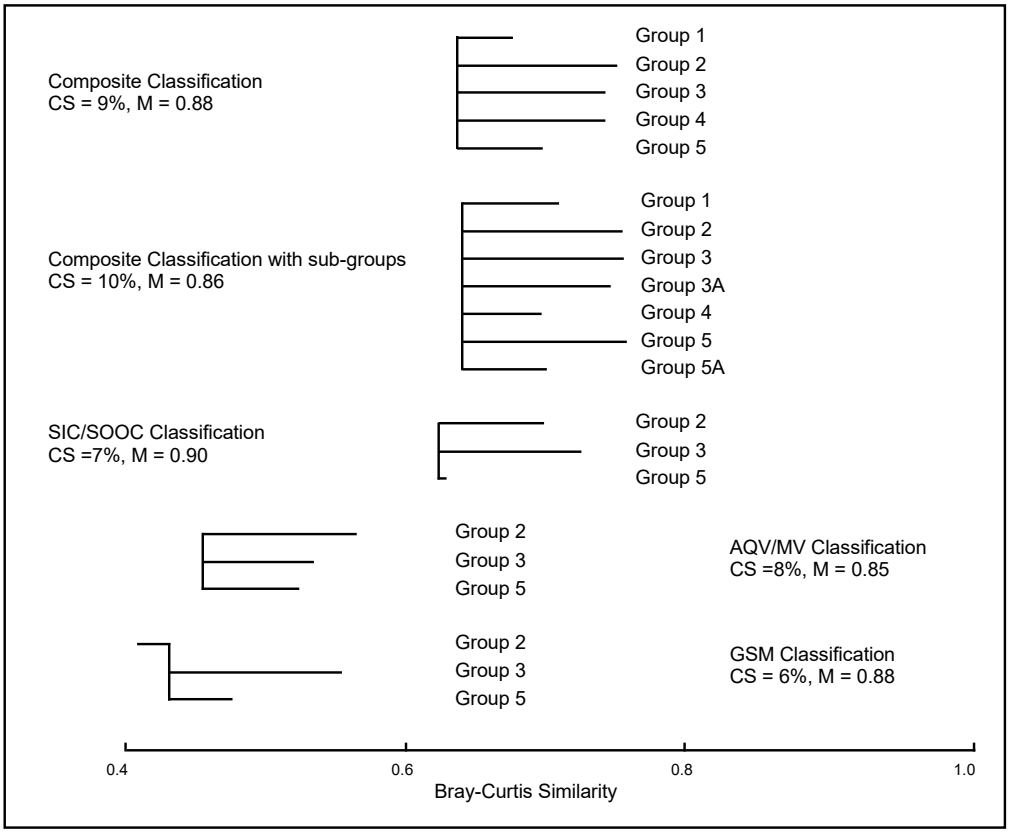


Figure 6.5 Mean similarity dendrograms of five alternate classifications for macroinvertebrate assemblages of Mpumalanga. The vertical lines represent the mean between-class similarity (*bbar*) and the horizontal lines terminate at the mean within-class similarity (w_1). M = *bbar/wbar*, where *wbar* is the overall weighted mean of all within-class similarities. CS (classification strength) = *Wbar-Bbar.*

Environmental variables identified as contributing to the differentiation of sites into the respective groups within each classification included ones at each scale, namely at the scale of catchment, site and habitat, in addition to several water chemistry variables. When the number of predictor variables was limited to one per ten sites altitude and longitude were identified as important predictors in all classifications except for the GSM classification, in which latitude was important. Altitude and longitude were highly correlated (Pearson product-moment correlation, $P < 0.05$). Latitude was only weakly correlated with canopy cover. Shallow-water habitat type was an important predictor variable in the composite classifications, as was shallow-water habitat depth in the GSM classification. Deep-water habitat depth was an important predictor variable in the AQV/MV classification. Geological type was an important predictor variable in the composite with sub-group, SIC/SOOC and AQV/MV classifications. Canopy cover was

identified as an important predictor in the SIC/SOOC classification. The percentages of boulder and mud were important predictor variables in the composite classification, whilst percentage mud, percentage G/S/M, percentage cobble/pebble and percentage sand were important predictor variables in the SIC/SOOC, AQV/MV and GSM classifications respectively. Temperature was an important water chemistry variable in the composite classifications and was correlated with both altitude and longitude.

When habitat and water chemistry variables potentially affected by anthropogenic activity were included in the DFA analysis, pH, silicate and turbidity became important predictor variables (Table 6.3). The classification of sites into Groups based on macroinvertebrate assemblage data was validated by examining the percentage of sites within each Group that were correctly classified on the basis of the above environmental variables. On this basis, the composite and SIC/SOOC classifications had the lowest error rates of 12% each, followed by GSM (18%), AQV/MV (19%) and composite with sub-groups (24%).

6.5 DISCUSSION

Understanding the factors contributing to the spatial distribution of 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, instream habitat and water chemistry, and many variables within each of these components interact with one another and with biotic components of aquatic systems to create spatially complex biotic assemblages.

Several environmental variables have been shown to contribute to observed spatial patterns in macroinvertebrate assemblages, including ones at each of the scales assessed. Of the catchment variables, location, in particular longitude, was shown to be an important discriminator of macroinvertebrate assemblages, and in Mpumalanga, longitude is highly correlated with altitude. Geographic co-ordinates are considered important (Wright 1995, Turak *et al*. 1999, Smith *et al.* 1999), particularly if the region under consideration is large. For example latitude, which ranged from 14ºS to 35ºS in Western Australia (area of 2 525 000 km^2), accounted for most variation between groups (Smith *et al*. 1999). Altitude was an important variable in all but one classification and reflects broad biogeographic patterns and longitudinal zonation, with most faunal differences occurring between upland and

Table 6.3 Subset of environmental variables that provided maximum discrimination between Groups within each separate classification. Error refers to the percentage of sites that were misclassified into Groups in the Discriminant Function Analysis (DFA) using the cross-validation option. Columns in bold are the result of DFA in which those variables commonly associated with anthropogenic activity were excluded, whilst those in italics included these variables (details in Table 6.2). The number of sites (n) in each classification class is given.

Variable Types	Variables	Composite		Composite with sub-groups		SIC/SOOC		AQV/MV		GSM	
	n (excluding outliers)	59		59		51		52		51	
	Error (%)	**12**	*16*	**24**	*25*	**12**	*12*	**19**	*15*	**18**	*25*
Catchment variables	Longitude	**3**	*3*	**3**	*3*	**2**	*2*	**2**	*2*		
	Latitude									**2**	*3*
	Altitude	**1**	*1*	**1**	*1*	**1**	*1*	**1**	*1*		
	Stream order			**5**	*5*						
Site variables	Shallow-water habitat									**4**	
	Shallow-water habitat-type	**2**	*2*	**2**	*2*					**5**	*4*
	Deep-water habitat							**4**	*5*		
	Geological type		*5*	**6**		**5**	*4*	**5**			
	Canopy cover					**4**	*5*				
Habitat variables	% Boulder	**6**									
	% Cobble/pebble		*6*							**1**	*1*
	% Sand									**3**	
	% Mud	**5**				**3**					
	% G/S/M							**3**	*3*		
Water chemistry variables	pH						*3*				
	Temperature	**4**		**4**	*6*						*2*
	Turbidity								*4*		*5*
	Silicate		*4*		*4*						

lowland sites. Harrison (1965b) described two biogeographic sub-groups in this region: a highveld, temperate species assemblage in the uplands and a tropical or warm stenothermal species assemblage of the lowveld. Longitudinal zonation of biota often occurs in response to changing physical and chemical characteristics of rivers as one moves downstream (Vannote *et al*. 1980). Marchant *et al.* (1999) found longitudinal gradients to be very important in affecting biotic associations. These longitudinal gradients are known

to correlate with several other physical and chemical variables such as geomorphology, discharge and temperature. Marchant *et al.* (1999) concluded that longitudinal gradients appear to be independent of scale, i.e. gradients were apparent when single rivers as well as broad geographic regions were examined. As Statzner & Higler (1986) have suggested, physical characteristics of flow are important environmental factors governing the longitudinal zonation of lotic assemblages. Whilst flow characteristics and specific measures of stream hydraulics were not considered in the present study, factors associated with flow characteristics, such as substratum type, were examined and shown to be contribute to differences in macroinvertebrate assemblages. Catchment-scale variables therefore appear to influence macroinvertebrate distribution substantially.

The nature and characteristics of the substratum were identified as important predictors both at the site and habitat-level. The type of shallow-water habitat, i.e. cobble riffle or bedrock rapid, and the depths of the shallow- and deep-water habitats contributed to the observed macroinvertebrate distributions. Habitat, which refers to the environment in which an aquatic organism lives, and may incorporate aspects such as substrate-type, and hydraulic and chemical conditions, strongly affects the distribution of macroinvertebrates since many organisms have specific substrate or hydraulic requirements. Riffle areas with a cobble substratum are known to support assemblages distinct from those of rapids with a bedrock substratum (e.g. Wohl *et al.* 1995). Whilst water in both habitats is fast-flowing and surface water passing over the substratum is broken, the complexity of the substrate varies considerably, with cobbles structurally more complex than bedrock. Structural complexity has been shown to influence the abundance of stream invertebrates strongly (e.g. Palmer *et al*. 1997). The interstitial spaces present within a cobble bed provide instream habitat for aquatic organisms. Hydraulic patterns are also more complex in cobble bed systems compared to bedrock ones, with a variety of flow types persisting within the system. Bedrock is a physically less complex substrate and organisms inhabiting it are generally adapted to maintaining their positions in a stream subjected to fast flow. Examples are the blackflies, which have a brush-type collecting apparatus for filtering water flowing over them, together with posterior hooks and silk threads that serve to attach the organisms to the surface of the rocks.

The relative percentages of boulder, cobble/pebble, sand and mud were all important environmental variables influencing macroinvertebrate distribution. This again reflects the

physical complexity of substrate type and, in the case of sand, the relatively unstable nature of the substrate (Richards *et al*. 1997) that limits habitation by aquatic organisms. Fine materials, such as mud or silt, whilst providing habitat for organisms like oligochaetes that are suited to such habitats, limit habitation by other organisms by the infilling of interstitial spaces within the cobble bed. The percentage of mud was identified as an important environmental variable in the SIC/SOOC classification, supporting the observation of Chutter (1970) that the presence or absence of fine material, which is generally determined by stream hydraulics, is an important factor determining species distributions. The percentage of cobble/pebble and sand were important variables in the GSM classification suggesting that the specific substrate-type, i.e. gravel, sand or mud, and the extent to which it resembles the cobble/pebble substrate-type, affects the macroinvertebrate assemblages. In combining the separate substrate types of gravel, sand and mud, information may be lost in that if the GSM biotope-group comprises mainly coarse gravel rather than sand or mud, the biotope, in terms of its macroinvertebrate assemblages, may begin to resemble SIC/SOOC. The percentage of the G/S/M biotope-group was an important predictor variable in the AQV/MV classification and perhaps reflected the availability of a means of attachment for macrophytes growing on the stream margins.

A site-level variable shown to be an important predictor variable in the SIC/SOOC classification is that of canopy cover. The extent of riparian vegetation, in particular the extent to which it provides a canopy for the stream, affects aspects such as water temperature (Graynorth 1979) and the extent and type of allochthonous material entering the stream. The SIC/SOOC biotope is relatively shallow and is therefore susceptible to temperature changes and to elevated summer temperatures in open-canopy systems. Closed-canopy streams, more common in upper catchment areas, often have lower stream temperatures and narrower temperature ranges than open-canopy streams, since solar radiation is reduced in closed-canopy streams and they have a greater shade ratio (Collier 1995). The amount of detritus entering a closed-canopy system may be expected to be greater than that of an open-canopy one. Invertebrates, termed shredders, that utilise this coarse detritus, may in turn be more abundant in closed- than open-canopy systems. In this way, canopy cover may be reflected as differences in macroinvertebrate assemblages, particularly when assemblages associated with specific biotope-groups such as stones-in-current are examined.

Geological type was identified as an important environmental variable in three of the classifications, and may also be considered to be a catchment-scale variable. The geological or lithostratigraphic characteristics of the catchment and the site affects intrinsic water chemistry, in particular the concentration of total dissolved solids, anions and cations and pH. The nature of the rocks over which the water flows imparts to the water its chemical composition. For example, igneous rocks contain calcium and magnesium and water flowing over or through them picks up measurable quantities of these elements, and of nutrients such as phosphates, nitrates and silicates (Day & King 1995). Waters affected by igneous rocks are dominated by calcium and/or magnesium cations and bicarbonate ions. Geological type, therefore, through its effect on ambient water chemistry, is an important variable linked to macroinvertebrates.

Of the water physico-chemical variables, temperature was identified as contributing to the observed groupings of macroinvertebrate assemblages in the composite classifications, and was correlated with both altitude and longitude. The thermal characteristics of running waters are dependent on various hydrological, climatic and structural features of the region, catchment area and river (Dallas & Day 1993). Hawkins *et al.* (1997) determined that stream temperatures were most strongly related to differences in channel morphology and hydrology among montane streams in California, United States. They found this to be particularly important under low-flow conditions during summer, since summertime temperatures may limit the presence or overall abundance of some species in a stream and this effect may carry over to long-term and large-scale biogeographic patterns. All organisms have a range of temperatures at which optimal growth, reproduction and general fitness occur and many life cycle characteristics of aquatic organisms are cued into temperature. Temperature changes affect metabolic processes and life cycle patterns by altering reproductive periods, rates of development and emergence times of aquatic organisms. Oxygen solubility and the toxicity of certain chemicals are also related to water temperature. Temperature, therefore, has the potential to affect the distribution of aquatic organisms, and in this study, the significant correlations between temperature and altitude, and temperature and stream order, suggest that differences in temperature are a reflection of longitudinal zonation.

Incorporating variables commonly affected by anthropogenic activity resulted in pH, turbidity and silicate being included in the list of predictor variables. The pH of natural

water is determined by geological and atmospheric influences, most fresh waters being relatively well buffered and more or less neutral, with pH ranges from 6 to 8 (Dallas & Day 1993). Some streams are naturally far more acidic than others and their biotas are adapted to these conditions. pH is determined largely by the concentration of hydrogen ions (H^+), and alkalinity by the concentrations of hydroxyl (OH^-), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions in water. The rate of change of pH is determined by the buffering capacity (usually by the carbonate-bicarbonate system) of the water, and is more rapid in poorly buffered waters. Changing the pH of water changes the concentration of both H^+ and OH^- ions, which affects the ionic and osmotic balance of aquatic organisms. pH also determines the chemical species (and thus potential toxicity) of numerous substances in water. The range of pH noted in this study was between 7.2 and 8.5, and median values were significantly different between Groups in the SIC/SOOC classification (Kruskal Wallis test statistic: $H = 22.18$, $p < 0.001$), and in particular differences were apparent between Groups 2 and 3 and 3 and 5. Differences in pH may be contributing to observed differences in macroinvertebrate assemblages, although other studies have recorded widely fluctuating inter-annual pH without a concomitant change in macroinvertebrate assemblages (e.g. Smith *et al.* 1999) and have recommended the exclusion of variables such as pH, alkalinity and nutrient concentrations from analyses aimed at identifying predictor variables.

Turbidity, identified as an important predictor variable in the AQV/MV and GSM classifications when all variables were included in the DFA, describes water-colour and clarity and affects light penetration in river systems. Natural turbidity in rivers is governed by basic hydrology and geomorphology of the particular region and it is naturally seasonal with elevated levels often associated with high-flow periods following land erosion by wind and rain (Dallas & Day 1993). Continuous high-level inputs of suspended material may have serious consequences for the riverine biota, since light penetration is reduced, primary production decreases and food availability to organisms higher in the food chain is diminished. Suspended material that settles out may smother and abrade riverine plants and animals and community composition may change depending on which organisms are best able to cope with this alteration in habitat. Whilst turbidity was identified as a variable in the AQV/MV and GSM classifications, significant differences between Groups were only observed in the AQV/MV Groups ($H = 7.45$, $p < 0.05$) and total range in turbidity across all sites was only < 1 to 4.7 NTU. This range is comparatively low and is unlikely to have had a

significant effect of the aquatic organisms, although experimental evidence to test this potential effect would be useful.

High levels of silicates are often associated with a preponderance of sandy substrate and the incorporation of silicate as a predictor variable may reflect the relative proportion of sandy substrate at a site. Silicates may also influence macroinvertebrate assemblages indirectly through their utilisation by diatoms, themselves a food source to certain aquatic invertebrates.

In conclusion, several environmental variables contributed to the observed distribution of macroinvertebrate assemblages in reference sites in Mpumalanga. These environmental variables varied to some extent when macroinvertebrate assemblages from different biotope-groups were examined. Whilst based on correlative data only, environmental variables were identified are all scales, from catchment, to site, to habitat and included water chemistry variables. This supports the observation of Turak *et al.* (1999) that at least one representative from each of five categories of environmental attributes, namely latitude, location (latitude and longitude), river size (e.g. distance from source, stream width), substratum (e.g. cover of bedrock, boulder, cobble) and water chemistry (alkalinity) appear to be needed to make good family-level prediction of macroinvertebrate fauna.

Of the different classifications, the composite one based on macroinvertebrate assemblages from all three biotope-groups seemed to be the most robust, in terms of both classification strength and the percentage of misclassification of sites. In the present study 53 of the 59 sites used in the composite analysis had all three biotope-groups available for sampling, and thus biotope-group differences, may have been less important than in instances where one or more biotope is absent. Where biotope availability is clearly different between sites, it may be necessary to undertake separate analyses for each biotope-group. Of the separate biotope-group analyses, the classification of reference sites based on macroinvertebrate assemblages of the SIC/SOOC biotope-group, whilst having a low classification strength, also had a low percentage error with respect to misclassification of sites. The likelihood of misclassification of sites in this classification is, therefore, low. Overall similarity of sites within this biotope-group was substantially higher than for the other two biotope-groups. Similar studies elsewhere have found that classifications based

on riffle biotopes with the SIC/SOOC biotope-group produced the most robust and consistent results (Parson & Norris 1996, Turak *et al.* 1999) since, in terms of the macroinvertebrate assemblage, this biotope is less variable than either the AQV/MV and GSM biotope-groups.

Environmental variables identified as providing the greatest discrimination between groups in the composite classification were altitude, shallow-water habitat type, longitude, temperature, the percentage mud and percentage boulder. Those in the SIC/SOOC classification were altitude, longitude, percentage mud, canopy cover and geological type. The structure of macroinvertebrate assemblages is therefore a function of both large-scale variables measured at the level of catchment, and smaller-scale variables measured at the level of site or habitat. From the perspective of classifying reference sites, this knowledge is useful in that it confirms the utility of a spatial framework within which reference sites are selected and bioassessment is undertaken. The number of variables, at the scale of site and habitat, that were identified as important environmental predictors contributing to the discrimination of macroinvertebrate assemblages in both the composite classification and biotope-specific classifications, highlights the importance of considering additional factors such as substratum that influence macroinvertebrate assemblages and contribute to the observed heterogeneity of lotic systems.

CHAPTER 7. VARIABILITY OF MACROINVERTEBRATE ASSEMBLAGES AT UPLAND SITES OF THE WESTERN CAPE

7.1 INTRODUCTION

River ecosystems are known to be complex systems affected by a multitude of biotic and abiotic factors acting and interacting at different scales. In the preceding chapters it has been shown that upper parts of catchments are distinct from lowland ones with respect to their macroinvertebrate assemblages and SASS scores. It has also been shown that the availability of biotopes for sampling and the biotope preferences of certain taxa influence the macroinvertebrate assemblages recorded at a site, in addition to affecting SASS scores. Seasonal differences in the relative occurrence of particular taxa, i.e. temporal variability, have been shown to be a consideration in the Western Cape, with certain taxa more commonly recorded in one or other season. Using data from Mpumalanga it has been shown that environmental factors at all scales, ranging from those at the scale of catchment to those at the scale of habitat, play a role in determining the resultant macroinvertebrate assemblages.

Upper catchments are considered to be highly variable systems and, in particular, mountain stream channels are chaotically and complexly structured (Grant *et al.* 1990 cited by Hawkins *et al.* 1997). Hawkins *et al.* (1997) suggest that in mountainous landscapes local conditions may be strong enough to mask patterns, e.g. regional patterns, which would have otherwise emerged in more homogenous landscapes. Such local conditions may include differences in temperature, flow and the availability of habitat or biotopes.

Mountains in the Western Cape, a region known for its high degree of endemism in aquatic biota (Harrison & Agnew 1962, Wishart & Day in press), generally comprise hard, resistant, quartzitic sandstones of the Table Mountain Group and waters flowing over such strata are characteristically acidic and low in nutrients and dissolved solids. The acid stream fauna of the upper catchments largely belongs to the palaeoendemics referred to as the South Temperate Gondwanian fauna (Harrison 1978) and is essentially restricted to perennial systems in high rainfall areas. Recent studies on the genetics and morphological systematics of several aquatic taxa endemic to the Western Cape (Stevens & Picker 1999, Stewart &

Griffiths in press) have led to taxonomic revisions and the identification of suites of new species. Often the distributions of many of these taxa are spatially distinct. This has led King & Schael (2001) to coin the phrase "catchment signatures" when referring to macroinvertebrate assemblages, since each catchment appears to have a characteristic macroinvertebrate species assemblage distinct from those of other catchments in the Western Cape.

Of the subregions examined in previous chapters, upland sites of the Western Cape proved to be the most variable, both spatially and temporally, with respect to macroinvertebrate assemblages. Given such spatial and temporal variability, is it possible to define ecological reference conditions for these streams or is the variability such that it masks the detection of a disturbance when acting as a benchmark with which a monitoring site is compared? Upper-catchment areas in the Western Cape have been subjected to various impacts, including those resulting from afforestation, aquaculture (Brown 1997), agricultural activities and inter-basin water transfers (Snaddon & Davies 1998) as well as structural modifications due to physical alteration of the channel and bank. Upland areas also make a significant contribution to overall catchment biodiversity (Furse 2000) and, whilst species richness may be relatively low in upland areas, they are important in terms of rarity with some taxa confined to single headwaters (Palmer *et al.* 1994). Whilst upper-catchment areas are relatively less disturbed than lowland ones, it is nonetheless important to derive baselines from which to gauge the degree of impairment of these sites when exposed to damaging anthropogenic activities. By examining the influence of variability in a region where variability is greatest, insight should be gained into the effects of variability when defining reference conditions and interpreting bioassessment data.

