AN EVALUATION OF SASS (SOUTH AFRICAN SCORING SYSTEM) AS A TOOL FOR THE RAPID BIOASSESSMENT OF WATER QUALITY

by

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TABLE OF CONTENTS

ABSTRACT

CHAPTER 1

Introduction 1

CHAPTER 2

The effect of water quality variables on riverine ecosystems: a review 7

2.1. Introduction 7

2.2. The effect of water quality on riverine ecosystems 8

2.3. Categories of water quality variables 9

2.3.1. Physical variables 10

2.3.2. Inorganic chemical variables 12

2.3.3. Organic compounds 16

2.4. Conclusions 18

CHAPTER 3

Assessment of the effects of changes in water quality on riverine ecosystems 19

3.1. Introduction 19

3.2. Assessment of water quality by means of physical and chemical data 20

3.3. Biological assessment 22

3.3.1. Selection of organisms for use in biological assessment 22

3.3.2. Approaches to biological assessment using benthic macroinvertebrates 24

3.4. Conclusions 38

CHAPTER 4

Description of the catchment, study-sites and sampling methodology 39

4.1. Introduction 39

4.2. Description of the Berg River catchment 40

4.3. Study-site selection and description 43

4.4. Collection methods 46

4.4.1. Benthic macroinvertebrates 46
4.4.2. Physical and chemical methods and analyses 50
4.4.3. Data analysis 52

CHAPTER 5

Variability and sample replication associated with two benthic macroinvertebrate sampling methods and a comparison between the quantitative and qualitative methods 55
5.1. Introduction 55
5.2. Results 56
5.2.1. Quantitative benthic sampling 57
5.2.2. Rapid bioassessment (qualitative sampling) 73
5.2.3. Comparison between quantitative benthic sampling and rapid bioassessment . . 82
5.3. Discussion 88
5.3.1. Variability and sample replication 88
5.3.2. Site differentiation 92
5.3.3. Mesh diameter 93
5.3.4. Diversity 94
5.4. Conclusions 95

CHAPTER 6

SASS and water quality: potential problems associated with this rapid bioassessment technique 97
6.1 Introduction 97
6.1.1. History of score allocation 101
6.2. SASS and water quality 102
6.2.1. Interpretation of scores 102
6.2.2. Total Score and Average Score per Taxon 105
6.3. Potential problems associated with SASS 107
6.3.1. Biotope availability 108
6.3.2. Temporal variability 111
6.3.3. Longitudinal changes 120
6.4. Regional differences and reference sites 122

6.5. Objective score allocation 123
6.6. Conclusions 127

CHAPTER 7
General discussion and conclusions 129

REFERENCES 139

ACKNOWLEDGEMENTS 151

APPENDIX A
Site code, river, site description, biotopes sampled and co-ordinates of 49 sites in the south-western Cape assessed using SASS 153

APPENDIX B
Physical attributes and concentrations of chemical constituents measured at 41 sites in the south-western Cape 155

ABSTRACT

The South African Scoring System (SASS) is a rapid bioassessment technique based on one component of riverine biotas, the benthic macroinvertebrates. Each taxon is assigned a tolerance/sensitivity score, which are summed to provide a Total Score. The Average Score per Taxon (ASPT) is calculated by dividing this Total Score by the number of taxa. This study was undertaken to evaluate the SASS technique as a tool for the assessment of water quality. Three study-sites, which differed in water quality, in the south-western Cape were selected for a detailed investigation into sample variability and replication of two methods of biological assessment, namely quantitative box-sampling and SASS. The ability of each method to differentiate between these sites was determined. The more general application of SASS in the south-western Cape was examined at forty nine sites and potential problems associated with SASS, namely biotope availability, temporal variability and longitudinal changes were investigated.

A minimum of twelve and four quantitative samples is needed to ensure collection of 95% or 75% of benthic macroinvertebrate taxa respectively. Sampling within a single biotope component, such as a "riffle" or "run" would reduce the number of samples needed. A minimum of four and two SASS samples is needed to ensure collection of 95% or 75% of the taxa respectively. This technique is however designed such that only one sample is taken per site. The Total Score that one sample would produce as a percentage of the Total Score from 20 samples, were 28%, 59% and 45% for Sites 1, 2 and 3 respectively. Total Score increases with increasing sampling effort, whilst ASPT is relatively unaffected by sampling effort. ASPT should therefore be used in interpretation of scores. Variability, as determined by both quantitative sampling and rapid bioassessment, was greatest at the least impacted site. Such sites should be more intensively sampled, either by increasing the number of box-samples taken, or by increasing the time period for SASS sampling.

Both quantitative and SASS sampling resulted in Family-level faunal samples that grouped by site. SASS distinguish the same site differences as quantitative sampling. Distinguishing taxa from the quantitative (>950 μm) and SASS samples overlapped, with two or three distinguishing taxa common to both at each of the three sites. The >950 μm size fraction

generally included between 86 and 94% of the taxa at each site, and smaller size fractions influenced total abundance and number of taxa.