This chapter therefore focuses on spatial and temporal variability of macroinvertebrate assemblages at upland sites within the Fynbos bioregion of the Western Cape. It aims to examine the taxa comprising the macroinvertebrate assemblages and to identify differences in taxa amongst groups of sites. The extent to which sites within the two upland subregions (mountain streams and foothill-cobble beds) are similar will be evaluated, in terms of both their macroinvertebrate assemblages and their SASS scores. Given the importance of biotope availability (Chapter 4) and the variation in the availability of aquatic and marginal vegetation at upland sites, analyses are undertaken separately for sites with and without vegetation biotopes. Environmental variables characterising each site are

examined as an aid to understanding any observed spatial variability. Lastly, the influence of spatial and temporal variability on defining ecological reference conditions for upland sites are examined by comparing several monitoring sites with the generated reference condition.

7.2 STUDY AREA

Twenty-one minimally-disturbed sites in mountain stream and six in foothill-cobble bed subregions situated on 24 rivers in the Fynbos bioregion were assessed in spring. Eight of these were assessed in November 1994 and the remainder in November 1995 (details of sampling dates are provided in Appendix A). Validation was conducted using an additional two mountain stream and two foothill-cobble bed reference sites, together with numerous assessments undertaken at different times at some of the previously assessed upland sites. Eighteen mountain stream sites and 22 foothill sites situated on 24 rivers, and exposed to different levels of disturbance, were used as monitoring sites for comparing with reference sites.

Table 7.1 Mountain stream and foothill-cobble bed sites assessed in the Western Cape indicating subregion, river and type. The codes for sites on each river are given in parenthesis and relate to Table 2.1 and Figures 2.1, 2.2 and 2.3 in Chapter 2. Reference (CM, CC or SC) and test/monitoring sites (TM or TC) are listed separately.

Subregion	River and site code
Mountain Stream	Assegaaibosch (CM01), Berg (CM02, CM03), Eerste (CM04) Lang (CM05), Palmiet (CM07), Elandspad (CM09), Wit (CM11), Rooiels (CM12), Houtbaais (CM14, CM15), Rietvlei (CM16), Boesmans (CM17), Baviaans (CM18), Boesmanskloof (CM19), Riviersonderend (CM20), Duiwelsbos (CM21), Hermitage (CM22), Meulkloof (CM23), Grootkloof (CM24), unspecified (CM13), Perdekloof (CM25), Swartboskloof (CM26), Dwars (TM01), Elandskloof (TM02), Hartbees (TM03), Koekoedou (TM04), Kraalstroom (TM05, TM06, TM07, TM08, TM09), Modder (TM10), Raaswater (TM11), Riviersonderend (TM12), Silvermine (TM13, TM14, TM15), Speksriviersкloof (TM16), Vals (TM17), Valsgat (TM18).
Foothill-cobble bed	Berg (CC01), Holsloot (CC02), Molenaars (CC03, CC04, CC05), Sandriftskloof (CC06), Dutoits (CC07), Duiwenshoek (SC01), Berg (TC01, TC02, TC03, TC04), Breede (TC05, TC06, TC07), Buffelsjag (TC08), Dwars (TC09), Eerste (TC10, TC11, TC12, TC13), Franshhoek (TC14), Hex (TC15, TC16), Hoeks (TC17), Keisers (TC18), Kruis (TC19), Lanzerac (TC20, TC21), Wemmers (TC22).

7.3 MATERIALS AND METHODS

7.3.1 Benthic macroinvertebrates: SASS4 sampling

Benthic macroinvertebrates were sampled using the qualitative rapid bioassessment method, SASS4 (South African Scoring System). A detailed description of the SASS method is given in Chapter 2.

7.3.2 Environmental variables

The environmental variables measured at each reference site were the same as those listed in Table 6.2 (Chapter 6). Variables were divided into four types, namely catchment variables such as longitude, latitude, altitude, distance from source and stream order; site variables such as channel pattern, hydrological type, stream width, habitat depths, geological and vegetation types and canopy cover; habitat variables such as substratum richness, composition and dominance, the percentage of each substratum type, percentage embeddedness, the SASS biotopes present (simplified into SIC/SOOC only or SIC/SOOC plus AQV/MV) and the percentage cover of algae and macrophytes; and water chemistry variables including pH, temperature, conductivity, dissolved oxygen, alkalinity and major anions and cations, expressed as cation and anion ratios. The presence of instream vegetation (*Isolepis* sp.), which provides an important instream habitat for aquatic organisms, was included as an additional site variable. Details pertaining to the analysis procedures of the chemical variables are given in Chapter 2. Each data point reflects an instantaneous measurement taken at the time of the sampling. Variables were tested for normality and, where necessary, data were log-transformed to approximate normality prior to analyses.

7.3.3 Data analysis

Analysis of macroinvertebrate assemblages

Cluster analysis and non-metric multidimensional scaling (MDS) were used to examine similarities amongst sites based on macroinvertebrate assemblage composition (Clark & Warwick 1994). Data were transformed using the presence/absence transformation (*PRIMER Version 5*) and the Bray-Curtis coefficient was used on these transformed data.

Hierarchical agglomerative clustering, using group-average linking, was used on the data matrix. Ordination of samples by MDS was undertaken, and stress values used to assess the reliability of the MDS ordination. Three separate analyses were done. The first included all uplands sites, the second only mountain stream sites and the third only foothill-cobble bed sites. The distinguishing taxa responsible for the similarity within groups of sites and the dissimilarity amongst groups of sites were established using SIMPER (*PRIMER Version 5*). Those taxa responsible for 90% within-group similarity or dissimilarity were identified. One-way Analysis of Similarities (ANOSIM, *PRIMER Version 5*) was used to test whether or not there were significant differences in macroinvertebrate assemblages among subregions.

Environmental variables

The environmental variables distinguishing each Group were identified using stepwise Discriminant Function Analyses (DFA, *Statistica Version 5.5 for Windows*). A "Group" is the term used to describe a group of sites that have similar macroinvertebrate assemblages. Prior to DFA, variables were analysed using a non-parametric analysis of variance (Kruskal-Wallis: KW) using Group membership as the factor variable. In general, environmental variables that showed significant differences ($p < 0.05$) among Groups were chosen for further analyses. DFA was run separately for the mountain stream and foothill-cobble bed subregions. A stepwise approach is recommended for finding the minimum subset of environmental variables that provides adequate prediction of Group membership (Parsons & Norris 1996). Several combinations of environmental variables were tested in the stepwise DFA and the combination which produced the lowest error in predicting Group membership of a site in the DFA was selected as the subset of environmental variables which best discriminated between Groups. Since the number of sites assessed was limited ($n = 21$), but the need to restrict the number of predictor variables was recognised (Smith *et al*. 1999), a maximum of four predictor variables were included, i.e. one per five sites.

SASS4 Scores, Number of Taxa and ASPT

Variability in SASS scores was examined by calculating median, minimum, maximum, 90% percentile and 5% percentile values for SASS4 Scores, number of taxa and ASPT values. This was done for all upland reference sites using the same spring data as used for the assemblage analysis. Values were also calculated separately for mountain stream and

foothill-cobble bed reference sites. SASS scores recorded at sites in each subregion were compared statistically using the non-parametric Kruskal-Wallis test.

7.4 RESULTS

7.4.1 Analysis of macroinvertebrate assemblages

Upland sites

The 27 upland sites formed three Groups with Groups 1 and 2 comprising sites within each of the two subregions (Figure 7.1). Sites were at least 50% dissimilar. Group 3 sites clustered with Group 2 sites on the ordination plot (3D stress = 0.16). Based on macroinvertebrate assemblages, sites in Group 1 were 61% similar and those in Group 2 were 56% similar. One site, CM21, clustered with Group 2 sites, but was grouped with Group 1 sites in the MDS ordination (indicated with an arrow in Figure 7.1). Most distinguishing taxa were common to both Groups, with those taxa where the number of types or species within a taxon are considered, being important, e.g. Baetidae, Hydropsychidae and Trichoptera (cased caddis) (Table 7.2). In addition, Gyrinidae and Philopotamidae were identified as characterising Group 2. The Global R value of the ANOSIM analysis indicated that macroinvertebrate assemblages from mountain streams and foothill-cobble bed sites were not significantly different (Global R = - 0.094).

Mountain stream subregion

When mountain stream sites were analysed separately from foothill-cobble bed sites, macroinvertebrate assemblages formed three Groups, with Group 1 sub-dividing into further sub-groups 1a and b, and Group 2 forming sub-group 2a (Figure 7.2). One site, CM24, was 58% dissimilar for all other sites. Average dissimilarity between Groups 1 and 3, and 2 and 3 was 51%, whilst average dissimilarity between Groups 1 and 2 was 47%. MDS ordination supported the observed ordination (3D stress = 0.16). Within-group similarity of macroinvertebrate assemblages at sites in Groups 1, 2 and 3 was 60%, 61% and 54% respectively. At the sub-group level, within-group similarity increased to between 63 and 68%. Distinguishing taxa characterising Group 1 exclusively (Table 7.2) included Heptageniidae, Helodidae, Hydraenidae and Trichoptera (cased caddis 2 Types). Of these, Heptageniidae, together with Blephariceridae were identified as distinguishing taxa for sub-group 1a. Those characterising Group 2 included Limnichidae and

Athericidae, whilst those exclusively characterising Group 3 included Caenidae, Gyrinidae, Ecnomidae, Philopotamidae, Aeschnidae, Chlorolestidae, Coenagrionidae and Hydrachnellae. Taxa notably not represented in Group 3 were Notonemouridae and Corydalidae, as well as Philopotamidae in Group 1.

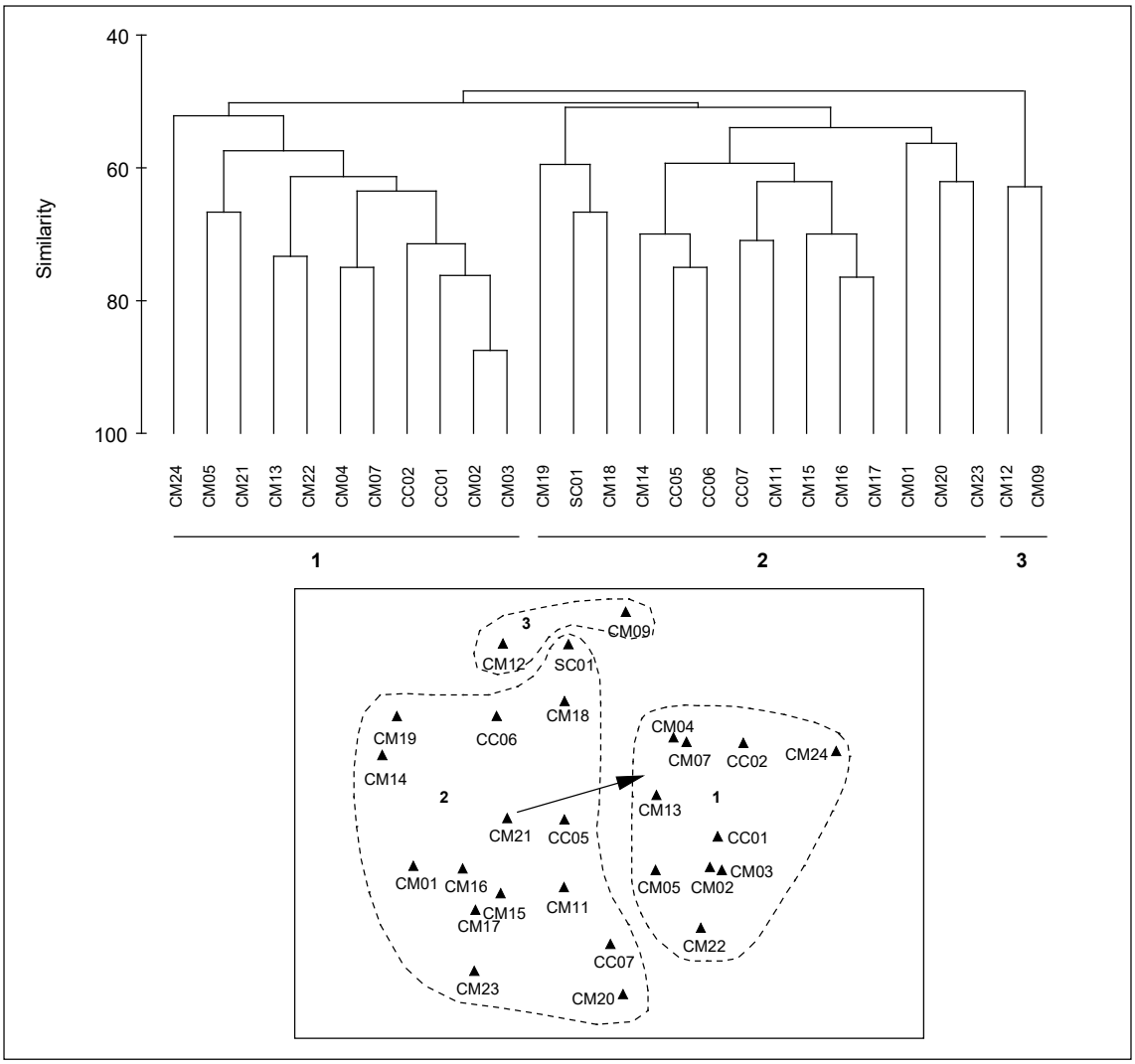


Figure 7.1 Dendrogram and MDS ordination showing the classification of upland sites in the Western Cape based on taxa recorded in spring at 21 mountain stream and six foothill-cobble bed sites. Codes: primary: C = Cape Fold Mountains, S = Southern Coastal; secondary: M = mountain stream and C = foothill-cobble bed.

Foothill-cobble bed

Of the six sites assessed, clustering occurred at approximately 52% similarity level, with sites forming two Groups based on their faunal assemblages (Figure 7.3). Stress values were very low (3-D Stress = 0.01). Distinguishing taxa characterising Group 1 exclusively

(Table 7.2) included Baetidae 3 types, Heptageniidae, Gyrinidae, Philopotamidae, Trichoptera (cased caddis 3 types) and Aeschnidae. Those characterising Group 2 included Notonemouridae, Helodidae, Trichoptera (cased caddis 2 types) and Tipulidae.

Table 7.2 Taxa contributing to within-group similarity of Groups identified from upland sites, mountain stream sites and foothill-cobble bed sites in the Western Cape using SIMPER analyses. Those taxa contributing to the first 50% of the similarity are indicated by ♦; the remaining taxa contributing to the next 40% (i.e. 90% in total) of the similarity are indicated by □.

	Upland sites Groups		Mountain stream Groups			Mountain stream sub-groups			Foothill-cobble bed Groups	
	1	**2**	**1**	**2**	**3**	**1a**	**1b**	**2a**	**1**	**2**
Similarity (%)	**61.0**	**56.0**	**60.4**	**61.1**	**54.0**	**66.1**	**63.5**	**67.7**	**66.9**	**62.3**
Number of distinguishing taxa	*14*	*16*	*15*	*14*	*18*	*15*	*10*	*12*	*13*	*11*
Notonemouridae	□	□	□	□		□	♦			□
Baetidae 1 Types				□				□		
Baetidae 2 Types		□			□	□				
Baetidae 3 Types	□		□		□		□		□	
Caenidae					□					
Teloganodidae	♦	♦	♦	□	♦	♦	♦	□	□	□
Heptageniidae	□	□	□			♦			□	
Leptophlebiidae	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
Elmidae/Dryopidae	□	♦	□	♦	□	□	□	□	♦	□
Gyrinidae		□			□				□	
Helodidae Larvae	♦	□	♦			□	□	□		♦
Hydraenidae	□	□	♦			□	□			
Limnichnidae				□						
Corydalidae	♦	□	♦	♦		♦	♦	□	♦	♦
Ecnomidae					□					
Hydropsychidae 1 Type		□		□	□			♦	□	
Hydropsychidae 2 Types	□		□		□	♦				□
Philopotamidae		□		□	□			♦	♦	
Case Caddis 2 Types	□		□							□
Case Caddis 3 Types		♦	□	♦	♦	□		♦	♦	
Athericidae				□						
Blephariceridae						□				
Chironomidae	♦	□	□	□	♦	□	□	□	□	□
Simuliidae	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
Tipulidae	□	□	□	□		□		♦		♦
Aeschnidae					□				□	
Chlorolestidae					□					
Coenagrionidae					□					
Hydrachnellae					□					

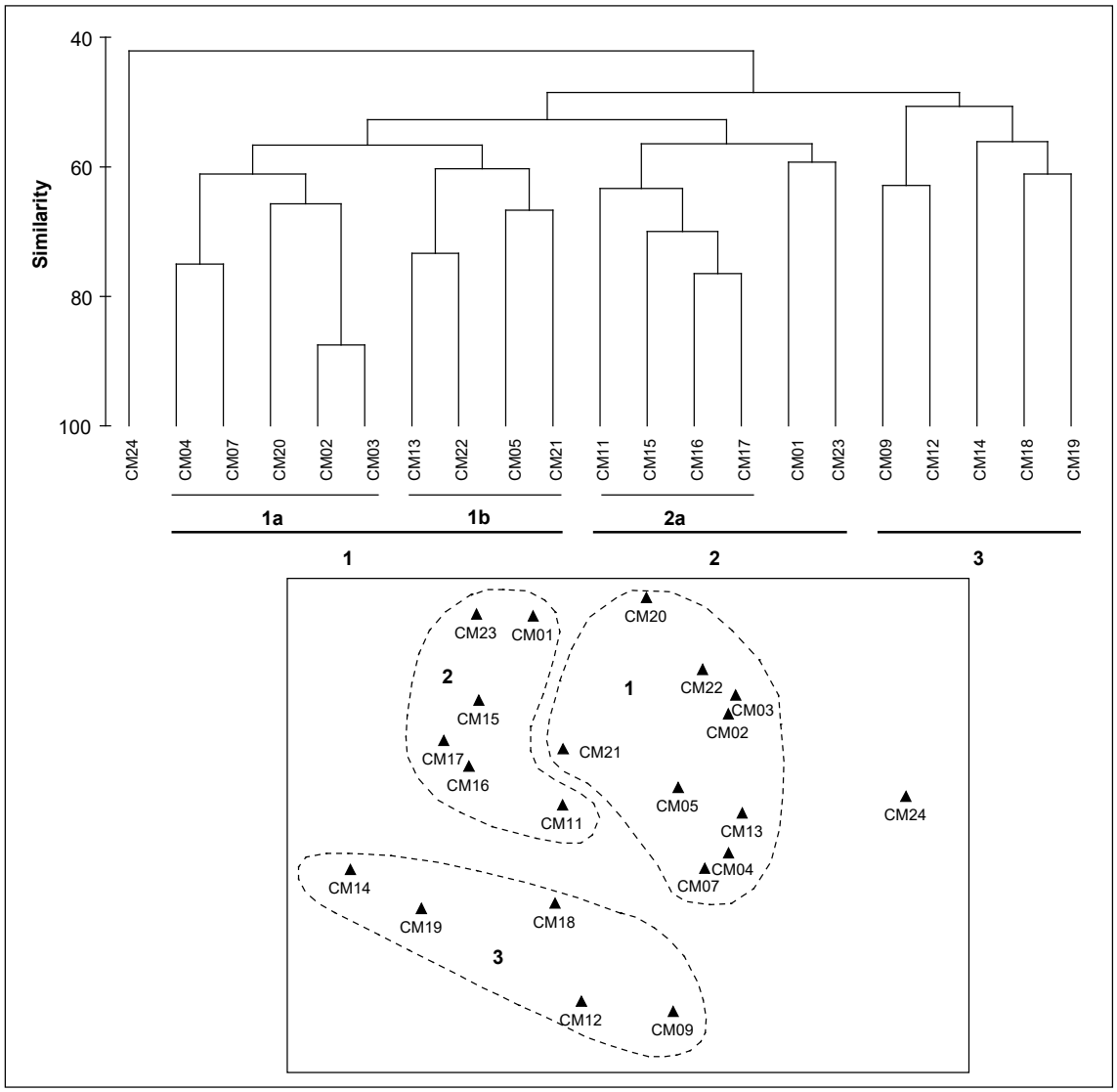


Figure 7.2 Dendrogram and MDS ordination showing the classification of mountain stream sites in the Western Cape based on taxa recorded in spring.

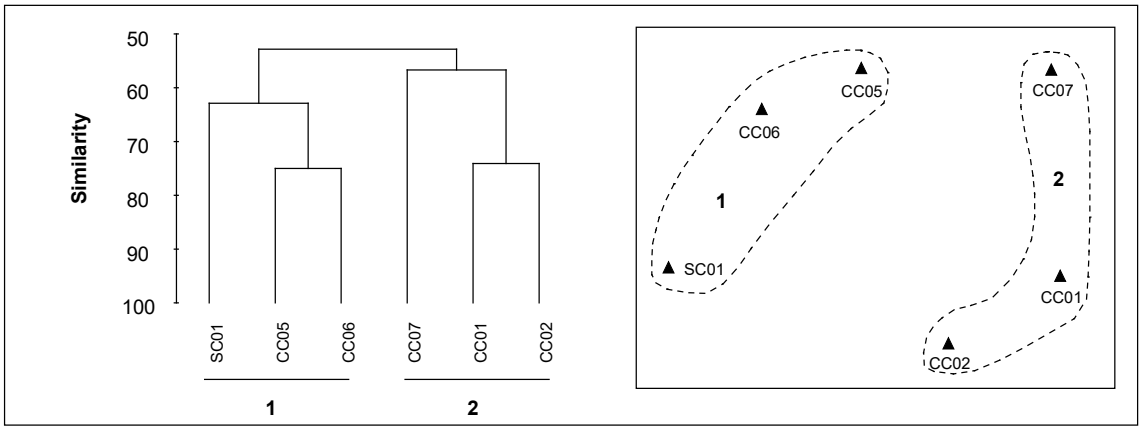


Figure 7.3 Dendrogram and MDS ordination showing the classification of foothill-cobble bed sites in the Western Cape based on taxa recorded in spring.

7.4.2 Environmental variables

A subset of environmental variables that produced the lowest error in predicting Group membership of a site in the DFA was identified for mountain stream and foothill-cobble bed classifications (Table 7.3). The environmental variables within the subset have been ranked such that the variable with the greatest predictive potential (PP) is ranked 1, the one with the second highest PP is ranked 2, the third 3, etc., up until the maximum number of variables identified as predictors for each classification. Distance from source, stream order and cation ratio were significantly different among Groups in the mountain stream classification (Kruskal-Wallis). In DFA, both distance from source and cation ratio were identified as important predictors, together with pH and longitude. Thus catchment level and water chemistry variables were included as predictor variables. Considering mountain stream sub-groups separately, i.e. 1a, 1b, 2a and 3, distance from source was again important, together with stream width, % bedrock, and longitude. DFA of Groups in the foothill-cobble bed classification suggests that pH and stream width are important variables in discriminating between Groups. The number of sites is, however, severely limited making any correlative information preliminary in nature.

Table 7.3 Subset of environmental variables that provided maximum discrimination between Groups within the classifications of mountain stream sites and foothill-cobble bed sites. Error refers to the percentage of sites that were misclassified into Groups in the DFA using cross-validation.