SASS was able to distinguish between sites with different water quality. Ranges of Total Scores and ASPT values within each water quality category were higher in the south-western Cape than those ranges calculated on a national scale. Sites grouped on the basis of the degree of impact and water quality, and subsequently assessed using SASS, showed a clustering of sites within each water quality group. There was a strong positive correlation ($r=0.77$, $p<0.05$) between ASPT and Total Score. Severely impacted sites were concentrated at the lower end of the scale, moderately impacted site in the middle, and unimpacted sites at the upper end of the scale. The unimpacted sites were the most dispersed, suggesting greater variability in both ASPT and Total Score at such sites.

The influence of the variety of biotopes available for habitation by macroinvertebrates on SASS scores was investigated. Taxa present in the stones-in-current (SIC) biotope constituted the highest percentage of Total Score, followed by those taxa present in marginal vegetation, stones-out-of-current, aquatic/instream vegetation and sand in decreasing percentages respectively. Whilst Total Score and the number of taxa varied between biotopes, ASPT values remained relatively constant. Temporal differences in SASS scores were examined on a monthly, a seasonal (from historical data) and an annual basis. Minor variations in Total Score and ASPT values were mostly at unimpacted sites, although these variations did not mask the effects of impaired water quality. Further assessments are needed to establish if the seasonal and annual difference in SASS scores are the result of intrinsic changes within the aquatic ecosystem, or the result of impaired or improved water quality. Total Score varied longitudinally down the river, but was generally highest in the upper mountain zones and stony-foothill zones. ASPT progressively decreased from the upper sites to the lower ones.

The importance of establishing reference sites, preferably within an ecoregion framework, such that regional and zonal differences can be accounted for, is emphasised. SASS is ideally suited to provide data for the selection of reference sites against which other sites can be compared. The deviation of the observed SASS score from the expected score at a realistically comparable reference site will provide a means of assessing the degree of impact.

Rehabilitative measures can then be undertaken and audited using SASS within this reference system.

A comparison of current SASS tolerance/sensitivity score and composite maximum values calculated for 50 benthic macroinvertebrate taxa indicated that certain taxa are overscored and others are underscored. The importance of incorporating a feedback loop to facilitate continual refinement of SASS scores is recommended.

CHAPTER 1

INTRODUCTION

The average annual rainfall in South Africa is approximately 497 mm. Sixty-five percent of the country receives less than 500 mm and twenty-one percent receives less than 200 mm of rain per annum however (DWA 1986). The average annual potential evaporation ranges from 1100 mm to 3000 mm, which is considerably higher than the annual rainfall (DWA 1986). This high evaporation potential over most of the country significantly affects surface runoff from rainfall and causes high evaporation losses from surface waters. The arid nature of South Africa, with its comparatively high temperatures, seasonal or unpredictable rainfall and scarcity of permanent bodies of standing water, has resulted in rivers becoming the focus for exploitation of surface water. Whilst cartographically rivers in South Africa are numerous, many of these are short in length, or ephemeral, or both. Water is therefore clearly a limited resource.

The increasing rate of water consumption is the result of population growth, industrialisation and urbanisation of the economy, and greater demand for irrigation and stockwater. These factors have lead to increased water abstraction and reduced water quality (defined as the combined characteristics of the chemical constituents and physical attributes of a water sample), both of which have resulted in increased pressure on South Africa's rivers. A river is particularly susceptible to pollution as it is a confined, uni-directional system and a "drain" for the landscape. Activities in the catchment are reflected in changes in the associated riverine ecosystems and alterations or perturbations, even in the upper reaches, may have an effect down the entire length of the river. The relative scarcity of available water, the increasing water demands, and pollution problems need to be incorporated into management plans so that the conservation of rivers and their associated biota are ensured. The conservation of rivers as environments for riverine biotas also preserves the water as a

resource for man (Hawkes 1979). The management of water as a scarce resource needs to be based on sound scientific principles.

The Water Act (1956, Act 54 of 1956) was aimed at the control of industrial use of water and the treatment and disposal of effluent. Historically, water pollution in South Africa has been controlled by applying a uniform effluent standard. Because of the scarcity of water within South Africa, the Water Act required that all effluent be returned to the water body from which the water was originally abstracted. The purification of effluent resulting from the use of any water for industrial purposes, which did not meet prescribed standards, was obligatory (DWA 1986). Two types of effluent standards were developed by the Department of Water Affairs in consultation with the South African Bureau of Standards (SABS): a General Effluent Standard which was applied universally and a Special Effluent Standard which was applied to streams in sensitive catchments, essentially trout streams. The Water Amendment Act (1984, Act 96 of 1984) broadened water quality management by regulating pollution both from industrial effluents and from sources other than effluent, e.g. water which arises as a by-product from industrial and mining activities, and seepage or stormwater runoff from a site (DWAF 1991). Following the continued deterioration of South Africa's water resources, the Department of Water Affairs and Forestry (DWAF 1991) has revised its policy of water quality management and adopted a resource-based approach. The Receiving Water Quality Objectives (RWQO) approach was adopted for non-hazardous substances and the Pollution Prevention approach for hazardous substances (DWAF 1991). The RWQO's approach aims to allocate waste loads for non-hazardous substances on a catchment-by-catchment basis. Water quality management objectives are based on the specific requirements of each user of the resource. These requirements are evaluated and measured against present and potential catchment conditions. Water "users" recognised in the Water Act (1956, Act 54 of 1956) are domestic, industrial, agricultural, recreational and nature conservation/ecological (= environment). In theory this approach promises to be of benefit in terms of conserving the rivers as environments for riverine biotas. By assessing each catchment individually, the specific attributes, problems and requirements of rivers within the catchment can be determined. Rivers of particular conservation importance can be granted a "hands-off" status, whilst rivers already in an irreparable ecological state can be managed as such. The concept when applied, for example, to industrial effluents, which