		Mountain stream		Foothill-cobble bed
Variable Types	**Variables**	**No sub-groups**	**Sub-groups**	
	n (excluding outliers)	20	18	6
	Error (%)	20%	17%	17%
Catchment variables	Longitude	4	4	
	Distance from source	1	1	
Site variables	Stream width		2	2
Habitat variables	% Bedrock		3	
Water chemistry variables	pH	3		1
	Cation ratio	2		

7.4.3 SASS4 Scores, Number of Taxa and ASPT

Median, minimum, maximum, 90th percentile and 5th percentile values for SASS4 Scores, number of taxa and ASPT values are given in Table 7.4. Generally all three metrics were slightly higher in mountain streams than in foothill-cobble bed sites but this was not significant (Kruskal Wallis $p > 0.05$). One mountain stream site (CM24) was excluded from analyses as it was identified as an outlier by the multivariate analysis (Figure 7.2), most likely because it had 95% bedrock with <5% cobble substrate available. Whilst assessment of seasonal differences in SASS scores was restricted by scarcity of data, particularly for foothill-cobble bed sites, significant differences in ASPT were noted between mountain stream sites in spring and autumn (Kruskal Wallis Test statistic: $H = 10.37$, $P < 0.05$). SASS4 Scores were similar in both seasons, but fewer taxa were recorded in spring than in autumn, resulting in significantly higher ASPT values in spring. Separate values are thus given for spring and autumn in the mountain stream subregion (Table 7.4). Differences in SASS scores at sites with or without aquatic/marginal vegetation, as assessed by comparing mountain stream and foothill-cobble bed sites in spring, revealed that both the SASS4 Score and number of taxa were higher at sites with vegetation, whilst ASPT was significantly higher at sites without vegetation (Kruskal Wallis: $H = 6.83$, $p < 0.05$). More detailed examination of mountain stream sites in autumn showed that both SASS4 Score and number of taxa were significantly higher at sites with vegetation (Kruskal Wallis: SASS4 Score: $H = 4.82$, Number of Taxa: $H = 5.63$, $p < 0.05$), whilst ASPT was higher at sites without vegetation, although this was not significant.

Derivation and validation of biological bands

Previous analysis (Chapter 4, Figure 4.8) has revealed the significant positive relationship between SASS4 Score and the number of biotopes sampled. Similarly, ASPT was negatively correlated with the number of biotopes sampled. On this basis, and given the observed differences in SASS scores at sites with and without vegetation, biological bands have been derived with ASPT plotted as a function of SASS4 Score (Figure 7.4) for 27 uplands sites. Sites have been plotted separately on the basis of season, subregion and type, i.e. reference or monitoring site. Since differences in SASS scores between upland subregions have been shown not to be significant, values based on all upland reference sites were used for deriving biological bands.

Table 7.4 Median, minimum, maximum, 90th and 5th percentiles and ranges for SASS4 Score, number of taxa and ASPT at upland sites assessed in spring in the Western Cape. Results are also given separately for mountains stream and foothill-cobble bed sites in spring, and mountain stream sites in autumn, and for upland sites with and without vegetation biotopes.

		SASS4 Score	Number of Taxa	ASPT
Upland sites, i.e. mountain stream and foothill-cobble bed sites combined (n = 26), spring, excluding CM24	Median	147	16	8.8
	Minimum	103	13	7.9
	Maximum	181	23	10.4
	90th percentile	166	19	9.5
	5th percentile	107	13	7.9
	Range	78	10	2.5
Mountain stream sites, spring, excluding CM24 (n = 20) (Values which differed when CM24 was included are given in parenthesis)	Median	150	17 (16)	8.8
	Minimum	107 (81)	13 (10)	7.9
	Maximum	181	23	10.4
	90th percentile	167	19	9.3
	5th percentile	113 (107)	13	8.0 (8.1)
	Range	74 (59)	10 (13)	2.5 (2.1)
Mountain stream sites, autumn, excluding CM24 (n = 11)	Median	161	19	8.0
	Minimum	103	12	6.9
	Maximum	239	30	9.1
	90th percentile	223	27	8.6
	5th percentile	103	12	6.9
	Range	136	18	2.2
Foothill-cobble bed sites, spring, (n = 6)	Median	126	15	8.6
	Minimum	103	13	7.9
	Maximum	161	19	9.5
	90th percentile	161	19	9.5
	5th percentile	103	13	7.9
	Range	58	6	1.5
Sites with SIC/SOOC biotopes only, spring (n = 15)	Median	142	14	9.1
	Minimum	116	13	8.4
	Maximum	185	22	10.4
	90th percentile	166	18	10.1
	5th percentile	116	13	8.4
	Range	69	9	2.0
Sites with SIC/SOOC biotopes and AQV/MV, spring (n = 29)	Median	151	17	8.8
	Minimum	103	12	7.6
	Maximum	194	24	10.3
	90th percentile	181	23	9.5
	5th percentile	107	13	7.9
	Range	91	12	2.7

A system modified from the RIVPACS and AusRivAS biological banding system (Furse 2000, Simpson & Norris 2000) has been developed. Whilst their systems are based on observed/expected ratios (O:E) and are largely generated using predictive models, the biological banding system shown in Figure 7.4 and Table 7.5, is based on absolute SASS4 Scores and ASPT values. Both methods use the variability in Expected values at reference sites to calculate band widths. These were calculated using median values, 90th and 5th percentiles of SASS4 Score and ASPT at reference sites, with band width calculated as the median minus the 5th percentile. Actual values for each band width are tabulated in Table 7.5. Deriving biological bands based on percentiles enables intrinsic variability in scores among reference sites to be incorporated.

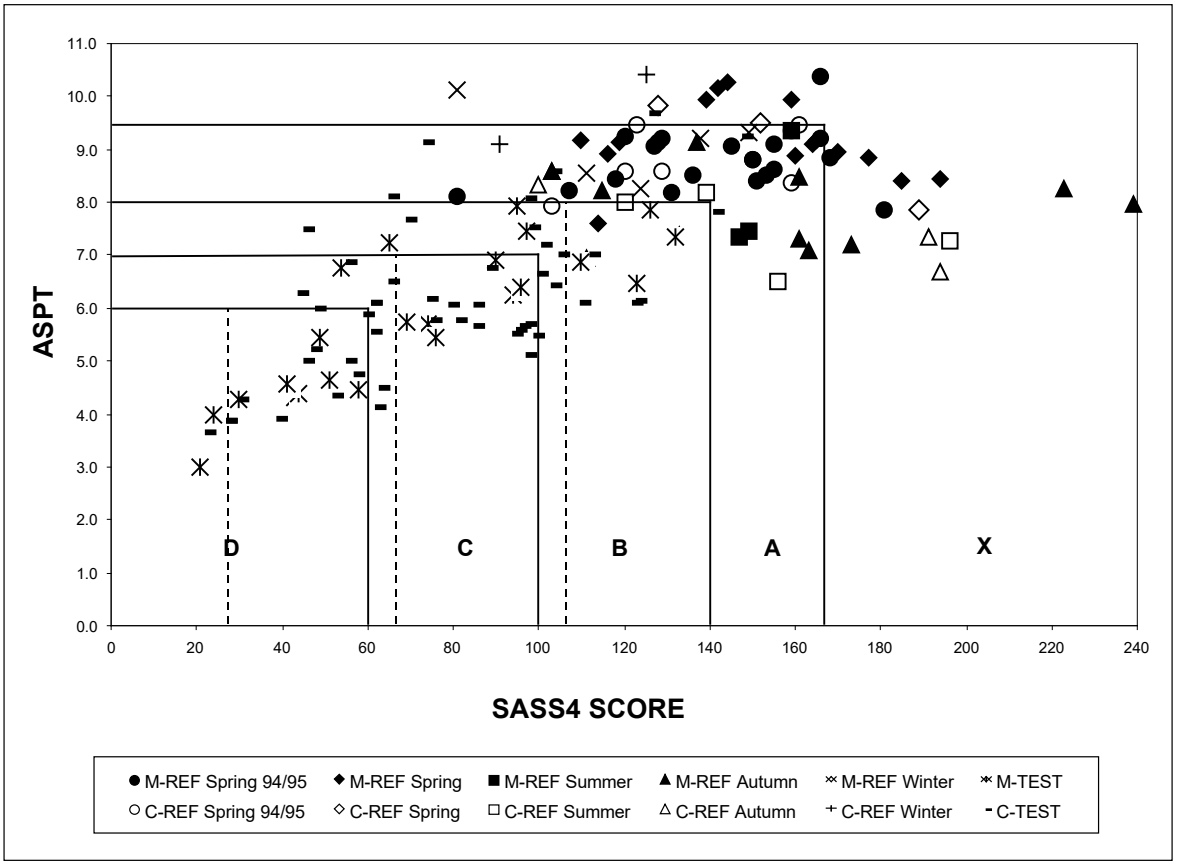


Figure 7.4 SASS4 Scores and ASPT values at reference and monitoring sites in mountain streams and foothill-cobble bed of the Western Cape. Validated biological bands X, A, B, C and D are indicated (solid lines) and original SASS4 Score bands, calculated using percentiles and median values, are shown as dotted lines. Sites are coded to indicate reference sites in mountain stream (M-REF) and foothill-cobble bed (C-REF) subregions and monitoring sites in mountain stream (M-TEST) and foothill-cobble bed (C-TEST) subregions.

The majority of reference sites fell within band A or X, regardless of season sampled. Several monitoring sites, which were known to be impacted by anthropogenic activities and thus considered to have reduced water quality, had SASS4 Scores exceeding 107, the lower limit of biological band A, i.e. reference site. The four mountain stream monitoring sites included three that were mildly affected by agricultural activity, and one that was a kilometer below an aquaculture farm and which was exhibiting signs of recovery towards pre-disturbance conditions (Dallas 1995, Brown 1997). On this basis it is suggested that the lower limit of SASS4 Score (107) as derived by using the 5th percentile value is not adequately separating reference from mildly impacted sites. In terms of ASPT, six sites in the foothill-cobble bed subregion exceeded the 5th percentile (> 7.9). All of these were on the upper Berg River, which is subjected to an inter-basin water transfer (IBT) scheme that operates during summer, the natural low-flow period. Three of these sites were sampled in winter when the IBT is not operational (Snaddon & Davies 1998). During this period, these sites resemble those sites above the IBT, which are included as reference sites for this region. Three sites were sampled in spring when the effects of the IBT may not yet have manifested themselves as a change in macroinvertebrate assemblages and hence as a reduction in SASS scores. All of these sites therefore act as reference sites during winter, and possibly spring, and thus their inclusion in the biological band A is perhaps to be expected. The reassignment of these sites from biological band A to B under IBT conditions suggests that this biological banding system is fairly sensitive to subtle changes in water quality.

On the basis of data validation using monitoring site data, it is apparent that the 5th percentile is inadequate to differentiate mildly-disturbed sites from reference sites, and it is proposed that the SASS4 Score delineating the lower limit of biological band A, i.e. reference, be increased from 107 to 140. This represents the 46.5th percentile value. This would ensure that disturbed sites were assigned to biological bands B, C or D. For practical reasons, the lower limit of biological band A for ASPT was increased from 7.9 to 8.0 and band width was increased from 0.9 to 1.0.

Table 7.5 Biological bands derived for SASS4 Score and ASPT based on reference data (*) for upland sites of the Western Cape sampled in spring 1994/5. Modified bands (#) are given for SASS4 Score and ASPT following validation with data from reference sites in other seasons and at monitoring sites.

Band	Description	SASS4 Score*	ASPT*	SASS4 Score#	ASPT#
X	**Richer than reference**: Greater than 90th percentile of reference sites; SASS4 Score and ASPT greater than expected; potential biodiversity "hot spot".	> 166	> 9.5	> 166	> 9.5
A	**Reference:** Within range of central 85% of reference sites (i.e. 5th to 90th percentiles); SASS4 Score and ASPT within range of 85% of reference sites.	107 - 166	7.9 - 9.5	140 - 166	8.0 - 9.5
B	**Below reference:** Below 5th percentile of reference sites; band width equal to median minus the 5th percentile; fewer taxa than expected. SASS4 Score and ASPT lower than expected; potential impairment of water quality and/or habitat with loss of pollution-sensitive taxa.	67 - 106	7.0 - 7.8	100 - 139	7.0 - 7.9
C	**Well below reference:** Below band B; same width as band B; many fewer taxa than expected; SASS4 Score and ASPT much lower than expected; substantial impairment of water quality and/or habitat; major loss of pollution-sensitive taxa.	27 - 66	6.1 - 6.9	60 - 99	6.0 - 6.9
D	**Impoverished:** Below band C to zero; few of the expected taxa remain; severe impairment; remaining taxa hardy and pollution-tolerant.	< 27	< 6.1	< 60	< 6.0

7.4.4 Expected SASS-taxa

Using the validated biological bands (Table 7.5, Figure 7.4) the relative frequency of occurrence of each SASS-taxon within each biological band, i.e. X, A, B, C and D, was calculated using all reference and monitoring site data within the respective band (Table 7.6). Thus, Notonemouridae occurred in 77% of the samples in band X, 64% of the samples in band A, 21% of the samples in band B, etc. Seventeen SASS-taxa showed a decrease in relative frequency of occurrence from biological bands X to D, i.e. as disturbance increased. Most of these were taxa inhabiting the stones-in-current or stones-out-of-current biotopes (see Tables 4.2 and 4.3, Chapter 4) and were taxa identified as characteristic of upland sites of the Western Cape (Table 7.2). Ten SASS-taxa increased in relative frequency of occurrence from biological bands X to D, i.e. as disturbance increased. The remainder showed neither an increase nor a decrease in the relative frequency of occurrence in response to increasing disturbance. Many of these taxa were more commonly recorded in aquatic or marginal vegetation (see Table 4.2, Chapter 4) and some were air-breathers (e.g. Hemipterans) and thus less dependent on water as a medium than organisms that are dependent on water for completion of part of their life cycle.

A table of "expected" or reference taxa for upland sites of the Western Cape has been formulated using information from chapters 4, 5 and 7 (Table 7.7). Given the substantial variability in macroinvertebrate assemblages at upland sites, the relative frequency of occurrence of each taxon, calculated using reference site data in biological bands X and A, is included. Thus, Notonemouridae occurred in 68% of the reference samples, Leptohphlebiidae occurred in 95%, and Amphipoda occurred in 18%, etc. This information is useful in that the presence of a taxon such as Leptophlebiidae, which has a high relative percentage occurrence, is to be expected at a reference site, and thus its absence at a monitoring site would indicate disturbance. On the other hand, the presence of a taxon such as Amphipoda, which has a low relative percentage occurrence, and is thus not always recorded at reference sites, is indicative of a site that is minimally-impacted. Its absence, however, does not necessarily indicate disturbance. Biotope and seasonal trends in the relative occurrence of each SASS-taxon are given as a guide for taking differences in the availability of biotopes and seasonal differences into account. Noting that a particular taxon showing a preference for a particular biotope or season does not necessarily imply that it is absent from other biotopes or in other seasons.

Table 7.6 Relative frequency of occurrence (expressed as a percentage) of each SASS-taxon in biological bands X, A, B, C and D. Increasing and decreasing trends are indicated with shading with highest frequencies darker and lower frequencies lighter. Individual frequencies that do not conform to the highlighted trend are not given in bold text. n = number of samples.

Biological Band	**X**	**A**	**B**	**C**	**D**
n	*26*	*53*	*19*	*30*	*20*
Notonemouridae	**77**	**64**	**21**	**10**	**0**
Baetidae 1 Type	4	15	11	13	20
Baetidae 2 Types	**31**	**34**	**37**	**47**	**55**
Baetidae 3 Types	**65**	**47**	53	**33**	**25**
Caenidae	**23**	**25**	**32**	**47**	40
Heptageniidae	**50**	**40**	**26**	33	**0**
Leptophlebiidae	**96**	**94**	**68**	**20**	**0**
Teloganodidae	**92**	**75**	**42**	**3**	**0**
Tricorythidae	0	0	5	3	0
Dytiscidae	46	17	32	27	15
Elmidae/Dryopidae	**77**	**74**	**63**	**33**	**5**
Gyrinidae	42	30	53	47	35
Helodidae larvae	**77**	**55**	**16**	**7**	**0**
Hydraenidae	**54**	**45**	**21**	**17**	**15**
Hydrophilidae	8	2	0	10	5
Limnichidae	**38**	**23**	**16**	**13**	**5**
Corydalidae	**73**	**75**	**58**	**30**	**15**
Ecnomidae	**46**	**25**	**11**	**10**	**0**
Hydropsychidae 1 Type	35	40	42	40	15
Hydropsychidae 2 Types	27	38	32	17	0
Hydropsychidae 3 Types	8	0	0	0	0
Hydroptilidae	15	2	5	10	0
Philopotamidae	**50**	**47**	**5**	**10**	**0**
Case Caddis 1 Type	15	21	26	37	5
Case Caddis 2 Types	31	23	26	0	0
Case Caddis 3 Types	**54**	**45**	**16**	**7**	**0**
Athericidae	**73**	**49**	**26**	40	**5**
Blephariceridae	**50**	**21**	**0**	**0**	**0**
Ceratopogonidae	12	**2**	**5**	**10**	**20**
Chironomidae	**88**	**91**	**95**	**100**	**100**
Culicidae	12	2	5	10	5
Dixidae	19	2	5	0	0
Empididae	**0**	**2**	**11**	**13**	**30**
Muscidae	**4**	**4**	**11**	**10**	**25**
Simuliidae	92	100	100	93	80
Syrphidae	0	0	0	0	5
Tabanidae	0	2	16	7	5
Tipulidae	31	**34**	**26**	**13**	**10**
Belastomatidae	8	8	16	3	0
Corixidae	23	9	16	33	50
Gerridae	23	11	5	3	15
Naucoridae	15	6	16	0	0
Nepidae	0	0	0	3	5
Notonectidae	15	8	0	3	0
Pleidae	4	0	11	3	0
Veliidae	38	25	42	27	15

Biological Band	X	A	B	C	D
Pyraustidae	8	6	16	7	0
Aeshnidae	38	34	26	33	15
Chlorolestidae	8	15	5	10	0
Coenagrionidae	42	17	21	30	5
Corduliidae	8	4	5	10	0
Gomphidae	19	15	42	47	10
Libellulidae	19	17	32	40	10
Platycnemididae	15	0	0	0	0
Zygoptera Juveniles	8	2	0	3	0
Hirudinea	0	0	0	3	0
Oligochaeta	46	**32**	**53**	**83**	**90**
Hydrachnellae	19	6	37	23	25
Amphipoda	**19**	**17**	**5**	**7**	**0**
Brachyura (Crabs)	27	26	42	43	30
Planariidae	**12**	**17**	**16**	**30**	**40**
Ancylidae	**0**	**0**	**16**	**40**	**30**
Lymnaeidae	**0**	**0**	**5**	**13**	**20**
Physidae	0	0	5	17	10
Planorbidae	0	2	0	0	0

Table 7.7 Reference SASS-taxa at upland sites of the Western Cape. The expected frequency of occurrence is expressed as a percentage. Biotope and seasonal trends are indicated with taxa most often recorded in a particular biotope shown (SI = stones-in-current, SO = stones-out-of-current, V = aquatic and marginal vegetation), or in a particular season (S = spring, A = autumn).

Order	SASS-taxon	% Frequency of occurrence	Biotope	Season
Plecoptera	Notonemouridae	68	SI	
Ephemeroptera	Baetidae 3 Types	53	SI, SO	S
	Heptageniidae	43	SI, SO	S
	Leptophlebiidae	95	SI, SO	
	Teloganodidae	81	SI, SO	S
Coleoptera	Elmidae/Dryopidae	75	SI	A
	Helodidae larvae	62	SI, SO	S
	Hydraenidae	48	SI	
	Limnichidae	28	SI	
Megaloptera	Corydalidae	75	SI	
Trichoptera	Ecnomidae	32	SO	A
	Philopotamidae	48	SI	A
	Case Caddis 3 Types	48	V	
Diptera	Athericidae	57	SI	
	Blephariceridae	30	SI	S
	Chironomidae	90	SI, V	
	Simuliidae	97	SI, V	
	Tipulidae	33	SI, SO	S
Crustacea	Amphipoda	18	SI, SO	S

Most of the taxa included in Table 7.7 are characteristic of minimally-disturbed upland sites and disappear or become rarer as disturbance increases. Others, such as Chironomidae and Simuliidae, are included since they are almost always present at upland sites, but do not necessarily disappear in response to disturbance. In these families, change often occurs at a resolution greater than family, with one species replacing another as disturbance increases (A.R. Harrison, Freshwater Research Unit, Department of Zoology, University of Cape Town).

A summary diagram is provided showing the SASS-taxa expected to decrease in response to disturbance, as well as those expected to increase in response to disturbance. These have been determined using relative frequency of occurrence data of each taxon at upland sites in the Western Cape (Figure 7.5). The disturbance is primarily that resulting from a reduced water quality at monitoring sites.

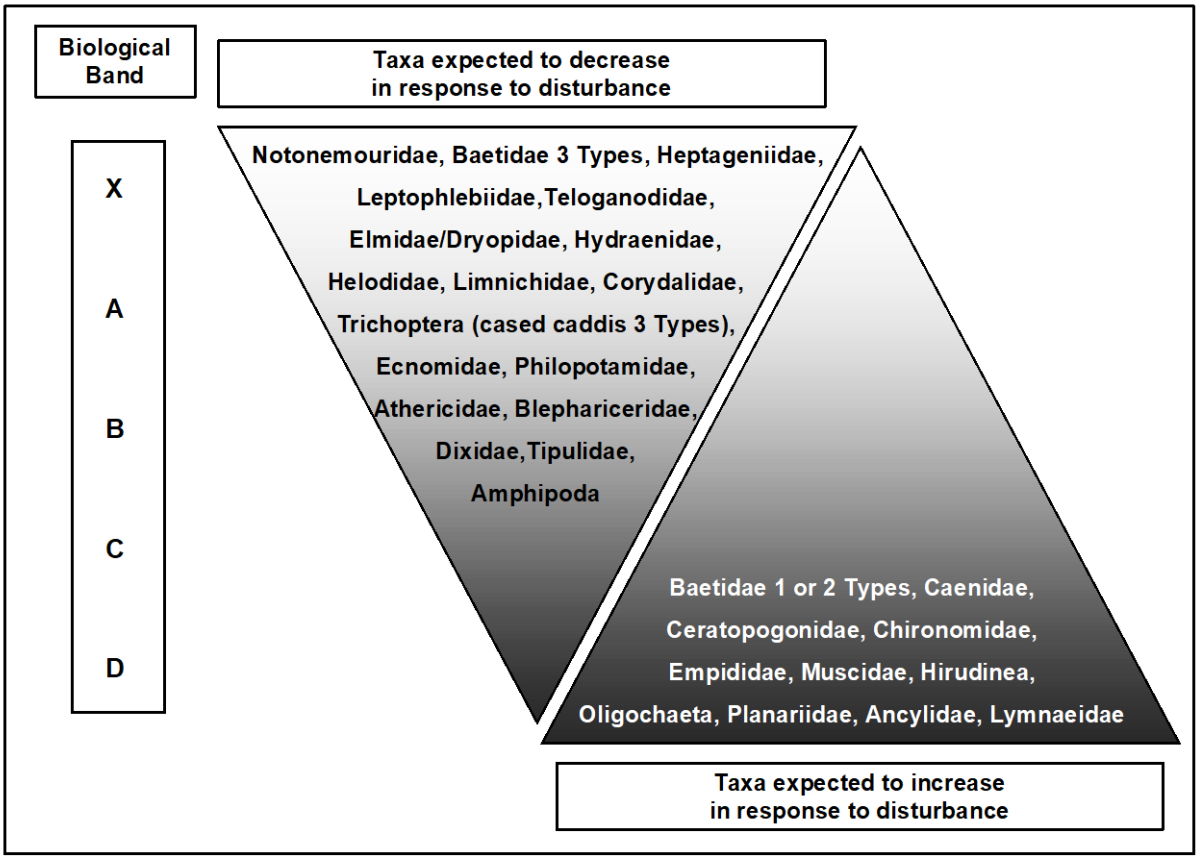


Figure 7.5 SASS-taxa shown to increase or decrease in response to increasing disturbance, primarily water quality impairment, at upland sites in the Western Cape. They have been determined on the basis of the relative frequency of occurrence of each SASS-taxon within biological bands X, A, B, C and D.

7.5 DISCUSSION

Understanding spatial heterogeneity in lotic systems, and the extent to which variability in macroinvertebrate assemblages affects the ability to define reference conditions, is important for the interpretation of bioassessment data. Upland sites are known for their physical complexity and, particularly in the Western Cape, for their variability with respect to substrate, hydraulic and biotope characteristics. It is often assumed that sites with similar abiotic characteristics will have similar biotic characteristics. Thus, if sites are in the same bioregion, subregion and river type (i.e. similar in terms of hydrological type, size, substratum etc.), it is assumed that their macroinvertebrate assemblages will also be similar. Homogeneity with respect to environmental factors is thereby assumed to be transmitted into homogeneity with respect to biotic assemblages. It has been shown, however, that this is not always the case, with factors such as biotic interactions (like predation: e.g. Cooper 1984, Crowl *et al.* 1997) influencing macroinvertebrate assemblages. Other factors related to biogeographic and evolutionary aspects may also play a role, particularly in a region like the Western Cape, which is known for its high degree of endemism. Of the 27 uplands sites assessed during this study, many were dissimilar in terms of their macroinvertebrate assemblages. Closer examination showed that this was partially a reflection of biotope availability, although sites with only stony biotopes present (i.e. SIC/SOOC only), were at least 47% dissimilar. Similarly, sites with both SIC/SOOC and AQV/MV were at least 50% dissimilar. The observed variability amongst upland sites may merely be an artifact of inadequate sampling but, given results of other studies within the region (e.g. King & Schael, Steven & Picker 1999, Stewart & Griffiths 2001), it seems likely that some form of "catchment signature" is present in the form of species-level, and possibly family-level, distinctiveness in macroinvertebrate assemblages within catchments.

Examination of the environmental variables revealed that in the mountain stream subregion, factors such as distance from source, cation ratio, pH and longitude all contributed towards predicting Group membership. Distance from source varied from 2 to 19 kilometres; pH varied from 4.1 to 6.6; cation ratio varied from 0.63 to 0.87, with the relative concentrations of sodium and calcium varying among sites; and longitude ranged from 18°30'E to 20°30'E, thus spanning approximately 170 km. Both pH and the cation ratio reflect geological or lithostratigraphic characteristics of the catchments. pH is also

influenced by the indigenous fynbos 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 & Day 1998). These humic substances are organic acids and, when they dissolve in water, pH is reduced. The range in pH in streams in the present study suggests that some are naturally far more acidic than others. Humic substances also give water its colour, and, whilst not measured in this study, water-colour varied from very dark brown to light yellow at different sites. Subtle differences in factors such as pH may in part explain observed differences in uplands sites of rivers, each of which acts as an isolated geographic entity.

On the basis of macroinvertebrate assemblages, therefore, uplands sites are significantly different from one another. When translated into SASS scores, in particular SASS4 Score and ASPT, however, differences are such that detection of a disturbance is not impeded. Overall variability in SASS scores at reference sites was high, with a range of 78 for SASS4 Score and 2.5 for ASPT. By interpreting monitoring-site data using biological bands based on both SASS4 Score and ASPT, variability in SASS scores at upland reference sites is taken into account. Interpretation based on the relationship between ASPT and SASS4 Score also presents a potential solution for incorporating between-site variation in the availability of biotopes. The main difference between upland sites is the presence or absence of aquatic and/or marginal vegetation. When available, aquatic vegetation, and in particular the aquatic sedge, *Isolepis* spp., which often occurs in-current, provides an important habitat for many taxa, including several species of cased caddisflies (pers. obs.). Marginal vegetation, when located in a lentic environment within the river, provides important habitat for those taxa that prefer slow-flowing, backwater habitats. The presence and type of vegetation are thus important determinants of macroinvertebrate assemblages at upland sites and the absence of vegetation at upland sites often translates into low numbers of taxa.

Validation of biological bands with monitoring-site data revealed the necessity to validate and modify the bands on the basis of empirical data. In upland sites of the Western Cape, perhaps because of the variability of reference sites in this region, it was necessary to increase the lower limit of the biological band A, i.e. reference, such that mildly impacted sites were assigned to band B. Following this adjustment, monitoring sites spanned the range of biological condition from band B to band D. Trends in the relative frequency of

occurrence of SASS-taxa from one biological band to another facilitates the identification of those taxa which decrease in response to disturbance, those that increase in response to disturbance, and those that are unaffected by disturbance. The majority of taxa that decreased where dwellers of the stones-in-current biotope and included several of the sensitive and high-scoring SASS taxa, suggesting that at uplands sites in the Western Cape, it is this biotope that is the most susceptible to disturbance. Those taxa that decreased included several of the more tolerant and low-scoring taxa such as Muscidae and Oligochaeta. A mayfly family, the Caenidae, also increased in response to increased disturbance, perhaps as a reflection of its ability to withstand increased levels of siltation and/or increased concentrations of total dissolved solids (TDS). This information enables comparisons to be made between macroinvertebrate assemblages from monitoring sites and reference sites and allows elucidation of taxa "lost" or "gained" in response to disturbance.

In conclusion, therefore, whilst macroinvertebrates at uplands sites are extremely variable in terms of their assemblages, when these assemblages are translated into SASS scores, variation is less pronounced. Several high-scoring, sensitive taxa are known to occur in low abundances, with instances where a single individual has been recorded. Given the spatial and temporal heterogeneity of uplands sites, the likelihood of missing one of these taxa is comparatively high. It seems, however, that when one taxon is not recorded, another may well be, and thus the effect on overall SASS score is negligible. By defining the reference condition as a band, thereby incorporating intrinsic variability, and by utilising the relationship between SASS4 Score and ASPT, the ability to detect a disturbance at a monitoring site is facilitated. Qualitative comparison of observed taxa, i.e. recorded at a monitoring site, with expected taxa, i.e. taxa identified as representative of a particular reference condition, enable spatial and temporal heterogeneity of macroinvertebrate assemblages, which are a distinct feature of upland sites of the Western Cape, to be taken into account.

CHAPTER 8. SYNOPSIS AND GENERAL DISCUSSION

Spatial and temporal heterogeneity are features of lotic systems (e.g. Palmer & Poff 1997) and spatial variability is reflected in the patchy distribution observed in components of riverine biotas such as macroinvertebrates (e.g. Pringle *et al.* 1988). Factors contributing to this variability operate at several scales, ranging from regional-level factors such as climate and geology (e.g. Richard *et al*.1997), to habitat-level factors (e.g. Armitage *et al.* 1995) acting on individual taxa, particularly those related to an individual's specific hydraulic and substrate requirements (Resh & Rosenberg 1984). Temporal variability is also dependent on regional-level factors such as climate, in that aspects such as flow pattern, discharge and water temperature (e.g. Hawkins *et al*. 1997) are largely determined by climate, whilst life history stages of individual taxa are often cued into seasonal variations in these factors (e.g. Yanoviak & McCafferty 1996).

Macroinvertebrates are commonly used in aquatic bioassessment (Rosenberg & Resh 1993), either in the formulation of biotic indices or in the development of predictive models. In both cases, understanding the extent of the spatial and temporal variability of macroinvertebrate assemblages is fundamental for effective bioassessment. Coupled with bioassessment are the identification and classification of reference sites (e.g. Reynoldson *et al*. 1997) and the definition of ecological reference conditions (e.g. Hughes 1995). Reference conditions, which enable the degree of degradation or deviation from natural conditions to be ascertained, are a critical interpretive component necessary for elucidation of bioassessment data and are thus important for effective management of aquatic resources. A highly variable biotic index at reference sites, for example, may reflect an insufficiently rigorous index, inadequate classification of reference sites, or variable levels of disturbance at a site (Hughes 1995).

For effective bioassessment and management of aquatic resources in South Africa, it is necessary to have an operational and scientifically-validated bioassessment tool, a spatial framework within which bioassessment is conducted, and regional reference conditions to facilitate data interpretation. The primary bioassessment tool, SASS (Chutter 1998), has been widely used and tested (e.g. Dallas 1995, 1997, Chutter 1998) and has proved to be a useful measure of water quality, as well as a more general measure of river condition. It is

regularly re-evaluated and, when necessary, modified such that it better reflects the water quality conditions it was designed to measure. South Africa is diverse in climate, geomorphology, geology and soils, and aquatic biotas vary in response to differences in these factors, as well as factors related to the evolutionary history and biogeography of the region. The hierarchical spatial framework developed in South Africa is an attempt to incorporate this diversity in a structured manner such that spatial heterogeneity is accounted for. To date, the utility of this framework has not been tested and the extent to which aquatic biotas vary regionally, whilst known intuitively by many aquatic ecologists, has not been evaluated within the context of bioassessment. The spatial framework also provides a structure within which reference sites are selected, and thus reference conditions defined. It is necessary to understand the extent of both spatial and temporal variability so that the utility of reference conditions as an interpretative tool can be evaluated. If a system is highly variable, it may indeed not be possible to define a reference condition, or it may be necessary to define several reference conditions for different types of rivers, even within a relatively discrete area. Fundamental to the definition of any reference condition is the selection of reference sites that should, ideally, be minimally-disturbed, be representative of the stream or river for which it provides a reference and have an appropriate variety of biotopes and substrates.

Central to this report is the question of whether ecological reference conditions are realistic and attainable entities, or whether intrinsic spatial and temporal heterogeneity of and variability in lotic systems are such that establishing reference conditions is not possible. The key questions posed, therefore, relate to the extent to which macroinvertebrate assemblages vary spatially and temporally, and the implications of this variability to bioassessment and defining reference conditions. The question has been addressed by examining regional variability of macroinvertebrate assemblages within the context of assessing the utility of the spatial framework for regional classification of reference sites; by examining variability at the level of habitat; by examining temporal variability; and by identifying the environmental variables contributing to the variability in macroinvertebrate assemblages. To answer these questions patterns of spatial and temporal heterogeneity were examined in two distinct geographic regions, and at the level of individual taxa, macroinvertebrate assemblages and the derived biotic index, i.e. SASS scores.

8.1 Spatial variability in macroinvertebrate assemblages at the regional level

The ultimate goal of regional classification is to generate homogenous groups of sites, which are expected to have greater similarity with sites in the same group, than with sites in a different group. The two alternative classification methods both have this as a goal, although the approaches vary, with the regional approach (e.g. Omerick 1987, Barbour *et al.* 1999) generating spatially discrete regions on an *a priori* basis, whilst the multivariate approach (e.g. Wright *et al.* 1993, Smith *et al*. 1999) allows biotic assemblages to generate the homogeneous groups. The underlying assumption is that natural variation is predictable among systems within the same region or homogenous group where environmental features are similar (Omerick & Bailey 1997). The validity of the regional classification system developed in South Africa was therefore assessed by comparing the regional and multivariate classifications. At the broadest scale examined in this study, macroinvertebrate assemblages showed distinct geographic separation into Western Cape and Mpumalanga regions. These differences were most distinct in upland areas, i.e. mountain streams and foothill-cobble bed, with lowland areas less regionally distinct (Chapter 3). Within regions, longitudinal zonation into upland and lowland areas was important, with sites grouping on the basis of broad geomorphological zones or subregions. Of the upland sites, differentiation into mountain streams and foothill-cobble beds was not apparent, although overall variability of assemblages within upland areas, in particular the Western Cape, was very high.

The distinctiveness of macroinvertebrate assemblages from the Western Cape and Mpumalanga is not unexpected given the different climatic conditions of the two geographic areas, with associated differences in geology (Day & King 1995), flow regime (King & Tharme 1994) and vegetation (Low & Rebelo 1996), together with biogeographic differences (Harrison1965b). That this distinction is most prevalent in upland areas is probably indicative of the large number of endemic taxa in mountain streams and cobble-bed foothills of the Western Cape (Harrison 1965a, b), whilst lowland reaches were dominated by more widespread, hardy species. Longitudinal zonation, apparent in both regions, is also a common feature of lotic systems, with macroinvertebrate assemblages responding to changes in, for example, stream hydraulics (Statzner & Higler 1986), temperature (Hawkins *et al.* 1997) and food resources along the longitudinal profile of a river (Vannote *et al*. 1980). The results of this study lend support to geomorphological

zonation, although difficulties may sometimes arise in separating observed longitudinal patterns that reflect changes in these factors, from those that reflect changes in water quality, particularly since many lowland rivers are disturbed.

In general, *a priori* regional classification of sites using the hierarchical spatial framework developed in South Africa provided a useful framework for preliminary classification of reference sites. Within-class variability (i.e. within a bioregion, ecoregion or bio-subregion etc.) was always lower than between-class variability (i.e. between bioregions, ecoregions, bio-subregions, etc.). Groups of sites based on *a posteriori* analysis of macroinvertebrate data, however, provided a more robust classification than any of the regional classifications. Spatial classifications therefore offer geographic partitions within which to expect somewhat similar conditions and regional reference sites selected within the context of the hierarchical spatial framework are likely to be more representative of specific river types than those selected without using the spatial framework. This lends support to studies elsewhere that have evaluated the ability of spatially-based regional classification systems to partition variability in lotic systems (e.g. Harding *et al.* 1997, Gerritsen *et al.* 2000). It also highlights the need for additional partitioning of variability at a lower scale (Johnson 2000) and for the classification of sites to be an iterative process that allows for subjective *a priori* regional classifications to be modified on the basis of independent, objective *a posteriori* classification of biological assemblages (Gerritsen *et al.* 2000). The lack of distinctiveness in macroinvertebrate assemblages from mountain streams and cobble-bed foothills, both of which are upland subregions, suggests that, from a practical perspective, and within the confines of bioassessment, mountain stream and foothill-cobble bed sites may be grouped together. This aspect was explored further in Chapter 7.

Some variability within both regional classes and groups of sites with similar macroinvertebrate assemblages (i.e. Groups) could not be accounted for at the regional or subregional levels, suggesting the presence of additional factors acting at a lower scale such as site or habitat. Aspects related to this were explored further in Chapter 4 (biotopes), Chapter 6 (environmental variables) and Chapter 7 (upland sites of the Western Cape). Further testing of the utility of regional classifications would also be useful since the limited data for the Western Cape prevented rigorous testing of regional classifications.

It would be advantageous to repeat the analyses once additional reference-site data have been collected.

8.2 Spatial variability in macroinvertebrate assemblages at the habitat level

Aquatic organisms have specific hydraulic and substrate requirements (e.g. Poff & Ward 1990), which often result in a patchy distribution of biota (e.g. Pringle *et al.* 1988) with spatial variability occurring at the level of habitat (e.g. Palmer *et al.* 1991, Wohl *et al*. 1995). Bioassessment that does not factor in these hydraulic and substrate requirements may not adequately reflect the conditions, such as water quality, that are being assessed. Historically, the merging of habitats or SASS-biotopes into a site-based assessment (e.g. Wright *et al.* 1984, Chutter 1998) did not take account of these differences. More recently, bioassessment has been undertaken such that habitats are sampled separately and comparisons are made at habitat rather than at site level. The extent to which macroinvertebrate assemblages varied amongst SASS-biotopes was, therefore, examined and evaluated in the light of defining reference conditions.

Spatial variability at the level of habitat (Chapter 4), specifically SASS-biotopes, revealed that several taxa exhibited a degree of biotope specificity, with some taxa recorded more frequently in one biotope rather than another. Several families recorded in the Western Cape, namely the Notonemouridae, Teloganodidae and Corydalidae, showed a preference for SIC/SOOC, whilst in Mpumalanga Heptageniidae, Psephenidae and Psychomyiidae showed a preference for SIC/SOOC. In Mpumalanga, both SIC/SOOC and AQV/MV supported several biotope-specific taxa. The relative importance of a biotope as a habitat for macroinvertebrates, as a reflection of both its availability and its utilisation by aquatic organisms, varied regionally. Marginal vegetation was more common at reference sites in Mpumalanga compared to the Western Cape and constituted a relatively important biotope for macroinvertebrates. Aquatic or instream vegetation, in the form of the sedge *Isolepis* spp., provided a unique and important habitat for several species of macroinvertebrates, including cased caddisflies, in the Western Cape. The importance of hydraulic condition coupled with substrate type became apparent with differences in taxa observed within a biotope-group. Marginal vegetation-in-current supported taxa, some of which were also recorded in stones-in-current, compared with marginal vegetation-out-of-current with which surface dwellers such as Gerridae and Veliidae were associated.

These associations were reflected in seasonal differences in the distinctiveness of biotopes, with distinctiveness more pronounced in autumn, under low-flow conditions, in comparison with less pronounced biotope specificity in spring in the Western Cape. Seasonal differences were not apparent in Mpumalanga, a summer-rainfall region. In the Western Cape, a winter-rainfall region, periods of lowest baseflow are coupled with high temperatures, whilst in Mpumalanga periods of lowest baseflow occur in winter, and are thus not coupled with high temperatures. The lotic environment in the Western Cape may therefore be thought of as a more stressful environment than that of Mpumalanga. Seasonal patterns, biotope specificity and overall variability of macroinvertebrate assemblages within uplands sites of this region may thus reflect adaptations of aquatic organisms to these harsh conditions over evolutionary time. Clearly, it is the combination of biotope availability, discharge, and perhaps temperature, particularly summer maxima, that influences the distribution of aquatic organisms even at the relatively coarse family-level within broad biotope and flow categories.

In terms of SASS Scores, SIC/SOOC was shown to be the most important SASS biotope-group and taxa associated with it contributed the highest percentage to site SASS Scores. SIC/SOOC was also the most consistent in terms of its associated macroinvertebrate assemblage. Taxa contributing to within-group similarity of SIC/SOOC biotope-group of the Western Cape included several high-scoring, sensitive taxa such as Notonemouridae, Heptageniidae, Corydalidae, Philopotamidae, Athericidae, Blephariceridae and Tipulidae. All three metrics, i.e. SASS4 Score, Number of Taxa and ASPT, differed significantly between biotope-groups, with highest scores consistently recorded in SIC/SOOC. The GSM biotope-group had the fewest taxa regularly associated with it, as well as the lowest SASS Scores, although when in-current, and where the substrate was predominantly gravel rather than sand or mud, the GSM biotope resembled the stones-in-current biotope in terms of its macroinvertebrate assemblages.

There was a significant positive relationship between SASS4 Score and number of taxa with number of biotopes sampled and a negative correlation between ASPT and number of biotopes sampled. This provides support for the concept, explored further in Chapter 7, of using the relationship between ASPT and SASS4 Score in interpretation of SASS data and in the derivation of biological bands. The implications of the observed biotope-related differences for defining reference conditions are that it is essential to sample biotopes

separately, and that within biotope-groups, flow conditions need to be considered. Specifically, note should be taken of whether stones are in- and/or out-of-current and vegetation is in- and/or out-of-current.

This report is based solely on correlative surveys that are considered essential for documenting broad geographic patterns of association of lotic biota (Power *et al.* 1988). Biotope-preferences, in particular, are based on correlative data, and whilst preferences were apparent in many taxa, it would be useful to test these preferences experimentally or expand the number of biotope-specific assessments taking into account the hydraulic conditions, specifically whether the biotope is in- or out- of current. Aquatic vegetation, i.e. *Isolepis* spp., in upland sites of the Western Cape, appears to provide an important habitat for aquatic organisms. The distribution of *Isolepis* in this region and information on the utilisation, including seasonal importance, of *Isolepis* by aquatic organisms would be very useful, particularly given the pressures exerted on Western Cape rivers with regards to flow regulation and water abstraction.

8.3 Temporal variability in macroinvertebrate assemblages

Lotic systems often exhibit daily, seasonal and annual periodicity, particularly in regions with highly seasonal climates such as South Africa. Seasonal variation in factors such as steam hydrology (e.g. McEravy et al. 1989), temperature (e.g. Hawkins et al. 1997) and biotope availability (e.g. Armitage & Pardo 1995) may lead to variation in the distribution and abundance of macroinvertebrates. Seasonal patterns in the distribution and abundance of macroinvertebrates reflect life history characteristics of individual taxa, and temporal differences in taxonomic makeup of macroinvertebrate assemblages within streams may be due to the differences among insect life cycles. Understanding the extent of these intrinsic seasonal differences is important so that an observed effect reflects a real change in, for example, water quality, rather than a seasonal pattern (e.g. Linke *et al.* 1999). The extent to which macroinvertebrate assemblages varied seasonally (Chapter 5) was investigated by examining seasonal differences in individual taxa, macroinvertebrate assemblages and SASS Scores. Generally, seasonal differences were less pronounced than biotope-related differences. A few individual taxa were more common in one or other season in the Western Cape and macroinvertebrate assemblages grouped by season, when assessments conducted in autumn and spring were considered. This was particularly apparent when

taxa associated with the SIC/SOOC biotope-group were examined. The lack of seasonal differences in Mpumalanga may be linked to the low-flow period coinciding with low temperatures, but insufficient information on aquatic insect life histories limits elucidation of the potential causes of this observation.