under the effluent standards practice was required to meet a particular effluent standard prior to discharging into a river, now takes into account the number of such discharges into a particular river as well as the current conditions and importance of the catchment.

A seven step procedure is undertaken when determining the RWQO's for a particular catchment (DWAF 1992). Firstly, general water quality guidelines are determined. Secondly, the system is characterised with regard to users, management, development and impacts. Thirdly, general water quality guidelines are translated into site- or region-specific guidelines. These guidelines are then integrated with the water quality situation prevailing in the catchment to obtain a management guide (fourth step). The fifth step involves the incorporation of external factors, such as economic, political, technological factors etc., and a strategic catchment plan is formulated. From this a detailed management plan is compiled, consisting of three components: a pro-active management plan that controls point source and diffuse source pollution; a reactive management plan that deals with instream management, treatment of water and management of consequences etc.; and a general management plan that incorporates education and training. The final step ensures that the potential impact of any new development is assessed. Site-specific effluent standards are then set in such a way that the Receiving Water Quality Objectives are met. This is termed a "waste load allocation".

This approach has only recently been initiated and on selected rivers only. Thus the overall effectiveness of the Department's water quality management policy needs to be evaluated. Case studies are needed on catchments where this change of policy has been implemented so that the effect of changed management practice on aquatic biota can be assessed. Within each catchment or region, baseline characteristics of chemical (e.g. pH, conductivity, cation and anion concentrations), physical (e.g. temperature, suspended solids, turbidity) and biological (e.g. biogeographic patterns, endemism) components need to be established for the resource-based approach. Comprehensive monitoring programmes have long been established for chemical and physical characteristics of the water and are conducted regularly at numerous sites on the country's rivers. Biological monitoring, defined here as the utilization of biota or a component of the biota to provide an indication of the quality of the riverine environment, has only recently become a point of focus of organisations interested in

ascertaining the biological characteristics and status of rivers in South Africa and the effects of the new water quality policy.

Biological monitoring has been acclaimed as being a more sensitive and reliable measure of environmental conditions (e.g. in terms of water pollution) than either physical or chemical measurements (Warren 1971, OHIO EPA 1987).

The biological monitoring system recently implemented by the Department of Water Affairs and Forestry is the South African Scoring System (SASS) (D. Roux, Department of Water Affairs & Forestry, pers. comm.). This technique was developed by Chutter (Chutter 1992, 1994, Chutter & Geuppert 1993, Moore & McMillan 1993) for use on rivers. As detailed in Chapter 5, SASS is a field-based, rapid bioassessment method. It assumes that benthic macroinvertebrates reflect water quality and it provides a Total Score and Average Score per Taxon (ASPT) based on the presence of certain benthic macroinvertebrates at a site. The Total Score is derived by summing the sensitivity/tolerance scores assigned to each macroinvertebrate taxon. The ASPT is calculated by dividing this Total Score by the number of taxa present at the site. The sensitivity/tolerance scores are largely based on expert knowledge of each taxon's sensitivity or tolerance to water quality impairment. Given that this method is destined to become an integral part of the national water quality monitoring network established by DWAF, and is already being utilized by various other organisations such as Cape Nature Conservation (B. Gale, Cape Nature Conservation, pers. comm.) and Umgeni Water (C. Dickens, Umgeni Water, pers. comm.), its effectiveness and reliability needs to be ascertained. Historically, assessments of riverine biota were often based on traditional box- or Surber-sampling methods (e.g. Harrison & Elsworth 1958). These are intensive in terms of labour and time (Resh & McElravy 1993), which makes it impractical for them to become part of a routine monitoring programme. The development of SASS has ensured that aquatic biota (i.e. benthic macroinvertebrates) form a component, together with chemical attributes and physical characteristics of the water, of river monitoring programmes within South Africa. The usefulness of SASS has already been demonstrated (Chutter 1994b) but the relationship of this rapid bioassessment method to the more traditional method of benthic macroinvertebrate sampling (i.e. box-sampling) has not been addressed. This relationship and potential problems associated with SASS form the focus of this study.