SASS Scores, specifically the number of taxa and ASPT, were significantly different among seasons in the Western Cape, with fewer taxa recorded in winter compared to summer and significantly higher ASPT values recorded in winter and spring in comparison to summer and autumn. Whilst more taxa were recorded in autumn than in spring, a higher proportion of sensitive and high-scoring taxa, including Teloganodidae, Heptageniidae, Helodidae, Blephariceridae and Amphipoda, were recorded in spring. Significant differences in SASS Scores were not apparent in Mpumalanga, with most taxa recorded in winter and ASPT slightly higher in winter versus spring. Examination of variation in SASS Scores at individual reference sites showed that, whilst macroinvertebrate assemblages were somewhat dissimilar between sampling occasions, SASS Scores, in particular ASPT, remained relatively stable over time.

In terms of defining reference conditions cognizance should be taken of the sampling season, particularly in regions that exhibit a relatively high degree of seasonal variability such as the Western Cape. Reference conditions need to take seasonal difference into account, particularly in that seasonal absences of certain taxa may affect the bioassessment results. Reference site classification based on seasonally-composite data for Mpumalanga, i.e. where data from three seasons (autumn, winter and spring) are combined (Chapter 3), seemed to produce a more robust classification than classification based on data from a single season (see also Turak *et al*. 1999), and provided a means of taking seasonal variability into account. Initial classification of reference sites based on composite data is therefore advisable.

In South Africa knowledge of the life histories of aquatic organisms is severely limited. Such information would provide valuable insight into observed seasonal variability and enable greater understanding of temporal heterogeneity in lotic systems. A need exists for long-term data to improve our understanding of natural variability in streams and to provide a baseline against which the effects of disturbance can be judged. Long-term data and variability estimates are considered essential for determining recovery rates as well as

disturbance effects in streams (Niemi *et al.* 1993). In South Africa we have some way to go in understanding the structure and functioning of our riverine ecosystems and a dearth of information exists on the life histories of aquatic organisms. Since this is integral to understanding seasonal trends in macroinvertebrate abundances, and to a degree the extent of biotope specificity, as well understanding and predicting the response of organisms to variation and change within and between lotic ecosystems (Power *et al.* 1988), further studies focusing on this aspect would be very useful. This type of information will enhance our understanding of the biota and on the processes acting on the biota.

8.4 Environmental variables

Thus, having established the existence of substantial spatial variability in macroinvertebrate assemblages at the regional, subregional and habitat levels, attention was focused on identifying the environmental variables contributing to the observed variability. It is widely recognised that river systems reflect the characteristics of the catchment (e.g. Hynes 1975), site (e.g. Reynoldson *et al.* 1997), instream habitat (e.g. Marchant *et al.* 1997) and water chemistry (e.g. Tate & Heiny 1995), and that many variables within each of these components interact with one another and with biotic components of aquatic systems to create spatially complex biotic assemblages. The goal of Chapter 6 was, therefore, to investigate the relationship between environmental variables and macroinvertebrate assemblages, with the aim of identifying the relative importance of variables at different scales in discriminating between identified groups of sites with similar macroinvertebrate assemblages in Mpumalanga. Environmental variables at all scales were identified as potential predictor variables. Of importance were the catchment-level variables altitude and longitude, providing support for the observed distinction between upland and lowland sites (Chapter 3). Temperature, a correlate of altitude, was also important, as was the depth of the shallow-water habitat (e.g. cobble riffle, bedrock rapid). Separate SASS biotope-group classifications showed much variation with respect to Group membership, although there was some agreement between classifications. The classification strength was greatest in the "composite with sub-groups" classification followed by the "composite classification", although when predictor variables were identified for each separate biotope-group classification, the composite and SIC/SOOC biotope-group classification had the lowest error rate, i.e. misclassification of sites. Biotope-group predictor variables varied to some degree with aspects such as geological-

type, canopy cover and the percentage of mud identified as important in the SIC/SOOC classification, in comparison to the depth of the deep-water habitat and the percentage of gravel/sand and mud in the AQV/MV classification. This finding provides some insight into the potential biotope-specific effects of different disturbances, with removal of riparian vegetation and siltation, for example, having a greater effect on shallow riffles than in, for example, pools. Neither longitude or altitude were important in the GSM classification suggesting that, on the basis of macroinvertebrate assemblages associated with this biotope-group, differentiation into upland and lowland areas was not evident.

From the perspective of classifying reference sites, this knowledge is useful in that it again confirms the utility of a spatial framework within which reference sites are selected and bioassessment is undertaken. The importance of considering additional factors such as substratum that influence macroinvertebrate assemblages, is highlighted by the number of river type variables, at the scale of site and habitat, that were identified as important discriminators of macroinvertebrate assemblages in both the composite classification and biotope-specific classifications.

8.5 Variability in macroinvertebrate assemblages within a region

The final chapter (Chapter 7) draws together aspects from all preceding ones, by examining spatial and temporal variability of macroinvertebrate assemblages within the most spatially and temporally variable group of sites identified in Chapter 3, namely upland sites of the Fynbos bioregion of the Western Cape. The degree of dissimilarity was a minimum of 47%, even when differences in the availability of biotopes, i.e. separating sites with- and without-vegetation, were included. Results confirmed that differences between sites in the two subregions, namely mountain streams and foothill-cobble beds, were not significant, although upland sites did form distinct Groups, particularly when mountain stream sites were considered in isolation. Each Group had a suite of SASS-taxa that distinguished it from other Groups. These taxa included some of those that characterised this subregion (Chapter 3) such as Heptageniidae, Corydalidae, Helodidae, Hydraenidae, Limnichidae, Ecnomidae, Philopotamidae, Trichoptera (cased caddis 2 Types), Athericidae and Blephariceridae.

Environmental variables identified as contributing to this observed grouping included factors such as distance from source, cation ratio, pH and longitude. These results show that even within a regionally-distinct group of reference sites, variability is such that separate groups are evident. The concept of "catchment signatures" (King & Schael 2001) whereby sites within a catchment are more similar to one another than to sites from other catchments, warrants further examination, especially since these findings have significant implications for river management. In particular, the extent to which it is possible to extrapolate from one upland site to another deserves investigation.

Of importance from a bioassessment perspective, SASS Scores calculated for these upland sites were less variable than the macroinvertebrate assemblages and did not preclude the detection of disturbance at monitoring sites. Biological bands derived for data interpretation that utilised the relationship between ASPT and SASS4 Score provided a means whereby variability resulting from differences in the availability of biotopes and seasonal differences could be taken into account. Examination of the relative frequency of occurrence of taxa within each biological band revealed three difference trends in response to increased disturbance. One group of taxa, many of which were high-scoring, sensitive taxa characteristic of minimally-disturbed upland sites, and many of which showed a preference for the stones-in-current biotope, decreased as disturbance increased. A second group of taxa, including several tolerant and low-scoring taxa such as Muscidae and Oligochaetes, increased in response to disturbance. These taxa are known for their tolerance to pollution, particularly organic pollution (e.g. Hynes 1960). A third group of taxa remained relatively unaffected by increased disturbance and included several hemipterans, dragonflies and damselflies. Many hemipterans are air-breathers and thus not that dependent on water as a medium. These taxa, particularly hemipterans and damselflies, are also largely associated with marginal vegetation and their presence at or absence from a site may more be a reflection of the presence or absence of marginal vegetation rather than of water quality. Marginal vegetation, whilst limited in upland sites of the Western Cape, is more readily available in Mpumalanga and in the lower reaches of the Western Cape and its importance as a habitat needs to be recognised, particularly since this habitat is often affected by modification to instream flows.

It would be useful to support these findings with experimental evidence, particularly with respect to observed spatial and temporal differences in the distribution of taxa at upland

sites of the Western Cape. An aspect not dealt with in this report but which has implications for the sensitivity of the bioassessment tool, SASS, relates to the incorporation of an abundance estimate in the biotic index. In the current SASS system, results are based on the presence or absence of each taxon. This precludes the elucidation of effects that do not lead to a loss or gain of species, but rather to a change in their relative abundances. Incorporating a rank abundance estimate may improve the sensitivity of the index and may also amplify seasonal differences, although this will need to be tested.

In conclusion, this study has shown that spatial and temporal heterogeneity are features of South African river systems. For effective management of these lotic systems it seems clear that intrinsic spatial and temporal heterogeneity and variability need to be understood and incorporated within the context of bioassessment. On the basis of the results of this study, it is possible to partition spatial variability such that defining reference conditions based on several similar reference sites is feasible. Adopting a regional framework, within which reference sites are selected and reference conditions defined, facilitates initial partitioning of variability resulting from differences at the regional and subregional levels.

Further spatial partitioning is necessary at the habitat level, specifically separation of SASS-biotopes during the bioassessment and analysis phase. In this way, differences in the availability of SASS-biotopes between reference and monitoring sites may be taken into account, and subsequent results will thus reflect conditions other than those resulting from habitat differences. Of significance is the variation observed in macroinvertebrate assemblages within SASS-biotope groups, which respond to differences in the hydraulic condition, specifically in response to whether the biotope is in- or out-of-current. Further consideration needs to be given to these differences and the possibility of limiting bioassessment to fewer, more specific biotope types, which have comparable hydraulic characteristics.

Temporal variability, whilst not as obvious as biotope differences, needs to be considered when defining reference conditions, with certain taxa more common in one or other season. The importance of seasonal differences was shown to vary between geographic regions, possibly in response to the harsher environment to which aquatic organisms are subjected, with greater stress prevalent in the Western Cape. Temporal variability did not, however, curtail the detection of disturbance at monitoring sites.

Notwithstanding the spatial and temporal variability, and the identification of environmental variables at all scales acting on and influencing macroinvertebrate distributions, it is possible to define a reference condition for macroinvertebrates. This study has shown that a reference condition comprised of biocriteria in the form of SASS scores and expected SASS-taxa allows the identification of disturbed sites. Development of biocriteria is an important process in the effective protection of aquatic ecosystems and the confidence with which a judgement of biological condition is made depends on the soundness and scientific validity of the bioassessment tool (e.g. the biotic index) and the reference condition defined.

The results of this study have contributed to our understanding of lotic systems in South Africa. It provides information of spatial and temporal variability in these systems and on the ability to define reference conditions, in spite of this variability. There is however, a clear need to expand the geographical range of reference sites and to initiate a long-term programme aimed specifically at defining reference conditions. Experience elsewhere (e.g. Wright 1995, Schofield & Davies 1996) demonstrates the importance of national co-operation and the participation of multiple departments and organisations in the water sector. The development of predictive models in the United Kingdom (e.g. Wright 1995) and Australia (e.g. Smith *et al.* 1999) has led to significant advances in the bioassessment field and thus the development of a prediction-based modelling system, similar to that of AusRivAs or RIVPACS, is strongly recommended for South Africa. The spatial and temporal complexity of macroinvertebrate assemblages and the uncertainty related to the measurement of them, make deriving sound reference conditions, in the absence of modelling, difficult, albeit possible. By ensuring that all biomonitoring practitioners adhere to the standard sampling protocol, which includes the collection of a subset of environmental variables and separate biotope-group sampling, we will be ensured of an extensive and useful dataset in the future. The vehicle for data storage has already been developed (Rivers Database: Fowler, Dallas *et al.* 2000). With national co-operation, it should, in the long term, be possible to develop a series of models based on River Health Programme data. These models will automate the allocation of a monitoring site to its appropriate group of reference sites, calculate the expected probabilities of each taxon occurring at the monitoring site, calculate the Observed/Expected ratios and thereby generate information on the extent to which the monitoring site has deviated from the

expected reference condition. This will greatly simplify data interpretation and reporting on the river health of lotic systems in South Africa.

The challenge for the future lies in protecting the ecological integrity and biodiversity of aquatic systems in the face of increasing pressures on our freshwater resources (Ward 1998).

CHAPTER 9. MANAGEMENT IMPLICATIONS AND RECOMMENDATIONS

This chapter is a "bulleted" summary of the preceding one in which recommendations are expanded on and general implications with respect to water resource management discussed. Generally, on the basis of the results of this study, it is possible to partition spatial variability such that defining reference conditions based on several similar reference sites is feasible.

9.1 Regional and subregional classification

- In general, *a priori* regional classification of sites, using the hierarchical spatial framework developed in South Africa, provided a useful framework for preliminary classification of reference sites.
- Within geographical regions, longitudinal zonation into upland and lowland areas was important, with sites grouping on the basis of broad geomorphological zones or subregions. Of the upland sites, differentiation into mountain streams and foothill-cobble beds was not apparent, although overall variability of assemblages within upland areas, in particular the Western Cape, was very high.
- Additional factors acting at a lower scale such as site or habitat influenced macroinvertebrate assemblages.

From a management perspective, the spatial framework provides a useful tool for initial grouping or separation of sites, and provides a starting point for the selection of reference and monitoring sites. It is, however, clear that additional factors, at the level of site and/or habitat, influence the macroinvertebrate assemblages recorded at a site. It is important for these site- and habitat-variables to be identified.

9.2 SASS-biotopes

- Spatial variability at the level of habitat, specifically SASS-biotopes, revealed that several taxa exhibited a degree of biotope specificity, with some taxa recorded more frequently in one biotope rather than another.

- The relative importance of a biotope as a habitat for macroinvertebrates, as a reflection of both its availability and its utilisation by aquatic organisms, varied regionally.
- The importance of hydraulic condition coupled with substrate type became apparent with differences in taxa observed within a biotope-group, i.e. stones-in-current versus stones-out-of-current.
- Seasonal differences in the distinctiveness of biotopes were observed in the Western Cape, with distinctiveness more pronounced in autumn, under low-flow conditions, in comparison with less pronounced biotope specificity in spring.
- In terms of SASS Scores, stones-in-current/stones-out-of-current (SIC/SOOC) was shown to be the most important SASS biotope-group and taxa associated with it contributed the highest percentage to SASS Scores calculated at the site level. SIC/SOOC was also the most consistent in terms of its associated macroinvertebrate assemblage.
- There was a significant positive relationship between SASS4 Score and number of taxa with number of SASS-biotopes sampled and a negative correlation between ASPT and number of SASS-biotopes sampled.

The importance of sampling SASS-biotopes separately is clearly demonstrated. This enables SASS data to be interpreted on a "per SASS-biotope" basis in instances where one or other SASS-biotope is absent from a monitoring or reference site. By sampling SASS-biotopes separately, differences in the availability of SASS-biotopes between reference and monitoring sites may be taken into account, and subsequent results will thus reflect conditions other than those resulting from habitat differences. Flow conditions and season are important additional factors that need to be taken into consideration when doing SASS, defining reference conditions and interpreting SASS data.

9.3 Temporal variability

- Generally, seasonal differences were less pronounced than biotope-related differences and were more prevalent in the Western Cape compared to Mpumalanga.
- SASS Scores, specifically the number of taxa and ASPT, were significantly different among seasons in the Western Cape, with fewer taxa recorded in winter compared to summer and significantly higher ASPT values recorded in winter and spring in

comparison to summer and autumn. Whilst more taxa were recorded in autumn than in spring, a higher proportion of sensitive and high-scoring taxa were recorded in spring.

- Temporal variability did not, however, curtail the detection of disturbance at monitoring sites.

In terms of defining reference conditions cognizance should be taken of the sampling season, particularly in regions that exhibit a relatively high degree of seasonal variability such as the Western Cape. When identifying expected or reference taxa for a seasonally variable region, details pertaining to the seasonal trends in individual taxa should be provided, since seasonal absences of certain taxa may affect the bioassessment results. Initial classification of reference sites based on seasonally-composite data provides a more robust classification of reference sites and is to be recommended.

9.4 Environmental variables

- Environmental variables at all scales were identified as potential predictor variables and were thus considered important in grouping sites with similar macroinvertebrate assemblages.
- Catchment-level variables included altitude and longitude, lending support to the observed distinction in macroinvertebrate assemblages between upland and lowland sites.
- Temperature, a correlate of altitude, was important, as was the depth of the shallow-water habitat (e.g. cobble riffle, bedrock rapid).
- Biotope-group predictor variables varied to some degree with aspects such as geological-type, canopy cover and the percentage of mud identified as important in the stony-habitat classification, in comparison to the depth of the deep-water habitat and the percentage of gravel/sand and mud in the vegetation classification.

The utility of a spatial framework within which reference sites are selected and bioassessment is undertaken is confirmed by these results. The importance of additional factors such as substratum that influence macroinvertebrate assemblages, is highlighted by the number of river type variables, at the scale of site and habitat, that were identified as important discriminators of macroinvertebrate assemblages in both the composite

classification and biotope-specific classifications. The importance of identifying these factors is again highlighted.

9.5 Variability in macroinvertebrate assemblages within a region

- Whilst macroinvertebrate assemblages were variable among sites within a region, SASS Scores calculated for these upland sites were less variable and did not preclude the detection of disturbance at monitoring sites.
- Biological bands derived for data interpretation that utilised the relationship between ASPT and SASS4 Score provided a means whereby variability resulting from differences in the availability of biotopes and seasonal differences could be taken into account.
- This study has shown that a reference condition comprised of biocriteria in the form of SASS scores and expected SASS-taxa facilitates the identification of disturbed sites.

Development of biocriteria is an important process in the effective protection of aquatic ecosystems and the confidence with which a judgement of biological condition is made depends on the soundness and scientific validity of the bioassessment tool (e.g. the biotic index) and the reference condition defined.

9.6 The protocol for deriving reference conditions

The protocol developed in Dallas (2000b) formed a sound basis for data analyses when applied to another region, i.e. the Western Cape. Each of the steps described in the protocol are important when reference conditions are established. Of significance are the regional differences in the relative importance of biotopes, biotope preferences of individual taxa, and biotope and seasonal differences in macroinvertebrate assemblages. In the Western Cape, data limitations prevented the calculation of ratios. Instead absolute values were used and biological bands were derived based on the relationship of ASPT to SASS4 Score. This proved to be a useful means for data interpretation and subsequent detection of disturbance at a monitoring site.

9.7 Recommendations

- Further testing of the utility of regional classifications would be useful since the limited data for the Western Cape prevented rigorous testing of regional classifications. It would be advantageous to repeat the analyses once additional reference-site data have been collected.
- Biotope-preferences, in particular, are based on correlative data, and whilst preferences were apparent in many taxa, it would be useful to test these preferences experimentally or expand the number of biotope-specific assessments taking into account the hydraulic conditions, specifically whether the biotope is in- or out- of current. Further consideration needs to be given to these differences and the possibility of limiting bioassessment to fewer, more specific biotope types, which have comparable hydraulic characteristics.
- Aquatic vegetation, i.e. *Isolepis* spp., in upland sites of the Western Cape, appears to provide an important habitat for aquatic organisms. The distribution of *Isolepis* in this region and information on the utilisation, including seasonal importance, of *Isolepis* by aquatic organisms would be very useful, particularly given the pressures exerted on Western Cape rivers with regards to flow regulation and water abstraction.
- In South Africa knowledge of the life histories of aquatic organisms is severely limited. Such information would provide valuable insight into observed seasonal variability and enable greater understanding of temporal heterogeneity in lotic systems.
- There is a clear need to expand the geographical range of reference sites and to initiate a long-term programme aimed specifically at defining reference conditions. Experience elsewhere demonstrates the importance of national co-operation and the participation of multiple departments and organisations in the water sector.
- Regional experts, who are familiar with the region, provide an excellent starting point for identification of river types and potential reference sites.
- The development of predictive models as in the United Kingdom and Australia is strongly recommended for South Africa.

- By ensuring that all biomonitoring practitioners adhere to the standard sampling protocol, which includes the collection of a subset of environmental variables and separate biotope-group sampling, we will be ensured of an extensive and useful dataset in the future.

REFERENCES. LITERATURE CITED IN ALL CHAPTERS

ALLAN, J.D., ERICKSON, D.L. & FAY J. (1997). The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology* **37**: 149-161.

ALLANSON B.R., HART R.C., O'KEEFFE J.H. & ROBARTS R.D. (1990). *Inland waters of Southern Africa: An ecological perspective. Monographiae Biologicae* 64. Kluwer Academic Publishers, Dortrecht, 458pp.

ANONYMOUS (2001). *State of the Rivers Report: Crocodile, Sabie-Sand and Olifants River Systems.* Water Research Commission Report No. TT 147/01, Pretoria, South Africa.

ARMITAGE P.D. , MOSS D., WRIGHT J.F. & FURSE M.T. (1983). The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Water Research* **17:** 333-347.

ARMITAGE, P.D. & PARDO I. (1995). Impact assessment of regulation at the reach level using macroinvertebrate information from mesohabitats. *Regulated Rivers: Research & Management* **10**: 147-158.

ARMITAGE, P.D., PARDO I. & BROWN A. (1995). Temporal constancy of faunal assemblages in 'mesohabitats' - application to management? *Archiv für Hydrobiologie* **133** (3): 367-387.

BAILEY R.C., KENNEDY M.G., DERVISH M.Z. & TAYLOR R.M. (1998). Biological assessment of freshwater ecosystems using a reference conditions approach: comparing predicted and actual benthic invertebrate communities in Yukon streams. *Freshwater Biology* **39**: 765-774.

BARBOUR M.T. & GERRITSEN J. (1996). Sub-sampling of benthic samples: a defense of the fixed-count method. *Journal of the North American Benthological Society* **15** (3): 386-391.

BARBOUR M.T., GERRITSEN J. & GRIFFITH G.E. (1996). A framework for biological criteria for Florida streams using benthic macroinvertebrates. *Journal of the North American Benthological Society* **15** (2): 185-211.

BARBOUR M.T., GERRITSEN J., SNYDER B.D. & STRIBLING J.B. (1999). *Rapid Bioassessment Protocols for Use in streams and wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish*, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C., U.S.A

BARBOUR M.T., STRIBLING J.B. & KARR J.R. (1995). Multimetric approach for establishing biocriteria and measuring biological condition. In: *Biological assessment and criteria. Tools for water resource planning and decision making* (Eds. Davies W. S. & Simon T. P), Lewis Publishers, Florida, U.S.A

BEISAL J.N., USSEGLIO-POLATERA P., THOMAS S. & MORETEAU J.S. (1998). Stream community structure in relation to spatial variation: the influence of mesohabitat characteristics. *Hydrobiologia* **38**: 73-88.

BOURNARD M., CELLOT B., RICHOUX P. & BERRAHOU A. (1996). Macroinvertebrate community structure and environmental characteristics along a large river: congruity of patterns for identification to species or family. *Journal of the North American Benthological Society* **15** (2): 232-253.

BREWIN P.A., NEWMAN T.M.L & ORMEROD S.J. (1995). Patterns of macroinvertebrate distribution in relation to altitude, habitat structure and land use in streams of the Nepalese Himalaya. *Archiv für Hydrobiologie* **135** (1): 79-100.

BRITTON D.L. (1991). The benthic macroinvertebrate fauna of a South African mountain stream and its response to fire. *South African Journal of Aquatic Science* **17** (1/2): 51-64.

BROWN C.A. (1997). *Modelling and managing the effects of trout farms on Cape rivers*. Unpublished Doctoral thesis, Zoology Department, University of Cape Town, South Africa. 175pp.