The following specific objectives were formulated:

1. To determine the variability between samples taken within one biotope at a particular site using the two different methods, i.e. traditional quantitative box-sampling and qualitative rapid bioassessment using SASS (South African Scoring System), and thus
2. to ascertain the number of samples that should be taken using each method, in the case of quantitative sampling to allow adequate representation of the benthic macroinvertebrate community at that site, and in the case of SASS, to allow adequate representation of the SASS scores.
3. To investigate the influence of mesh diameter of the sampling equipment on the adequate sampling of benthic macroinvertebrate communities. Objectives 2 and 3 are designed to ascertain the most labour- and time-effective manner in which benthic macroinvertebrate collections can be undertaken.
4. To ascertain if the SASS method is reliable in reflecting water quality and in relation to this to undertake a preliminary investigation into the potential problems associated with the SASS method.

The thesis has been structured as follows:

- Chapter 1. A general introduction
- Chapter 2. A review of the effect of water quality variables on riverine ecosystems.
- Chapter 3. A review of assessment methodologies, in particular biological assessment, used to determine the effects of changes in water quality on riverine ecosystems.
- Chapter 4. A description of the study catchment, selection of study-sites, and methods used for biological sampling, in particular a detailed account of the rapid bioassessment method developed in South Africa [South African Scoring System (SASS)], and the techniques used for the analyses of chemical attributes and physical characteristics of water.
- Chapter 5. Variability and sample replication associated with two benthic macroinvertebrate sampling methods and a comparison of the two methods.

- Chapter 6. SASS and water quality: potential problems associated with this rapid bioassessment method.
- Chapter 7. General discussion and conclusions.

CHAPTER 2

THE EFFECT OF WATER QUALITY VARIABLES ON RIVERINE ECOSYSTEMS: A REVIEW

This chapter is a précis of a review written and published in 1993 (Dallas & Day 1993). In the review, chapters one, two and three were written jointly, chapters four, five, and eight to eighteen were written by Dallas, and chapters six and seven by Day. The chapter numbers are given in parentheses in each section heading and the entire review is available on request.

2.1. INTRODUCTION

The structure and composition of biotic communities in riverine ecosystems are determined by a number of interacting factors, including the flow regime, energy and/or food source, habitat structure, biotic interactions, and water quality (defined as the combined characteristics of the chemical constituents and physical attributes of a water sample) of the water body. Figure 2.1. indicates the complexity of the various physical, chemical and biological factors that influence and determine the resultant biotic community. The flow regime is determined by characteristics of the catchment such as catchment size, relief and rainfall; and by factors such as runoff patterns, volume, high-low extremes and the relative contribution of ground water. Fluctuating water levels are an integral part of all stream ecosystems and aquatic organisms have evolved to compensate for changing flow regimes (Karr & Dudley 1981). The form and source of energy, e.g. allochthonous (imported) versus autochthonous (produced *in situ*) organic matter, is important in determining biotic community structure (Cummins 1973, Karr & Dudley 1981) as are nutrient availability, primary and secondary production and seasonal patterns in these factors (e.g. King *et al.* 1987). Habitat structure incorporates geomorphological features such as gradient, channel