BROWN C.A., EEKHOUT S. & KING J.M. (1996). *National Biomonitoring programme for riverine ecosystems: technical considerations and protocol for the selection of reference and monitoring sites*. National Biomonitoring Programme for Riverine Ecosystems: Report Series No 3. Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria, South Africa.

BRYCE S.A. & CLARKE S.E. (1996). Landscape-level ecological regions: linking state-level ecoregion frameworks with stream habitat classifications. *Environmental Management* **20** (3): 297-311.

BUNN S.E. (1986a). Spatial and temporal variation in the macroinvertebrate fauna of streams of the northern jarrah forest, Western Australia: community structure. *Freshwater Biology* **16**: 67-91.

BUNN S.E. (1986b). Spatial and temporal variation in the macroinvertebrate fauna of streams of the northern jarrah forest, Western Australia: functional organisation. *Freshwater Biology* **16**: 621-632.

BUNN S.E., EDWARD D.H. & LONERAGAN N.R. (1988). Processing of leaf litter in a northern jarrah forest, Western Australia. I. Seasonal differences. *Freshwater Biology* **162**: 201-632.

CAIRNS J., MCCORMICK P.V. & NIEDERLEHNER B.R. (1993). A proposed framework for developing indicators of ecosystem health. *Hydrobiologia* **263**: 1-44.

CAO Y., BARK A.W. & WILLIAMS W.P. (1997). Analysing benthic macroinvertebrate community changes along a pollution gradient: a framework for the development of biotic indices. *Water Research* **31** (4): 884-892.

CARLISLE D.M. & CLEMENTS W.H. (1999). Sensitivity and variability of metrics used in biological assessments of running waters. *Environmental Toxicology & Chemistry* **18** (2): 285-291.

CARTER J.L., FEND S.V. & KENNELY S.S. (1996). The relationship among three habitat scales and stream benthic invertebrate community structure. *Freshwater Biology* **35** (1): 109-124.

CHESSMAN B.C. (1994). The use of macroinvertebrates for the rapid biological assessment of streams in the Sydney Region, New South Wales, Australia. In: *Classification of river, and environmental health indicators. Proceedings of a Joint South African/Australian workshop*. (Ed. Uys, M.C.). February 7-14 1994, Cape Town, South Africa. Water Research Commission Report No. TT 63/94. Water Research Commission, Pretoria, South Africa.

CHESSMAN B.C. (1995). Rapid assessment of rivers using macroinvertebrates: a procedure based on habitat-specific sampling, family level identification and a biotic index. *Australian Journal of Ecology* **20**: 122-129.

CHESSMAN B.C., GROWNS J. E. & KOTLASH A. R. (1997). Objective derivation of macroinvertebrate family sensitivity grades numbers for SIGNAL biotic index: application to the Hunter River System, New South Wales. *Marine and Freshwater Research* **48**: 159-172.

CHUTTER F.M. (1970). Hydrobiological studies in the catchment of the Vaal Dam, South Africa. Part I. River zonation and the benthic fauna. *Internationale Revue der Gesamten Hydrobiologie* **55**: 445-496.

CHUTTER F.M. (1972). An empirical biotic index of the quality of water in South African streams and rivers. *Water Research* **6**: 19-30.

CHUTTER F.M. (1998). *Research on the rapid biological assessment of water quality impacts in streams and rivers*. Water Research Commission Report No 422/1/98 . Water Research Commission, Pretoria, South Africa. 230pp.

CLARK K.K. & WARWICK R.M. (1994). *Changes in marine communities: an approach to statistical analysis and interpretation.* Natural Environmental Research Council, United Kingdom, 144pp.

COGERINO L., CELLOT B. & BOURNAUD M. (1995). Microhabitat diversity and associated macroinvertebrates in aquatic banks of a large European river. *Hydrobiologia* **304** (2): 103-115.

COLLIER K.J. (1995). Environmental factors affecting the taxonomic composition of aquatic macroinvertebrate communities in lowland waterways of Northland, New Zealand. *New Zealand Journal of Marine and Freshwater Research* **29** (4): 453-465.

COLLIER K.J., CHAMPION P.D. & CROKER G.F. (1999). Patch- and reach-scale dynamics of a macrophyte-invertebrate system in a New Zealand lowland stream. *Hydrobiologia* **392** (2): 89-97.

COLLIER K.J., WILCOCK R.J. & MEREDITH A.S. (1998). Influence of substrate type and physico-chemical conditions on macroinvertebrate faunas and biotic indices in some lowland Waikato, New Zealand, streams. *New Zealand Journal of Marine and Freshwater Research* **32** (1): 1-19.

COOPER S.D. (1984). The effects of trout on water strider in stream pools. *Oecologia* **63**: 376-379.

COOPER S.D., BARMUTA L., SARNELLE O., KRATZ K. & DIEHL S. (1997). Quantifying spatial heterogeneity in streams. *Journal of the North American Benthological Society* **16** (1): 174-188.

COOPER S.D., DIEHL S., KRATZ K. & SARNELLE O. (1998). Implications of scale for patterns and processes in stream ecology. *Australian Journal of Ecology* **23**: 27-40.

CROWL T.A., TOWNSEND C.R., BOUWES N. & THOMAS H. (1997). Scales and causes of patchiness in stream invertebrate assemblages: top-down predator effects. *Journal of the North American Benthological Society* **16** (1): 277- 285.

DALLAS H.F. (1995). *An evaluation of SASS (South African Scoring System) as a tool for the rapid bioassessment of water quality.* Unpublished MSc. thesis, Zoology Department, University of Cape Town, South Africa.

DALLAS H.F. (1997). A preliminary evaluation of aspects of SASS (South African Scoring System) for the rapid bioassessment of water quality in rivers, with particular reference to the incorporation of SASS in a national biomonitoring programme. *Southern African Journal of Aquatic Sciences* **23** (1): 79-94.

DALLAS H.F. (2000a). *Ecological Reference condition project: Field-manual. General Information, catchment condition, invertebrates and water chemistry*. National Biomonitoring Programme for Riverine Ecosystems: Report Series No 10. Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria, South Africa.

DALLAS H.F. (2000b). *The derivation of ecological reference conditions for riverine macroinvertebrates*. National Biomonitoring Programme for Riverine Ecosystems: Report Series No 12. Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria, South Africa.

DALLAS H.F. & DAY J.A. (1993). *The effect of water quality variables on riverine ecosystems: a review.* Water Research Commission Technical Report TT 61/93, Pretoria, South Africa. 240pp.

DALLAS H.F., DAY J.A., MUSIBONO D.E. & DAY E.G. (1998). *Water quality for aquatic ecosystems: tools for evaluating regional guidelines*. Water Research Commission Report No. 626/1/98. Water Research Commission, Pretoria, South Africa. 240pp.

DALLAS H.F., DAY J.A. & REYNOLDS E.G. (1995). *The effect of water quality variables on riverine biotas.* Water Research Commission Report No 351/1/94 . Water Research Commission, Pretoria, South Africa. 230pp.

DALLAS H.F. & FOWLER J. (2000). *Delineation of river types for rivers of Mpumalanga, South Africa: the establishment of a spatial framework for the selection of reference sites*. National Biomonitoring Programme for Riverine Ecosystems: Report Series No 9. Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria, South Africa.

DAVIES B.R. & DAY J.A. (1998). *Vanishing* waters. University of Cape Town Press, South Africa.

DAY J.A., DALLAS H.F. & WACKERNAGEL A. (1998). Delineation of management regions for South African rivers based on water chemistry. *Aquatic Ecosystem Health and Management* **1**: 183-197.

DAY J.A. & KING J.M. (1995). Geographical patterns, and their origins, in the dominance of major ions in South African rivers. *South African Journal of Science* **91**: 299-306.

DEPARTMENT OF WATER AFFAIRS (1986). *Management of water resources of the Republic of South Africa*. Government Printers, Pretoria, South Africa.

DICKENS C.W.S. & GRAHAM P.M. (1998). Biomonitoring for effective management of wastewater discharges and the health of the river environment. *Aquatic Ecosystem Health and Management* **1**: 199-217.

DOWNES B.J., LAKE P.S., SCHREIBER E.S.G. & GLAISTER A. (1998). Habitat structure and regulation of local species diversity in a stony, upland stream. *Ecological Monographs* **68** (2): 237-257.

EEKHOUT S. E., BROWN C. A. & KING J. M. (1996). *National Biomonitoring Programme for Riverine Ecosystems: Technical considerations and protocol for the selection of reference and monitoring sites.* National Biomonitoring Programme for Riverine Ecosystems: Report Series No 3. Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria, South Africa. 68pp.

EEKHOUT S., KING J. M. & WACKERNAGEL A. (1997). *Classification of South African rivers. Volume 1*. Department of Environment Affairs and Tourism, Pretoria, South Africa. 125pp.

FEMINELLA J.W. (2000). Correspondence between stream macroinvertebrate assemblages and 4 ecoregions of the southeastern USA. *Journal of the North American Benthological Society* **19** (3): 442-461.

FIELD J.F., CLARK K.R. & WARWICK R.M. (1982). A practical strategy for analysing multispecies distributions patterns. *Marine Ecology Progress Series* **8**: 37-52.

FORE L. S., KARR J. R. & WISSEMAN R. W. (1996). Assessing invertebrate responses to human activities: evaluating alternative approaches. *Journal North American Benthological Society* **15** (2): 212-231.

FOWLER J., H. F. DALLAS & M. P. JANSSENS (2000). *Rivers Database: A User Manual.* National Biomonitoring Programme for Riverine Ecosystems: Report Series No 11. Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria, South Africa.

FRISSELL C.A., LISS W.J., WARREN C.E. & HURLEY M.D. (1986). A hierarchical framework for stream habitat classification: viewing streams in a watershed context. *Environmental Management* **10** (2): 199-214.

FURSE M.T. (2000). The application of RIVPACS procedures in headwater streams - an extensive and important natural resource. In: *Assessing the biological quality of fresh waters: RIVPACS and other techniques* (Eds. Wright J.F., Sutcliffe D.W. & Furse M.T.), Freshwater Biological Association, United Kingdom.

FURSE M.T., MOSS D., WRIGHT J.F. & ARMITAGE P.D. (1984). The influences of seasonal and taxonomic factors on the ordination and classification of running water sites and on their prediction of macro-invertebrate communities. *Freshwater Biology* **14**: 257-280.

GERRITSEN J., BARBOUR M.T. & KING K. (2000). Apples, oranges, and ecoregions: on determining pattern in aquatic assemblages. *Journal of the North American Benthological Society* **19** (3): 487-496.

GOLTERMAN H.I., CLYMO R.S. & OHNSTAD M.A.M. (1978). *Methods for chemical and physical analysis of fresh waters.* IBP Handbook No. 8. Blackwell Scientific Publications, Oxford, United Kingdom.

GRAYNORTH E. (1979). Effects of logging on stream environments and faunas in Nelson. *New Zealand Journal of Marine and Freshwater Research* **13**: 79-109.

GROWNS J. E., CHESSMAN B. C., JACKSON J. E. & ROSS D. G. (1997). Rapid assessment of Australian rivers using macroinvertebrates: cost and efficiency of six methods of sampling. *Journal North American Benthological Society* **16** (3): 682-693.

GUEROLD F. (2000). Influence of taxonomic determination level on several community indices. *Water Research* **34** (2): 487-492.

HARDING J.S., WINTERBOURN M.J. & MCDIFFETT W.F. (1997). Stream faunas and ecoregions in South Island, New Zealand: do they correspond? *Archiv für Hydrobiologie* **140** (3): 289-307.

HARRISON A.D. (1959). *General statement on South African Hydrological Regions*. *Report No 1, Project 6.8H*. Internal Report, National Institute for Water Research, CSIR, Pretoria, South Africa.

HARRISON A.D. (1965a). River Zonation in South Africa. *Archives fur Hydrobiologia* **61** (3): 380-386.

HARRISON A.D. (1965b). Geographical distribution of riverine invertebrates in Southern Africa. *Archiv für Hydrobiologie* **61** (3): 387-394.

HARRISON A.D. 1978. Freshwater invertebrates (except molluscs). In: *Biogeography and ecology of Southern Africa*. (Ed. Werger M.J.A.). *Monographiae Biologicae* 31, W. Junk, The Hague, Netherlands.

HARRISON A.D. & AGNEW J.D. (1962). The distribution of invertebrates endemic to acid streams in the Western and Southern Cape Province. *Annals of the Cape Provincial Museums* II, CSIR Reprint No. RW 121, Pretoria, South Africa.

HARRISON S.S.C. (2000). The importance of aquatic margins to invertebrates in English chalk streams. *Archiv für Hydrobiologie* **149** (2): 213-240.

HAWKES H.A. (1975). River zonation and classification. In: *River ecology* (Eds. Whitton B.A.). Blackwell, London, United Kingdom.

HAWKES H.A. (1979). Invertebrates as indicators of water quality. In: *Biological Indicators of water quality*. (Eds. James A. & Evison L.). John Wiley & Sons, Chichester, United Kingdom.

HAWKES H.A. (1997). Origin and development of the Biological Monitoring Working Party score system. *Water Research* **32** (3): 964-968.

HAWKINS C.P., HOGUE J.N., DECKER L.M. & FEMINELLA J.W. (1997). Channel morphology, water temperature, and assemblage structure of stream insects. *Journal of the North American Benthological Society* **16** (4): 728-749.

HAWKINS C.P. & NORRIS R.H. (2000). Performance of different landscape classifications for aquatic bioassessments: introduction to the series. *Journal of the North American Benthological Society* **19** (3): 367-369.

HAWKINS C.P. & VINSON M.R. (2000). Weak correspondence between landscape classification and stream invertebrate assemblages: implications for bioassessment. *Journal of the North American Benthological Society* **19** (3): 501-517.

HELLAWELL J.M. (1986). *Biological indicators of freshwater pollution and environmental management*. Elsevier Applied Science, London, United Kingdom. 546pp.

HEWLETT R. (2000). Implications of taxonomic resolution and sample habitat for stream classification at a broad geographic scale. *Journal of the North American Benthological Society* **19** (2): 352-361.

HILSENHOFF W.L. (1988). Rapid field assessment of organic pollution with a family-level biotic index. *Journal of the North American Benthological Society* **7**: 65-68.

HUGHES R.M. (1995). Defining acceptable biological status by comparing with reference conditions. In: *Biological assessment and criteria: tools for water resource planning and decision making.* (Eds W.S. Davies & T.P. Simon), pp 31-47. Lewis Publishers, Florida, U.S.A.

HUMPHRIES P. (1996). Aquatic macrophytes, macroinvertebrate associations and water levels in a lowland Tasmanian river. *Hydrobiologia* **321** (3): 219-233.

HYNES H.B.N. (1960). *The biology of polluted waters.* Liverpool University Press, Liverpool, United Kingdom. 202pp.

HYNES H.B.N. (1975). The stream and its valley. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* **12**: 1-57.

JOHNSON R.K. (2000). Spatial congruence between ecoregions and littoral macroinvertebrate assemblages. *Journal of the North American Benthological Society* **19** (3): 475-486.

JACOBSON D., SCHULTZ R. & ENCALADA A. (1997). Structure and diversity of stream invertebrate assemblages: the influence of temperature with altitude and latitude. *Freshwater Biology* **38**: 247-261.

JOHNSON S.L. & VAUGHN C.C. (1995). A hierarchical study of macroinvertebrate recolonization of disturbed patches along a longitudinal gradient in a Prairie river. *Freshwater Biology* **34** (3): 531-540.

JOUBERT A.R. & HURLY P.R. (1994). Grouping South African rivers using flow-derived variables. In: *Classification of river, and environmental health indicators. Proceedings of a Joint South African/Australian workshop*. (Ed. Uys, M.C.). February 7-14 1994, Cape Town, South Africa. Water Research Commission Report No. TT 63/94. Water Research Commission, Pretoria, South Africa.

KARR J.R. (1981). Assessment of biotic integrity using fish communities. *Fisheries* **66**: 21-27.

KARR J.R. (1999). Defining and measuring river health. *Freshwater Biology* **41**: 221-234.

KARR J.R. & DUDLEY D.R. (1981). Ecological perspectives on water quality goals. *Environmental Management* **1**: 55-68.

KAY W.R., SMITH M.J., PINDER A.M., MCRAE J.M., DAVIS J.A. & HALSE S.A. (1999). Patterns of distribution of macroinvertebrate families in river of north-western Australia. *Freshwater Biology* **41** (2): 299-316.

KEMPER N.P. (1999). RVI: *Riparian Vegetation Index.* Water Research Commission. Report, WRC Project No. K5/850, Pretoria, South Africa.

KEMPER N.P & KLEYNHANS C.J. (1998). *Methodology for the preliminary present status of river.* Unpublished report for the Institute for Water Quality Studies, Department of Water Affairs & Forestry, Pretoria, South Africa.

KERANS B.L., KARR J.R. & AHLSTEDT S.A. (1992). Aquatic invertebrate assemblages: spatial and temporal differences among sampling protocols. *Journal North American Benthological Society* **11** (4): 377-390.

KING J.M. (1981). The distribution of invertebrate communities in a small South African river. *Hydrobiologia* **83**: 43-65.

KING J.M., DAY J.A., HURLY P.R., HENSHALL-HOWARD M-P. & DAVIES B.R. (1988). Macroinvertebrate communities and environment in a southern African mountain stream. *Canadian Journal of Fisheries and Aquatic Science* **45** (12): 2168-2181.

KING J.M., DE MOOR F.C. & CHUTTER F.M. 1992. Alternative ways of classifying rivers in Southern Africa. In: *River conservation and management.* (Eds. Boon P.J., Calow P. & Petts G.E.) John Wiley & Sons, United Kingdom.

KING J.M. & SCHAEL D.M. (In press). *Assessing the ecological relevance of a spatially-nested geomorphological hierarchy for river management.* Water Research Commission Report 754/x/x. Pretoria, South Africa.

KING J.M & THARME R.E. (1994). *Assessment of the instream flow incremental methodology, and initial development of alternative instream flow methodologies for South Africa.* Water Research Commission Report 295/1/94. Water Research Commission, Pretoria, South Africa.

KLEYNHANS C.J. (1999). The development of a fish index to assess the biological integrity of South African rivers. *Water SA* **25** (3): 265-278.

KLEYNHANS C.J., SILBERBAUER M. & KEMPER N. (1998a). *Preliminary ecoregion level 1 classification for South Africa.* Unpublished report for the Institute for Water Quality Studies, Department of Water Affairs & Forestry, Pretoria, South Africa.

KLEYNHANS C.J., SILBERBAUER M. & KEMPER N. (1998b). *Preliminary ecoregion level 2 classification for the Olifants River catchment.* Unpublished report for the Institute for Water Quality Studies, Department of Water Affairs & Forestry, Pretoria, South Africa.

LAKE P.S., SCHREIBER E.S.G., MILNE B.J. & PEARSON R.G. (1994). Species richness in streams: patterns over time, with stream size and with latitude. *Verhandlungen der Internationalen Vereinigung fűr Theoretische und Angewandte Limnologie* **25**: 1822-1826.

LAMMERT M. & ALLAN J.D. (1999). Environmental auditing: assessing biotic integrity of streams: effects of scale in measuring the influence of land use/cover and habitat structure on fish and macroinvertebrates. *Environmental Management* **23** (2): 257-270.

LINKE S., BAILEY R.C. & SCHWINDT J. (1999). Temporal variability of stream bioassessments using benthic macroinvertebrates. *Freshwater Biology* **42**: 575-584.

LOCH D.D., WEST J.L. & PERLMUTTER (1996). The effect of trout farm effluent on the taxa richness of benthic macroinvertebrates. *Aquaculture* **147**: 37-55.

LOW A.B. & REBELO A.G. (1996). *Vegetation of South Africa, Lesotho and Swaziland*. Department of Environmental Affairs & Tourism, Pretoria, South Africa.

MARCHANT R. (1988). Seasonal and longitudinal patterns in the macroinvertebrate communities of cobbles from the upper La Trobe River, Victoria, Australia. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* **23**: 1389-1393.

MARCHANT R., BARMUTA L.A. & CHESSMAN B.C. (1995). Influence of sample quantification and taxonomic resolution on the ordination of macroinvertebrate communities from running waters in Victoria, Australia. *Marine and Freshwater Research* **46** (2): 501-506.

MARCHANT R., HIRST A., NORRIS R.H., BUTCHER R., METZELING L. & TILLER D. (1997). Classification and prediction of macroinvertebrate assemblages from running waters in Victoria, Australia. *Journal North American Benthological Society* **16** (3): 664-681.

MARCHANT R., A. HIRST, R. NORRIS & L. METZELING (1999). Classification of macroinvertebrate communities across drainage basins in Victoria, Australia. consequences of sampling on a broad spatial scale for predictive modelling. *Freshwater Biology* **41**: 253-268.

MARCHANT R., WELL F. & NEWALL P. (2000). Assessment of an ecoregion approach for classifying macroinvertebrate assemblages from streams in Victoria, Australia. *Journal of the North American Benthological Society* **19** (3): 497-500.

MAXTED J.R., BARBOUR M.T., GERRITSEN J., PORETTI V., PRIMSOE N., SILVIA A., PENROSE D. & RENFROW R. (2000). Assessment framework for mid-Atlantic coastal plain streams using benthic macroinvertebrates. *Journal of the North American Benthological Society* **19** (1): 128-144.

McELRAVY E.P., LAMBERTI G.A. & RESH V.H. (1989). Year-to-year variation in the aquatic macroinvertebrate fauna of a northern California stream. *Journal of the North American Benthological Society* **8** (1): 51-63.

MEYER J.L. (1997). Stream health: incorporating the human dimension to advance stream ecology. *Journal North American Benthological Society* **16** (2): 439-447.

MINNS C.K. (1995). Approaches for assessing and managing cumulative ecosystem change, with the Bay of Quinte as a case study: an assay. *Journal of Aquatic Ecosystem Health* **4**: 1-24.

MINSHALL G.W. (1984). Aquatic insect-substratum relationships. In: *The ecology of aquatic insects*. (Eds. Resh V.H. & Rosenberg D.M.), Praeger Scientific, New York, U.S.A.

MINSHALL G.W., CUMMINS K.W., PETERSEN R.C., CUSHING C.E., BRUNS D.A. SEDELL J.R. & VANNOTE R.L. (1985). Developments in stream ecosystem theory. *Canadian Journal of Fisheries and Aquatic Sciences* **42**: 1045-1055.

MONAGHAN K.A., PECK M.R., BREWIN P.A., MASIERO M., ZARATE E., TURCOTTE P. & OMEROD S.J. (2000). Macroinvertebrate distribution in Equadorian hill streams: the effects of altitude and land use. *Archiv für Hydrobiologie* **149** (3): 421-440.

MOSS D., WRIGHT J.F., FURSE M.T. & CLARKE R.T. (1999) A comparison of alternative techniques for prediction of the fauna of running-water sites in Great Britain. *Freshwater Biology* **41**: 167-181.