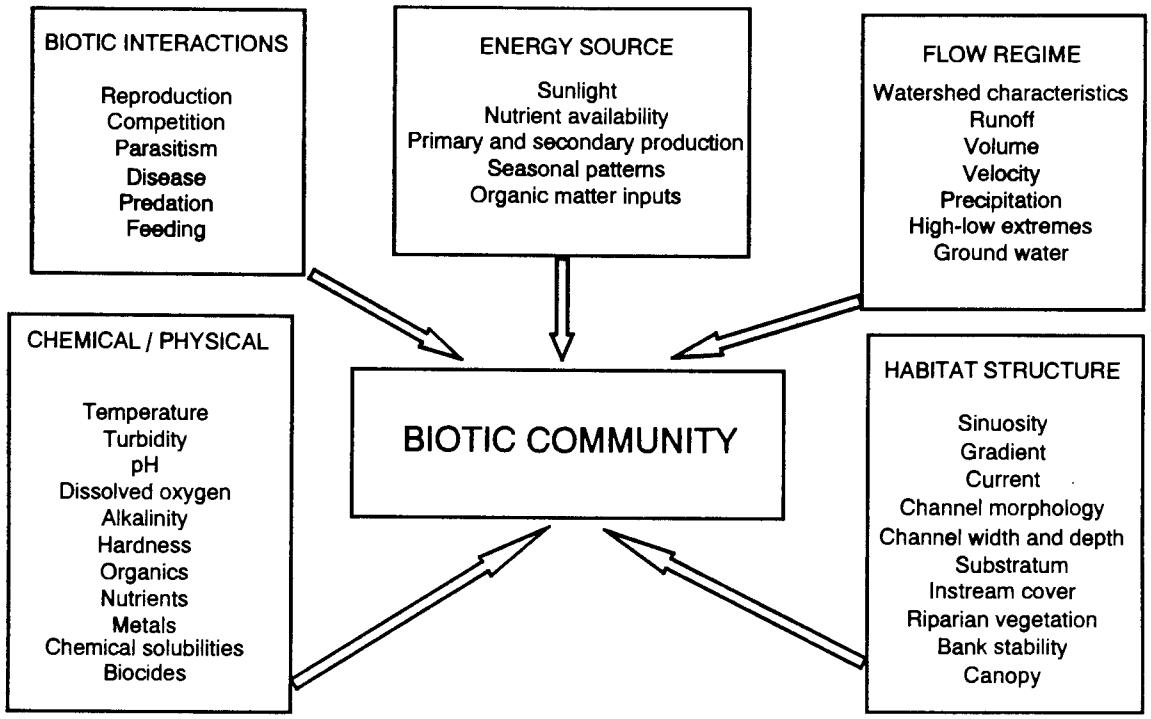


Figure 2.1. Some of the important chemical, physical and biological factors that influence and determine biotic communities [modified from Karr *et al.* (1986), cited by Rankin 1991].

morphology, channel width and depth, and substratum, in addition to factors related to the riparian zone such as riparian vegetation, canopy cover and bank stability. Substrate particle size determines the size of the interstitial spaces which, in turn, affects the type of organisms comprising the bottom-dwelling community (Karr & Dudley 1981). Biotic interactions include reproduction, feeding, competition, predation, parasitism and disease.

2.2. THE EFFECT OF WATER QUALITY ON RIVERINE ECOSYSTEMS

The physical attributes and chemical constituents of natural fresh waters are determined by climate, geology, geomorphology and biota and thus often vary regionally.

Climate affects water quality in a variety of ways. Mean annual precipitation, and seasonal variations in precipitation, determine the amount of water flowing in rivers at different times

and therefore dictate the degree of dilution of natural and anthropogenic constituents in the water. The converse effect occurs with evaporation, which concentrates the constituents in water and may alter the proportions of major ions and result in precipitation or crystallization of salts. Temperature determines the availability of nutrients and toxins, as well as the oxygen saturation level in the water body.

The geomorphology of the landscape determines the river gradient and therefore the amount of turbulence and hence oxygen concentration in the water. It also contributes to the degree of erosion within the catchment.

Different geological formations vary in chemical composition and thus soils derived from them contribute ions in different quantities and of different proportions to the waters flowing over or percolating through them.

Components of the biota, in particular phytoplankton and microbes, affect water quality. Photosynthesis and decomposition alter the pH and oxygen concentration of water. Riparian and catchment vegetation also influences the chemical composition of a water body. For instance, humic substances characteristic of the black acid streams of the Fynbos Biome in the south-western Cape are derived from catchment vegetation.

2.3. CATEGORIES OF WATER QUALITY VARIABLES

"Water quality" is the term used to describe the combined characteristics of the chemical constituents and physical attributes of a water sample in relation to a "user". A "user" utilizes the resource and has specific water quality needs and requirements. Water "users" recognized in the Water Act (1956, Act 54 of 1956) are domestic, industrial, agricultural, recreational and nature conservation/ecology (DWAF 1992). This section reviews the significance of a number of water quality variables which affect riverine ecosystems, and has been broadly divided into physical variables, and inorganic and organic chemical variables.

2.3.1. Physical variables

Temperature (Chapter 4)

The thermal characteristics of running waters are dependent on hydrology, climate and structural features of the catchment. Water temperature tends to increase longitudinally along a river course, i.e. rivers tend to be cooler in the upper reaches, and additionally, in regions of seasonal climates, running waters exhibit daily and annual periodicity.

All organisms have a temperature range, usually narrow, at which optimal growth, reproduction and fitness occur and a wider temperature range in which they can survive. Elevated water temperature reduces oxygen solubility, increases the toxicity of certain chemicals (Duffus 1980), and increases metabolic rate, including respiration and thus the amount of oxygen required by aquatic organisms. Since oxygen demand increases and supply decreases with increasing temperature, the biota is doubly stressed by elevated temperatures. Lowered water temperature reduces metabolic rate and thus decreases the speed at which animals can move, while increasing the length of time to maturity. Many life cycle characteristics, such as reproductive periods, development rates and emergence times, are cued to particular temperatures or natural seasonal changes in temperature (e.g. Nordlie & Arthur 1981, Wrenn *et al.* 1979, cited by Nordlie & Arthur 1981).

Anthropogenic changes in temperature in river systems may result from thermal pollution by heated industrial or power station discharges (Mann 1965), stream regulation (Ward & Stanford 1982), interbasin water transfer, and alterations in the amount and type of riparian vegetation (Graynorth 1979). The extent to which this alteration in the aquatic environment is reflected in the biotic community could be assessed using SASS.

Suspended solids, suspensoids and turbidity (Chapter 5)

Suspended solids (also known as suspensoids) include silt, dead organic matter and other small particles suspended in water. These particles have both physical and chemical effects. Suspensoids have a large surface-area-to-volume ratio and often carry an electrical charge. This results in a variety of dissolved substances, e.g. some nutrients, trace metals and

biocides, becoming adsorbed onto the surfaces of the particles. The consequences can be significant in that adsorbed substances may become unavailable, which is beneficial if they are toxic trace metal ions, but detrimental if they are nutrient molecules. These particles often settle and become part of the sediments. Adsorbed toxins in the sediments are essentially unavailable, although mobilization of the sediments during heavy spates may result in their release. Desorption of the attached molecules may also occur following chemical changes such as a decrease in pH.

Large numbers of small particles suspended in water may be visible as turbidity. Turbidity is defined as the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through a water sample (American Public Health Association 1989). The scattering of light is caused by suspended matter, such as clay, silt, finely dissolved organic and inorganic matter, plankton and other microscopic organisms. In standing waters, the reduction in light penetration decreases primary production and less food becomes available to organisms at higher trophic levels. Suspensoids that settle out of the water column may smother and abrade riverine plants and animals resulting in communities dominated by organisms that are able to cope with this alteration in habitat (Hellawell 1986). Predators that rely on sight to search for their prey may be unable to find food (Bruton 1985).

Turbidity in rivers often changes seasonally (e.g. Harrison & Elsworth 1958). The extent is governed by the hydrology and geomorphology of a region. Erosion of land surfaces by wind and rain is a continuous and historically natural process. Land-use practices such as overgrazing, non-contour ploughing and removal of riparian vegetation accelerate the rate of erosion, however, and result in increased quantities of suspensoids in receiving streams. Various other anthropogenic activities such as release of domestic sewage or industrial discharge, and physical perturbations (e.g. road and bridge construction; Taylor & Roff 1986) and reservoir management (Gray & Ward 1982a, 1982b) have been implicated in increasing the turbidity of streams. If anthropogenically-derived turbidity increases are as infrequent as natural flooding is, they may well be tolerated by the stream community. Continuously high levels of suspensoids may however have serious consequences for the riverine biota. The consequence of these continuous high levels may result in a change in

macroinvertebrate community structure, if certain taxa can be shown to be silt-tolerant or silt-sensitive.

2.3.2. Inorganic chemical variables

Total dissolved solids (TDS), conductivity and salinity (Chapter 7)

The total amount of material dissolved in a water sample is commonly measured as total dissolved solids (TDS), or conductivity. TDS represents the total quantity of dissolved material, organic and inorganic, ionized and un-ionized, in a sample of water. Conductivity is a measure of the ability of a sample of water to conduct an electrical current. TDS and conductivity usually correlate closely for a particular type of water. Natural TDS in rivers is determined largely by the degree of weathering, the chemical composition of rocks and by the relative influences of precipitation and rainfall in the catchment.

Anthropogenic activities such as irrigation, clear-felling, re-use of water and the release of industrial effluents, lead to increases in TDS. Information on the tolerances of riverine organisms to increased TDS is very limited and SASS has the potential to expand the knowledge of organisms' tolerances to elevated TDS concentrations. It is probable that rate of change rather than the absolute change in TDS has the greatest effect on the aquatic biota. Juvenile stages are often more sensitive than adults and effects may be more pronounced in upper mountain streams, where organisms are generally less tolerant to stress.

The major ions: Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, HCO_3^- and CO_3^{2-} (Chapter 7)

The ions that form the bulk of TDS are the cations sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}), and the anions chloride (Cl^-), sulphate (SO_4^{2-}), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}). These ions are generally not toxic *per se* and are required in certain quantities by the biota.

pH and alkalinity (Chapter 6)

The pH of water is a measure of the concentration of hydrogen (H^+) ions (Golterman *et al.*

1978). As $[H^+]$ increases, so pH decreases and the solution becomes acidic; as $[H^+]$ decreases, pH increases and the solution becomes alkaline. Alkalinity is determined as "acid neutralizing capacity", which in fresh waters is usually due largely to bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions. Since pH is by definition $-\log_{10}$ of hydrogen ion activity, a decrease in one pH unit means a ten-fold increase in $[H^+]$. The rate of change of pH is determined by the buffering capacity (e.g. by the carbonate-bicarbonate system) of the water.

The pH of natural waters is determined by geological and atmospheric influences. Most fresh waters are relatively well buffered and more or less neutral, with pH ranging between about 6 and 8. One of the main ways in which pH affects aquatic ecosystems is by determining the chemical species (and thus the availability and the potential toxicity) of many trace metals. Aluminium, for example, is highly toxic in very acid waters, where the low pH results in the formation of the toxic aquo-Al^{3+} ion.

Changing the pH of water changes the concentration of both H^+ and hydroxyl (OH^-) ions, which in turn affects the ionic and osmotic balance of aquatic organisms. Relatively small changes in pH are not normally lethal, although sublethal effects such as impaired growth rates and reduced fecundity may occur as a result of increased physiological stress placed on the organism by increased energy requirements (e.g. Berril *et al.* 1991). The lethal effects of decreased pH are frequently the result of the mobilization of toxic substances such as the bioavailable aquo-Al^{3+} ion. The testing of the ability of a method such as SASS to detect these subtle changes in pH would be useful, particularly in regions such as the south-western Cape where acid-adapted species are common.