MOSTERT S.A. (1983). Procedures used in South Africa for the automatic photometric determination of micronutrients in seawater. *South African Journal of Marine Science* **1**: 189-198.

NEWBOLD J.D., SWEENEY B.W. & VANNOTE R.L. (1994). A model for seasonal synchrony in stream mayflies. *Journal North American Benthological Society* **13**: 3-18.

NIEMI G.J., DETENBECK N.E., PERRY J.A. (1993). Comparative analysis of variables to measure recovery rates in streams. *Environmental Toxicology & Chemistry* **12**: 1541-1547.

NOBLE R.G. & HEMENS J. (1978). *Inland water ecosystems in South Africa - A review of research needs.* South African National Programmes Report No. 34. CSIR, Pretoria, South Africa.

NORRIS R.H. & GEORGES A. (1993). Analysis and interpretation of benthic macroinvertebrate surveys. In: *Freshwater biomonitoring and benthic macroinvertebrates*. (Eds. Rosenberg D. M. & Resh V. H.). Chapman Hall, New York, U.S.A. 488pp.

OMERNIK J.M. (1987). Ecoregions of the conterminous United States. *Annals of the Association of American Geographers* **77**: 118-125.

OMERNIK J.M. & BAILEY R.G. (1997). Distinguishing between watersheds and ecoregions. *Journal of the American Water Resources Association* **33**: 935-949.

PADMORE C.L. (1998). The role of physical biotopes in determining the conservation status and flow requirements of British rivers. *Aquatic Ecosystem Health and Management* **1**: 25-35.

PALMER C.G., O'KEEFFE J.H. & PALMER A.R. (1991). Are macroinvertebrate assemblages in the Buffalo River, southern Africa, associated with particular biotopes? *Journal of the North American Benthological Society* **10** (4): 349-357.

PALMER C. PALMER A. et al (1994). Macroinvertebrate community structure and altitudinal changes in the upper reaches of a warm, temperate Southern African river. *Freshwater Biology* **32**: 337-347.

PALMER M.A., HAKENKAMP C.C. & NELSON-BAKER K. (1997). Ecological heterogeneity in streams: why variance matters. *Journal of the North American Benthological Society* **16** (1): 189-202.

PALMER M.A. & POFF N.L. (1997). Heterogeneity in streams: the influence of environmental heterogeneity on patterns and processes in streams. *Journal of the North American Benthological Society* **16** (1): 169-173.

PARDO I. & ARMITAGE P.D. (1997). Species assemblages as descriptions of mesohabitats. *Hydrobiologia* **344**: 111-128.

PARSONS M. & NORRIS R.H. (1996). The effect of habitat-specific sampling on biological assessment of water quality using a predictive model. *Freshwater Biology* **36**: 419-434.

PINDER L.C.V., LADLE M., GLEDHILL T., BASS J.A.B. & MATTHEWS A.M. (1987). Biological surveillance of water quality - 1. A comparison of macroinvertebrate surveillance methods in relation to assessment of water quality, in a chalk stream. *Archiv für Hydrobiologie* **109**: 207-226.

PLAFKIN J.L., BARBOUR M.T., PORTER K.D., GROSS S.K. & HUGHES R.M. (1989). *Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish*. U. S. Environmental Protection Agency Report No. EPA/440/4-89-001. Assessment and watershed division, Washington, DC 20460. U.S.A.

POFF N.L. & WARD J.V. (1990). Physical habitat template of lotic systems: recovery in the context of historical pattern of spatiotemporal heterogeneity. *Environmental Management* **14** (5): 629-645.

POWER M.E., STOUT R.J., CUSHING C.E., HARPER P.P., HAUER F.R., MATTHEWS W.J., MOYLE P.B., STATZNER B & DE BADEN I.R. (1988). Biotic and abiotic controls in river and stream communities. *Journal of the North American Benthological Society* **7** (4): 456-479.

PRIDMORE R.D. & ROPER D.S. (1985). Comparison of the macroinvertebrate faunas of runs and riffles in three New Zealand streams. *New Zealand Journal of Marine and Freshwater Research* **19**: 283-291.

PRIMER (1999). Version 5. Plymouth Marine Laboratory, United Kingdom.

PRINGLE C.M., NAIMAN R.J., BRETCHKO G., KARR J.R., OSWOOD M.W., WEBSTER J.R., WELCOME R.L., & WINTERBOURN M.J. (1988). Patch dynamics in lotic systems: the stream as a mosaic. *Journal of the North American Benthological Society* **7**: 503-524.

QUINN J.M., COOPER A.B., DAVIES-COLLEY R.J., RUTHERFORD J.C. & WILLIAMSON R.B. (1997). Land use effects on habitat, water quality, periphyton, and benthic invertebrates in Waikato, New Zealand, hill-country streams. *New Zealand Journal of Marine and Freshwater Research* **31** (5): 579-597.

QUINN J.M. & HICKEY C.W. (1990). Characterisation and classification of benthic invertebrate communities in 88 New Zealand rivers in relation to environmental factors. *New Zealand Journal of Marine and Freshwater Research* **24**: 387-409.

RABENI C.F. & DOISY K.E. (2000). Correspondence of stream benthic invertebrate assemblages to regional classification schemes in Missouri. *Journal of the North American Benthological Society* **19** (3): 419-428.

RAVICHANDRAN S., RAMANIBAI R. & PUNDARIKANTHAN N.V. (1996). Ecoregions for describing water quality patterns in Tamiraparani basin, South India. *Journal of Hydrology* **178**: 257-276.

RESH V. H. & JACKSON J.K (1993). Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. In: *Freshwater biomonitoring and benthic macroinvertebrates*. (Eds. Rosenberg D.M. and Resh V.H.) Chapman and Hall, New York.

RESH V.H. & ROSENBERG D.M. (1984). *The ecology of aquatic insects*. Praeger, New York, U.S.A.

REYNOLDSON T.B. & METCALFE-SMITH J.L. (1992). An overview of the assessment of aquatic ecosystem health using benthic invertebrates. *Journal of Aquatic Ecosystem Health* **14**: 295-308.

REYNOLDSON T.B., NORRIS R.H., RESH V.H., DAY K.E. & ROSENBERG D.M. (1997). The reference condition: a comparison of multimetric and multivariate approaches to assess water-quality impairment using benthic macroinvertebrates. *Journal of the North American Benthological Society* **16** (4): 833-852.

REYNOLDSON T.B. & WRIGHT J.F. (2000). The reference condition: problems and solutions. In: *Assessing the biological quality of fresh waters: RIVPACS and other techniques* (Eds. Wright J.F., Sutcliffe D.W. & Furse M.T.), Freshwater Biological Association, United Kingdom.

RICHARDS C., HARO R.J., JOHNSON L.B., HOST G.E. (1997). Catchment and reach-scale properties as indicators of macroinvertebrate species traits. *Freshwater Biology* **37**: 219-230.

ROSENBERG D.M. & RESH V.H. (1993). *Freshwater biomonitoring and benthic macroinvertebrates*. Chapman & Hall, New York, U.S.A.

ROTHROCK J.A., BARTEN P.K. & INGMAN G.L. (1998). Land use and aquatic biointegrity in the Blackfoot River watershed, Montana. *Journal of the American Water Resources Association* **34** (3): 565-581.

ROUX D.J., KEMPSTER P.L., KLEYNHANS C.J., VAN VLIET H.R. & DU PREEZ H.H. (1999). Integrating stressor and response monitoring into a resource-based water-quality assessment framework. *Environmental Management* **23** (1): 15-30.

ROWNTREE K.M. & WADESON R.A. (1999). *A hierarchical geomorphological model for the classification of selected South African rivers.* Water Research Commission Report No. 497/1/99. Water Research Commission, Pretoria, South Africa. 333pp.

ROWNTREE K.M., WADESON R.A. & O'KEEFFE J. (1998). Geomorphological zonation for ecological river typing. In: *Proceedings of the Biennial Conference of the Southern African Association of Geomorphologists,* Grahamstown (Ed. Rowntree K.M.), June 28th - July 1st, South Africa.

RUSE L.P. (1996). Multivariate techniques relating macroinvertebrate and environmental data from a river catchment. *Water Research* **30** (12): 3017-3024.

SANDIN L. & JOHNSON R.K. (2000). Ecoregions and benthic macroinvertebrate assemblages of Swedish streams. *Journal of the North American Benthological Society* **19** (3): 462-474.

SCHOFIELD N.J. & DAVIES P.E. (1996). Measuring the health of our rivers. *Water* May/June: 39-43.

SCHOLTZ C.H. & HOLM E. (1985). *Insects of Southern Africa*. University of Pretoria, Pretoria, South Africa.

SCRIMGEOUR G.J. & WICKLUM D. (1996). Aquatic ecosystem health and integrity: problems and potential solution. *Journal of the North American Benthological Society* **15** (2): 254-261.

SIMPSON J.C. &. NORRIS R.H (2000). Biological assessment of river quality: developments of AUSRIVAS models and outputs. In: *Assessing the biological quality of fresh waters: RIVPACS and other techniques* (Eds. Wright J.F., Sutcliffe D.W. & Furse M.T.), Freshwater Biological Association, United Kingdom.

SMITH M.J., KAY W.R., EDWARD D.H.D., PAPAS P J., RICHARDSON K.J.,. SIMPSON J.C, PINDER A.M., CALE D.J., HOWITZ P.H.J., DAVIS J.A., YUNG F.H., NORRIS R.H. & HALSE S.A. (1999). AusRiVAS: using macroinvertebrates to assess ecological condition of rivers in Western Australia. *Freshwater Biology* **41** (2): 269-2.

SNADDON C.D. & DAVIES B.R. (1998.) A preliminary assessment of the effects of a small inter-basin transfer of water on discharge and invertebrate community structure. *Regulated Rivers: Research and Management* **14**: 421-441.

SNADDON C.D., STEWART B.A. & DAVIES B.A. 1991. The effect of discharge on leaf retention in two headwater streams. In: Cook B.A. 1991. *The systematics, distribution and aspects of the ecology of the freshwater amphipod genus Paramelita (Crangonyctoidea: Paramelitidae).* Doctoral Thesis, Department of Zoology, University of Cape Town, South Africa. 372pp.

SOUTH AFRICAN NATIONAL WATER ACT (1998).

STARKE J.D. (1993). Performance of the macroinvertebrate community index: effects of sampling method, sample replication, water depth, current velocity and substratum on index values. *New Zealand Journal of Marine and Freshwater Research* **27**: 463-478.

STATZNER B. & HIGLER B. (1986). Stream hydraulics as a major determinant of benthic invertebrate distribution patterns. *Freshwater Biology* **16**: 127-139.

STEVENS D.M. & PICKER M.D. (1999). A revision of *Aphanicercella* Tillyard (Plecoptera: Notonemouridae) including the *A. barnardii* (Tillyard) species-complex. *African Entomology* **7**: 197-209.

STEWART B.A. & GRIFFITHS C.L. (2001). Amphipods. In: *Guides to the freshwater invertebrates of southern Africa. Volume 4, Crustaceae III* (Eds. Day J.A., Stewart B.A., De Moor I.J & Louw A.E.). Water Research Commission, Pretoria, South Africa.

SWEENEY B.W. (1984). Factors influencing life-history patterns of aquatic insects. In: *The ecology of aquatic insects* (Eds. Resh V.H & Rosenberg D.M.). Praeger Publishers, New York, U.S.A.

TATE C.M & HEINY J.S. (1995). The ordination of benthic invertebrate communities in the South Platte River Basin in relation to environmental factors. *Freshwater Biology* **33** (3): 439-454.

THARME R.E (1996). *Review of international methodologies for quantification of the instream flow requirements of rivers*. Water Law Review: Report for policy development. Commissioned by the Department of Water Affairs & Forestry. Freshwater Research Unit, University of Cape Town, South Africa. 114pp.

THORNE R.J., WILLIAMS W.P. & CAO Y. (1999). The influence of data transformations on biological monitoring studies using macroinvertebrates. *Water Research* **33** (2): 343-350.

TOWNSEND C.R. (1989). The patch dynamics concept of stream community ecology. *Journal of the North American Benthological Society* **8** (1): 36-50.

TOWNSEND C.R., DOLEDEC S. & SCARSBROOK M.R. (1997). Species traits in relation to temporal and spatial heterogeneity in streams: a test of habitat templet theory. *Freshwater Biology* **37**: 367-387.

TURAK E, FLACK L.K., NORRIS R.H., SIMPSON J. & WADDELL N. (1999). Assessment of river condition at a large spatial scale using predictive models. *Freshwater Biology* **41**: 283-298.

VAN SICKLE J. (1997). Using mean similarity dendrograms to evaluate classifications. *Journal of Agriculture, Biological and Environmental Statistics* **2** (4): 370-388.

VAN SICKLE J. & HUGHES R.M. (2000). Classification strengths of ecoregions, catchments, and geographic clusters for aquatic vertebrates in Oregon. *Journal of the North American Benthological Society* **19** (3): 370-384.

VANNOTE R.L., MINSHALL G.W., CUMMINS K.W., SEDELL J.R. & CUSHING C.E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 130-137.

VEGTER J.R . (1995). *Geology map of South Africa with simplified lithostratigraphy for geohydrological use*. Water Research Commission TT 74/95, Pretoria, South Africa.

VOSCHELL J.R., SMITH E.P., EVANS S.K. & HUDY M. (1997). Effective and scientifically sound bioassessment: opinions and collaboration from Academe. *Human and ecological risk assessment* **3** (6): 941-954.

WADESON R.A. (1999). Resource Directed Measures for Protection of Water Resources: River Ecosystems. Unpublished report for the Department of Water Affairs & Forestry, Pretoria, South Africa.

WALLACE J.B., GRUBAUGH J.W. & WHILES M.R. (1996). Biotic Indices and stream ecosystem processes: Results from an experimental study. *Ecological Applications* **6** (1): 140-151.

WALTON C. (Ed.). (1994). *The illustrated atlas of southern Africa*. Reader's Digest Association of South Africa, Cape Town, South Africa.

WARD J.V. (1998). Riverine landscapes: biodiversity patterns, disturbance regimes , and aquatic conservation. *Biological Conservation* **83** (3): 269-278.

WARD J.V., VOELZ N.J. & POFF N.L. (1994). Gradient analysis of zoobenthos community structure along a mountain stream continuum. *Verhandlungen der Internationalen Vereinigung fűr Theoretische und Angewandte Limnologie* **25**: 1462-1464.

WARREN C.E. (1971). *Biology and water pollution control*. W.B. Saunders Co., Philadelphia, U.S.A.

WELLS J. (1992). *A pre-impoundment study of the biological diversity of the benthic macro-invertebrate fauna of the Sabie-Sand river system*. Unpublished MSc. Thesis, Department of Zoology, University of Cape Town, South Africa.

WHITE P.S. & PICKETT S.T.A. (1985). Natural disturbance and patch dynamics: an introduction. In: *The ecology of natural disturbance and patch dynamics* (Eds. Pickett S.T.A & White P.S.), Academic Press, New York, U.S.A.

WILEY M.J., KOHLER S.L. & SEELBACH P.W. (1997). Reconciling landscape and local views of aquatic communities: lessons from Michigan trout streams. *Freshwater Biology* **37**: 133-148.

WISHART M.J. & DAY J.A. (In press). Endemism in the freshwater fauna of the south-western Cape, South Africa. *Verhandlungen der Internationalen Vereinigung fűr Theoretische und Angewandte Limnologie.*

WOHL D.L., WALLACE J.B. & MEYER J.L. (1995). Benthic macroinvertebrate community structure, function and production with respect to habitat type, reach and drainage basin in the southern Appalachians (USA). *Freshwater Biology* **34** (3): 447-464.

WRIGHT I.A., CHESSMAN B.C., FAIRWEATHER P.G. & BENSON L.J. (1995). Measuring impacts of sewage effluent on the macroinvertebrate community of an upland stream: the effect of different levels of taxonomic resolution and quantification. *Australian Journal of Ecology* **20**: 142-149.

WRIGHT J.F. (1995). Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. *Australian Journal of Ecology* **20**: 181-197.

WRIGHT J.F. (2000). An introduction to RIVPACS. In: *Assessing the biological quality of fresh waters: RIVPACS and other techniques* (Eds. Wright J.F., Sutcliffe D.W. & Furse M.T.), Freshwater Biological Association, United Kingdom.

WRIGHT J.F., MOSS D., ARMITAGE P.D. & FURSE M.T. (1984). A preliminary classification of running-water sites in Great Britain based on macro-invertebrate species and the prediction of community type using environmental data. *Freshwater Biology* **14**: 221-256.

WRIGHT J.F., FURSE M.T., ARMITAGE P.D. (1993). RIVPACS- A technique for evaluating the biological quality of rivers in the U.K. *Freshwater Biology* **3** (4): 15-25.

WRIGHT J.F., FURSE M.T., ARMITAGE P.D. (1994). Use of macroinvertebrate communities to detect environmental stress in running water. In: *Water Quality and Stress Indicators in Marine and Freshwater Systems: Linking levels of organisation*. (Ed. Sutcliffe D.W.). Freshwater Biological Association, United Kingdom. pp.15-34.

WRIGHT J.F., MOSS D. & FURSE M.T. (1998a). Macroinvertebrate richness at running-water sites in Great Britain: a comparison of species and family richness. *Verhandlungen der Internationalen Vereinigung fűr Theoretische und Angewandte Limnologie* **26**: 1174-1178.

WRIGHT R.G., MURRAY M.P. & MERRIL T. (1998b). Ecoregions as a level of ecological analysis. *Biological Conservation* **86**: 207-213.

YANOVIAK SP.P. & McCAFFERY W.P. (1996). Comparison of macroinvertebrate assemblages inhabiting pristine streams in the Huron Mountains of Michigan, USA. *Hydrobiologia* **330** (3): 195-211.

ZAMORA-MUNOZ C., SAINZ-CANTERO C.E., SANCHEZ-ORTEGA A. & ALBA-TERCEDOR J. (1995). Are biological indices BMWP' and ASPT' and their significance regarding water quality seasonally dependent? Factors explaining their variation. *Water Research* **29** (1): 285- 290.

Appendix A. Sampling sites in the Western Cape showing river name, sub-region and sampling dates.

Code	River	Sub-region	Sampling Dates
CM01	Assegaaibosch	M	Sep-1994, Nov-1994, Mar-1995, Jul-1995
CM02	Berg	M	Sep-1994, Nov-1994, Mar-1995
CM03	Berg	M	Sep-1994, Nov-1994
CM04	Eerste	M	Feb-1994, Mar-1994, Sep-1994, Nov-1994, Mar-1995, Apr-1995, Jul-1995, Oct-2001
CM05	Lang	M	Mar-1994, Sep-1994, Nov-1994, Mar-1995, Jul-1995, Oct-2001
CM06	Window Stream	M	Sep-94
CM07	Palmiet	M	Feb-1994, Mar-1994, Sep-1994, Nov-1994, Jul-1995
CM08	Elandspad	M	Mar-94
CM09	Elandspad	M	Nov-1994, Oct-2001
CM10	Kraalstroom	M	Feb-1994, Mar-1994
CM11	Wit	M	Nov-1995
CM12	Rooiels	M	Nov-1995
CM13	Unspecified	M	Nov-1995
CM14	Houtbaais	M	Nov-1995
CM15	Houtbaais	M	Nov-1995
CM16	Rietvlei	M	Nov-1995
CM17	Boesmans	M	Nov-1995
CM18	Baviaans	M	Nov-1995
CM19	Boesmanskloof	M	Nov-1995
CM20	Riviersonderend	M	Sep-1994, Nov-1994, Mar-1995, Jul-1995, Nov-1995
CM21	Duiwelsbos	M	Nov-1995
CM22	Hermitage	M	Nov-1995
CM23	Meulkloof	M	Nov-1995
CM24	Grootkloof	M	Nov-1995
CM25	Perdekloof	M	Mar-1995
CM26	Swartboskloof	M	Oct-2001
SC01	Duiwenshoek	C	Nov-1995
CC01	Berg	C	Feb-1994, Sep-1994, Nov-1994, Mar-1995, Jul-1995, Sep-1995, Feb-1996, May-1996, Aug-1996
CC02	Holsloot	C	Nov-1995
CC03	Molenaars	C	Feb-1994, Mar-1994
CC04	Molenaars	C	Feb-1994, Mar-1994
CC05	Molenaars	C	Nov-1995, Oct-2001
CC06	Sandriftskloof	C	Nov-1995
CC07	Dutoits	C	Nov-1995
TM01	Dwarriega	M	Nov-1995
TM02	Elandskloof	M	Nov-1995
TM03	Hartebees	M	Nov-1995
TM04	Koekoedou	M	Nov-1995
TM05	Kraalstroom	M	Feb-1994, Mar-1994
TM06	Kraalstroom	M	Feb-1994, Mar-1994
TM07	Kraalstroom	M	Feb-1994, Mar-1994
TM08	Kraalstroom	M	Feb-1994, Mar-1994

Code	River	Sub-region	Sampling Dates
TM09	Kraalstroom	M	Feb-1994, Mar-1994
TM10	Modder	M	Nov-1995
TM11	Raaswater	M	Jan-2000
TM12	Riviersonderend	M	Nov-1995
TM13	Silvermine	M	Aug-00
TM14	Silvermine	M	Sep-200
TM15	Silvermine	M	Aug-2000
TM16	Spekriviersk loof	M	Nov-1995
TM17	Vals	M	Nov-1995
TM18	Valsgat	M	Nov-1995
TC01	Berg	C	Feb-1994, Mar-1994, Sep-1994, Nov-1994, Mar-1995, Jul-1995, Oct-1995
TC02	Berg	C	Sep-1994, Nov-1994, Mar-1995, Jul-1995, Jan-1996
TC03	Berg	C	Sep-1994, Nov-1994, Jul-1995
TC04	Berg	C	Nov-1994, Jul-1995, Oct-1995, Feb-1996, May-1996, Aug-1996
TC04	Berg	C	Jul-1995
TC05	Breede	C	Nov-1995
TC06	Breede	C	Nov-1995
TC07	Breede	C	Nov-1995
TC08	Buffelsjag	C	Nov-1995
TC09	Dwars	C	Sep-1994, Nov-1994, Mar-1995, Jul-1995
TC10	Eerste	C	Apr-1995
TC11	Eerste	C	Oct-1994, Apr-1995, Oct-2001
TC12	Eerste	C	Oct-1994
TC13	Eerste	C	Oct-1994
TC14	Franschhoek	C	Sep-1994, Nov-1994, Jul-1995
TC15	Hex	C	Nov-1995
TC16	Hex	C	Nov-1995
TC17	Hoeks	C	Nov-1995
TC18	Keisers	C	Nov-1995
TC19	Kruis	C	Nov-1995
TC20	Lanzerac	C	Oct-1994
TC21	Lanzerac	C	Oct-1994
TC22	Nuy	C	Nov-1995
TC23	Wemmers	C	Mar-1994, Sep-1994, Nov-1994, Jul-1995

APPENDIX B. INCORPORATING ABUNDANCE INTO SASS

B1 Introduction

SASS4 is a qualitative index that relies on the presence or absence of SASS taxa at a site and abundance, whilst noted as a rank value (A: 1-10, B: 11-100, C: 101-1000 and D: > 1000), is not incorporated in the index. Qualitative indices ignore quantitative changes in community structure, i.e. changes in the number of individuals within a taxon, and are therefore subject to the effect of sampling errors and the presence/absence of rare species (Cao *et al.* 1997).