Anthropogenic acidification of rivers is normally the result of point-source effluents such as those from the chemical and pulp-and-paper industries, mine drainage and acid precipitation ("acid rain"). Eutrophication can result in a significant increase in pH, since excessive plant production consumes carbon dioxide (CO_2) and so alters the equilibrium of the CO_2/HCO_3^- buffering system. Under eutrophic conditions, pH may fluctuate diurnally by two or three units, depending on the quantity of CO_2 consumed during photosynthesis relative to that produced during respiration and decomposition.

Dissolved oxygen (Chapter 10)

The concentration of dissolved oxygen is probably one of the most important abiotic determinants of the survival of most aquatic organisms. Under natural conditions the concentration of dissolved oxygen fluctuates diurnally, depending on the relative rates of photosynthesis and respiration. It is usually lowest near dawn, increasing during the day, peaking in the afternoon, and decreasing at night. Various factors determine the amount of oxygen that can be dissolved in water. These include the rate of aeration from the atmosphere (dependent on turbulence, oxygen deficit and atmospheric pressure); temperature; salinity; and respiration by all organisms and photosynthesis by plants. Oxygen levels decrease where organic enrichment occurs (biological oxygen demand) because aerobic decomposer micro-organisms require oxygen, and where certain oxygen-"consuming" chemicals are present (chemical oxygen demand).

The extent to which an organism is affected by dissolved oxygen concentrations is determined by its dependence on water as a medium. Fish are particularly susceptible (Alabaster & Lloyd 1980), as are larvae of stoneflies, caddisflies and mayflies, which respire with gills or by direct cuticular exchange (Nebeker 1972). The absence of such taxa as ascertained by SASS would indicate an impairment of the water quality. Low concentrations of dissolved oxygen may cause various sublethal effects such as changes in behaviour (Davis 1975), blood chemistry (Davis 1975), growth rate (Brungs 1971) and food intake (Stewart *et al.* 1967), as well as lethal effects.

The amount of oxygen dissolved in water may be reduced by increases in temperature and salinity, by oxygen-consuming chemical effluents and by high levels of organic waste.

Nutrients (Chapter 8)

Various elements, including carbon, oxygen, hydrogen, sulphur, potassium, nitrogen and phosphorus, are required for normal growth and reproduction in plants. Nitrogen and phosphorus are most commonly implicated in excessive plant growth resulting from nutrient enrichment (eutrophication) of aquatic systems. Most nutrients are not toxic (exceptions include nitrite and ammonia under certain circumstances), even in high concentrations. When present in aquatic systems in these high concentrations, they may however have a

significant impact on the structure and functioning of biotic communities. Climatic and catchment characteristics influence initial nutrient concentrations in rivers but these may be significantly altered by biotic activity.

Phosphorus is required in numerous life processes and is an integral part of DNA. In nature inorganic phosphorus occurs most commonly as the phosphate ion (PO_4^{3-}). Immediately available Soluble Reactive Phosphorus (SRP) is seldom found in quantity in non-polluted water as it is taken up by plants, or is adsorbed onto suspensoids or bonded to ions such as iron, aluminium, calcium and organic polyphenols (Addiscott *et al.* 1991).

Nitrogen occurs abundantly in nature and is an essential constituent of many biochemical processes. It occurs as nitrate, nitrite, ammonia and many nitrogen-containing organic compounds. Nitrate is seldom abundant in natural surface waters because it is incorporated into organic nitrogen in plant cells (Porter 1975) or is reduced by microbes and converted into atmospheric nitrogen. Nitrite is an intermediate in the inter-conversion of ammonia and nitrate, and is toxic to aquatic organisms at certain concentrations. Ammonia, either in the free un-ionized form (NH_3) or as ammonium ions (NH_4^+), is a common pollutant associated with sewage and industrial effluent. The toxicity of ammonia is directly related to the proportion of the un-ionized form (Williams *et al.* 1986), which increases in relative proportion as pH and temperature increase.

High levels of nutrients may enter rivers as point-source effluents from waste water treatment works, industry and intensive animal enterprises (Schofield *et al.* 1990), or diffusely as runoff from fertilized agricultural areas (Rosich & Cullen 1982) and urban storm water. Non-point-source pollution has always been problematic to quantify using chemical and physical methods. An assessment method based on a component of the biota, such as benthic macroinvertebrates, would facilitate detection of a deleterious effect. Repeated SASS sampling at sites within close proximity to one another can provide evidence of problem reaches.

Trace metals (Chapter 12)

Trace metals are those that occur in very small quantities in water. Natural sources of trace metals include geological weathering and atmospheric particulates, while greater quantities are produced in industrial effluents, agricultural runoff and acid drainage from mines. Most trace metals are highly toxic at even slightly elevated levels. Their toxicity is controlled by a number of chemical and physical factors, including the chemical species of the metal, the presence of other metals and organic compounds that may result in either synergistic or antagonistic interactions, the flow rate and volume of the receiving water, substratum type, dissolved oxygen, temperature, hardness, pH, and salinity.