Most biotic indices (e.g. BMWP, SASS) do not incorporate abundance and rely on changes in taxonomic richness, which generally decreases with decreasing water quality. However, in addition to decreasing taxonomic richness, the number of individuals and biomass may increase, or decrease, in response to disturbance (Norris & Georges 1993). This is dependent on the type of disturbance and the organisms involved. Ephemeroptera, Trichoptera and Plecoptera, for example, are sensitive to most types of pollution, so the number of individuals in these orders will decrease with a decrease in water quality. The numbers of some Diptera and tubificid worms may, conversely, increase in response to pollution. These relative increases and decreases in abundance are not integrated into SASS.

Incorporating an estimate of abundance in a semi-quantitative way, such as with rank abundances, may increase the sensitivity of the index, particularly for sites that are mildly disturbed. Starke (1998) also suggests that the inclusion of abundance would reduce the likelihood of "misrepresenting" the true character of a site in cases where taxa (normally in low densities) have drifted in from upstream.

Consider a minimally impacted site (Site A) at which several sensitive taxa such as Telagonodidae (abundance = B), Ephemerellidae (abundance = B), and Helodidae (abundance = A), are recorded, together with the more tolerant Chironomidae (abundance = A, a family which includes species which span the range of sensitivities from sensitive to tolerant) and Oligochaeta (abundance = A). In comparison, a mildly disturbed site (Site

B) may have the same taxa, but different abundances (Telagonodidae = A, Ephemerellidae = A, Helodidae = A, Chironomidae = B and Oligochaeta = B). SASS Scores at these sites using the qualitative SASS version would be SASS4 Score = 43, ASPT = 8.6. If, however, a weighting system (W) was introduced whereby the rank abundance was used to weight the sensitivity/tolerance score, SASS scores at Site A would be: W-SASS4 Score = 71, W-ASPT = 10.1, compared to at Site B: W-SASS4 Score = 46, W-ASPT = 6.7. Thus, whilst the same taxa were recorded at both sites, differences in their rank abundance resulted in substantial differences in their weighted SASS scores.

At mildly disturbed sites, where sensitive taxa may be present in low abundances, a qualitative index, based solely on presence/absence data, would thus not detect a disturbance that resulted in a decrease in the abundance of sensitive taxa. Incorporating an abundance estimate, which enabled the rating of sensitive taxa to become larger as their abundance increased, would reflect the observation that more sensitive taxa were present at minimally disturbed sites, and in greater abundance, and that their abundance was lower at disturbed sites.

Internationally, there is differing support for the incorporation of abundance in biotic indices. An abundance rating was applied in the original Biological Monitoring Working Party (BMWP) system in Great Britain (Hawkes 1997), from which SASS was modified. This rating was subsequently dropped from the BMWP system for the following reasons: 1) the derivation of abundance measurements from data derived from qualitative sampling methods could not be justified scientifically, 2) sampling and sample processing would be greatly simplified, and 3) and it would make little difference to the total score.

The SQMCI (Semi-Quantitative Macroinvertebrate Community Index) used in New Zealand incorporates coded-abundance data and responds to changes in community dominance (Stark 1998). Five categories of abundance are recorded in the SQMCI method, including "rare", "common", "abundant", "very abundant", and "very very abundant". The coded abundances for each, and which are subsequently used in the calculation of SQMCI are 1, 5, 20, 100 and 500. The equation is as follows: each taxon is assigned an abundance code that is then used in the following calculation:

$$SQMCI = \sum_{i=1}^{i=s} \frac{(n_i \times a_i)}{N}$$

where, s = the total number of taxa in the sample, n = coded-abundance for taxon i, a = is the score of the ith taxon, and N = total of the coded abundances for the entire sample.

Chessman (1995), in Australia, indicated that a weighted index (SIGNAL-W) could be calculated by multiplying the taxon score of each family present by a value to represent its occurrence level (1 = rare, 2 = scarce, 3 = common and 4 = abundant), summing the products, and dividing by the sum of the occurrence values. Quinn & Hickey (1990) compared MCI (Macroinvertebrate Community Index) and the quantitative equivalent QMCI used in bioassessment in New Zealand, and found that they were strongly correlated, with MCI more strongly correlated with water enrichment parameters. This suggests that MCI, which requires less effort, is a slightly more sensitive measure of water enrichment than QMCI.

It is clear, therefore, that there are various attitudes as to the value of incorporating an abundance estimate in biotic indices. This section aims to explore the relationship between SASS scores and the detection of disturbance at a site and to compare SASS4 results when abundance is included with those when it is excluded. The method of Chessman (1995) has been used, whereby a weighted SASS4 index has been calculated, prefaced with a "W", both as W-SASS4 and W-ASPT (i.e. weighted).

B2 Method

Weighting was applied to data for ninety-nine SASS4 assessments in the Western Cape, where rank abundances were predominantly A's (1-10 individuals) or B's (11-100 individuals), with some C (101-1000 individuals), but no Ds (>1000 individuals). Weighting was also applied to 216 SASS4 assessments conducted in Mpumulanga, where rank abundances ranged from predominantly As and Bs, to Cs and Ds. A weighted SASS4 Score, i.e. W-SASS4 Score, was calculated by multiplying the sensitivity/tolerance score of each SASS taxon present by a value to represent its abundance (A = 1, B = 2, C = 3 and

D = 4). The products were summed and dividing by the sum of the abundance values to get the weighted ASPT, i.e. W-ASPT.

B3 Results

There was a significant positive linear relationship between W-SASS4 Score and SASS4 Score and between W-ASPT and ASPT in both the Western Cape (Figure 1) and Mpumalanga (Figure 2). ASPT and W-ASPT plotted as a function of SASS4 Score and W-SASS4 Score respectively, suggest that of the two metrics, SASS4 Score is altered by the abundance weighting procedure, with the upper limit increasing (Figures 3 and 4). The range and maximum values of W-SASS4 Score were greater than for SASS4 Score (Table 1), whilst the range of W-ASPT and ASPT was more similar. When SASS data were interpreted on the basis of biological table derived for upland sites of the Western Cape (See Chapter 7, Table 7.5), 50% of the samples remained in the same biological band, whilst 46% moved up a band, 2% moved up two bands and 2% of the samples moved down a band. Closer examination of SASS4 Scores and ASPT values separately, rather than in combination as per Table 7.5, showed that on the basis of SASS4 Scores alone, only 41% of the samples remained in the same band, 48% moved up a band and 11% of the samples moved up two bands. On the basis of ASPT alone 73% of samples remained in the same band and 27% moved up a band.

Table B1. Minimum and maximum values, and ranges for SASS4 Score, W-SASS4 Score, ASPT and W-ASPT for samples in the Western Cape (n = 99) and Mpumalanga (n = 216).

Region	Metric	Minimum	Maximum	Range
Western Cape	SASS4 Score	26	177	151
	W-SASS4 Score	39	240	201
Mpumalanga	SASS4 Score	37	273	236
	W-SASS4 Score	42	353	311
Western Cape	ASPT	3.6	10.4	6.9
	W-ASPT	3.5	11.1	7.5
Mpumalanga	ASPT	5.0	8.5	3.5
	W-ASPT	5.1	8.8	3.7

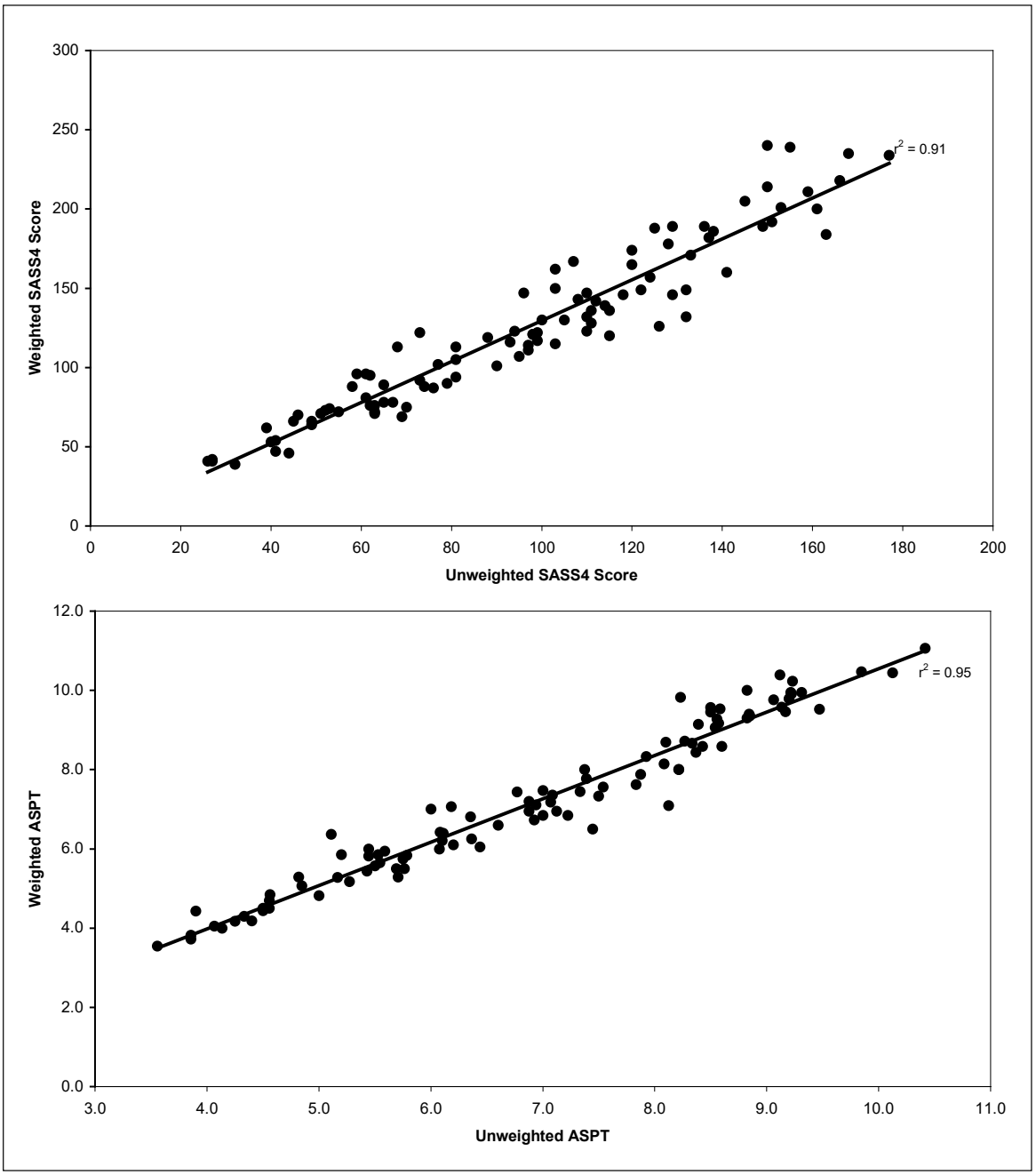


Figure B1. Linear relationship between W-SASS4 Score (weighted) and unweighted SASS4 Score, and between W-ASPT (weighted) and unweighted ASPT, based on 99 SASS4 samples in the Western Cape.

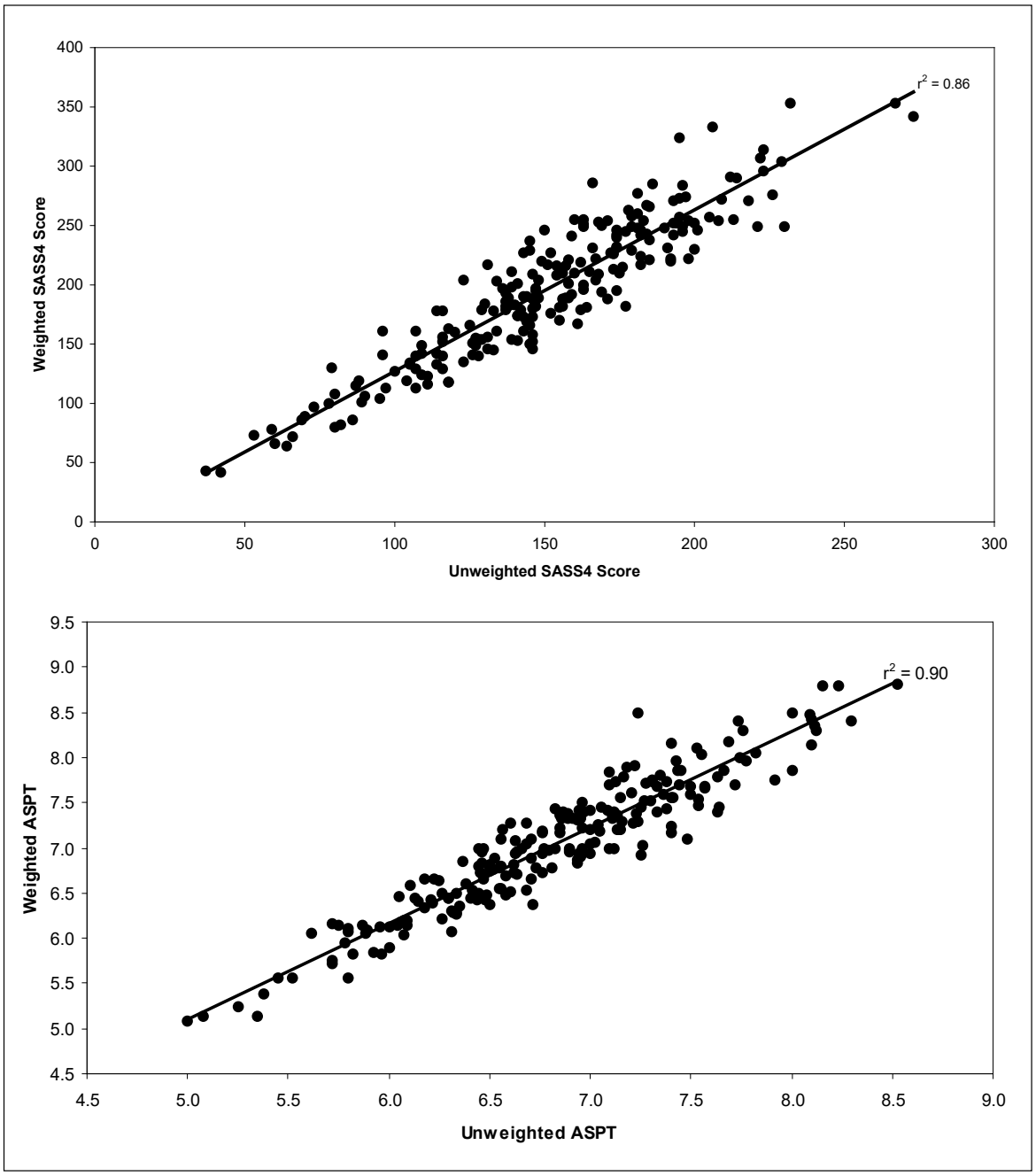


Figure B2. Linear relationship between W-SASS4 Score (weighted) and unweighted SASS4 Score, and between W-ASPT (weighted) and unweighted ASPT, based on 216 SASS4 samples in Mpumalanga.

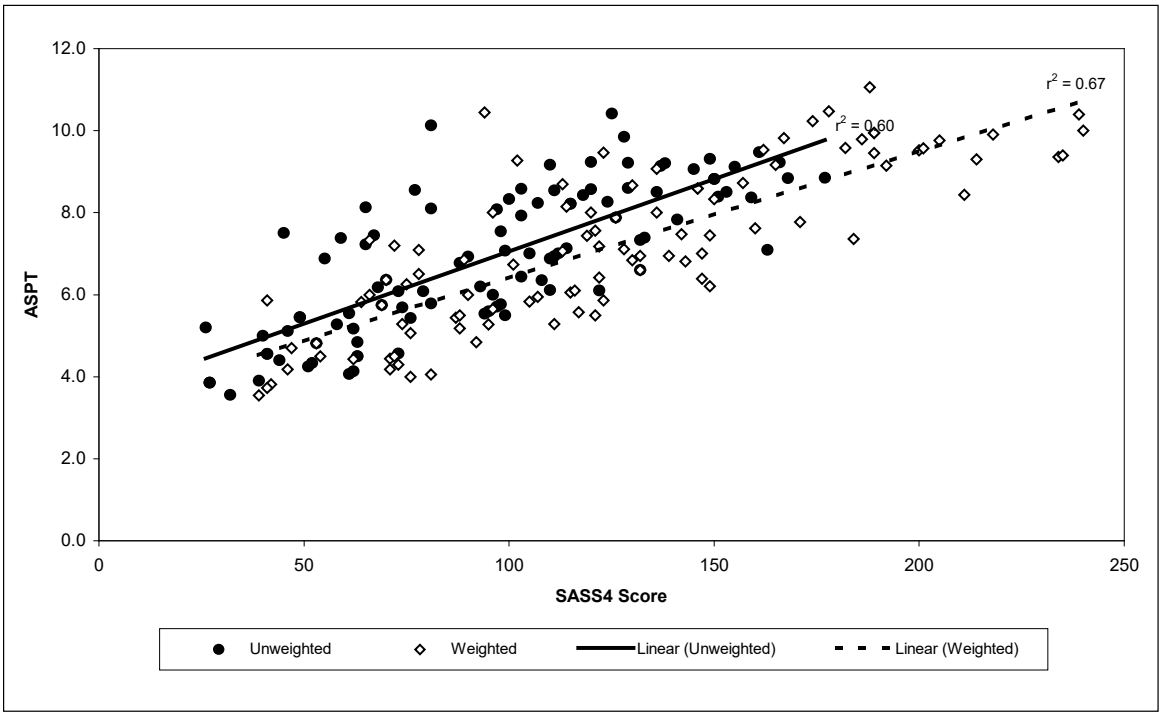


Figure B3. Linear relationship between ASPT and SASS4 Score for 99 SASS4 samples in the Western Cape. Unweighted and weighted SASS4 Scores and ASPT values are plotted separately and the r^2 values for the regression analyses are given.

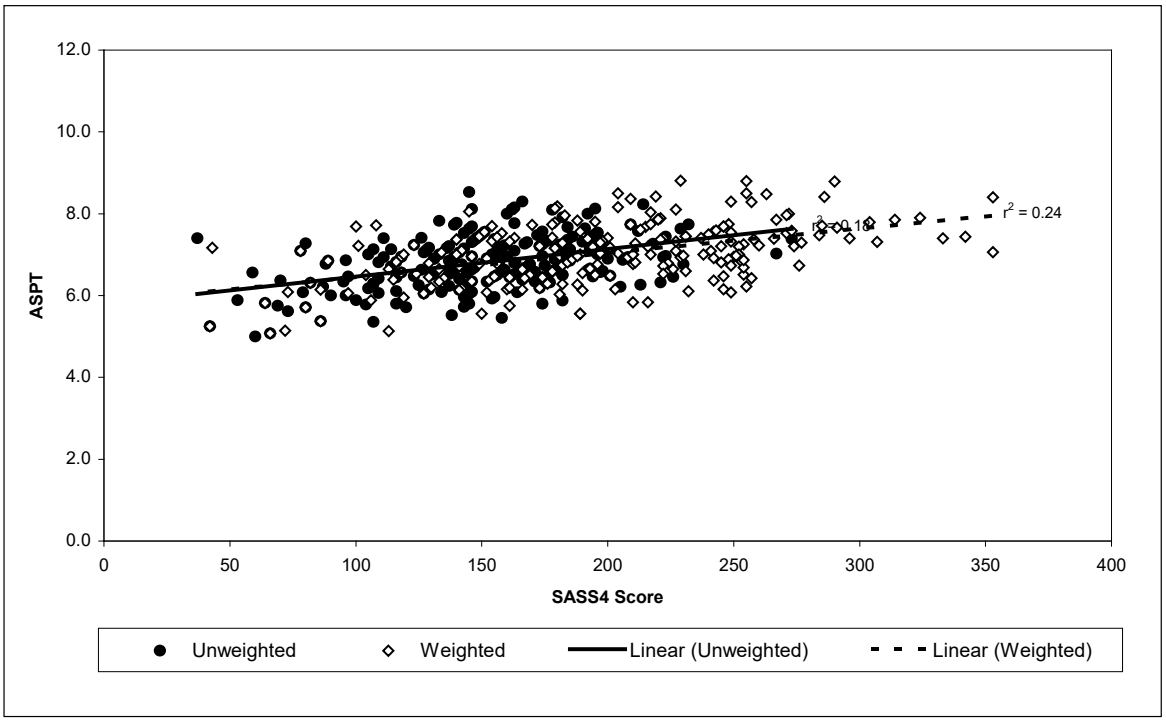


Figure B4. Linear relationship between ASPT and SASS4 Score for 216 SASS4 samples in Mpumalanga. Unweighted and weighted SASS4 Scores and ASPT values are plotted separately and the r^2 values for the regression analyses are given.

B4 Discussion

Examination of data in this study showed that there is a highly significant linear correlation between unweighted and weighted SASS Scores. This indicates that the inclusion of rank abundances did not alter the assessment of disturbance appreciably. The key difference was a broadening of the SASS4 Score range, particularly of the upper limit, suggesting that greater resolution may be attained between minimally disturbed sites and mildly disturbed sites, i.e. biological bands A and B.

Resistance from biomonitoring practitioners to the inclusion of an abundance estimate in biotic indices is often related to the additional effort required for collecting semi-quantitative or quantitative data. The incorporation of a rank abundance estimate in the calculation of SASS Scores does, however, not affect the sample collection process or duration. The most recent version of SASS, i.e. SASS 5, incorporates an estimate of abundance as follows: 1: 1 individual, A: 2 to 10 individuals, B: 11 to 100 individuals, C: 101 to 1000 individuals and D: > 1000 individuals. By including "singletons", i.e. taxa where only one individual is recorded, rare or potential "drift" taxa are taken into account.

On this basis, and on the basis of the results of this study, it seems that the inclusion of a rank abundance as a means of weighting SASS scores, will not greatly alter the detection of disturbance at a site. Rather, the adherence to the current practice of using the rank abundance estimates as additional descriptive and interpretive tools of the macroinvertebrate assemblage at a site is probably sufficient.