Biological factors (e.g. life history stage, age, sex, tolerance levels), influence an organism's susceptibility to trace metal toxicity. The overall ecological consequences of trace metal contamination of aquatic ecosystems is a reduction in species richness and diversity and a change in species composition. The selective elimination of less tolerant species, with the resultant reduction in competition and predation, may result in an increase in the abundance of more tolerant species. The ability of SASS to detect these changes in community structure warrants investigation. The degree of change is related to the concentration of the metal(s) and the type (e.g. chronic or acute, constant or intermittent) and timing of exposure.

2.3.3. Organic compounds

Organic compounds contain carbon and are mostly produced by living organisms. Naturally occurring organic compounds are not usually toxic.

Organic waste (= "oxygen-demanding waste"; Chapter 9)

Dissolved organic matter (DOM) and particulate organic matter (POM) are derived from biological activity, including the decomposition of dead material. In rivers, POM is an important source of food or energy for many benthic organisms, including detritivores and decomposer bacteria. Some of these bacteria require oxygen and when present in large quantities can entirely deplete the water of oxygen. Organic enrichment is probably the most common and the most extensively documented type of pollution in rivers. The organic

material in effluents such as sewage is generally not directly toxic to aquatic life. The resulting oxygen depletion may however significantly alter community structure by encouraging the survival of very hardy species and eliminating those sensitive to reduced oxygen.

The main sources of organic waste are effluents from domestic sewage, food processing plants, animal feedlots and abattoirs. Increases in turbidity (and hence reduced light penetration), suspended solids (and hence substrate modification) and nutrients (nitrogen, phosphorus, and hence increased potential for plant growth) often accompany oxygen depletion in organically polluted waters. Combined, these factors significantly affect species richness and diversity (Seager & Abrahams 1990, Whitehurst & Lindsey 1990), and community composition usually becomes less diverse, whilst biomass increases many fold. SASS will enable such changes in community structure to be recorded.

Biocides (Chapter 11)

The term "biocide" generally refers to toxic chemicals produced to eliminate pest organisms. Biocides potentially harmful to aquatic ecosystems include herbicides and fungicides, and insecticides such as organochlorines, organophosphates, carbamates, formamidines and pyrethroids. They may enter aquatic environments via different pathways: directly when aquatic pests (e.g. bilharzia snails) are being controlled; in industrial effluents and sewage; by leaching and runoff from soil (Wilcock *et al.* 1991); and by deposition of aerosols and particulates (Robinson 1973). The nature, modes of action and toxicity of biocides vary considerably. Generally, organochlorine insecticides (e.g. DDT, dieldrin) are the most hazardous with respect to the natural environment. They are persistent, largely insoluble in water, photostable, and highly toxic to many organisms (Livingston 1977). Organochlorine insecticides tend to accumulate in living organisms (many are fat-soluble) and thus become "biomagnified" through food chains. Because biocides are so varied in nature and are toxic in minute quantities, their chemical detection and quantification in aquatic systems is complex and expensive. The potential utilization of a method such as SASS, which could be used as a first-phase or early warning signal in areas suspected to be contaminated with biocides might be investigated.

Oils and greases

Oils, fats and greases (lipids) are long-chain organic molecules which are insoluble in water. At ambient temperatures oils are liquid and fats are solid, while greases have much wider melting-points, allowing them to be adhesive at ambient temperatures. Oils and greases are present in various effluents such as sewage. Their effect, in large quantities, is to coat the surfaces of organisms, thereby reducing the rate of diffusion of gases. Oils may also have toxic effects.

Surfactants

Man-made surfactants (surface-active agents) include soaps, detergents and oil dispersants, which dissolve or disperse lipids. Proteins and various other natural organic compounds are surface-active and cause foaming. It is however the synthetic surfactants that potentially affect aquatic ecosystems. They reduce the rate of re-aeration of water and thus reduce the amount of oxygen available to the biota. Their toxic properties result largely from their ability to dissolve lipids on the surfaces of organisms. Aquatic pollution by detergents is however not due only to their surface-active properties. Household detergents often contain large quantities of phosphate ions in order to enhance foaming and may therefore contribute to eutrophication.

2.4. CONCLUSIONS

The physical attributes and chemical constituents of a water body influence the biota (i.e. structure) and therefore the functioning of riverine ecosystems. It is important to ascertain intrinsic water quality conditions within a riverine system, prior to focusing on the effects of anthropogenic inputs. To facilitate scientifically-based management of an aquatic ecosystem, the baseline physical, chemical and biological characteristics need to be determined. Given the availability of this information, procedures can be adopted to assess the effects of an alteration in water quality on riverine biotas. The assessment of the effects of water quality on riverine ecosystems is discussed in the following chapter.

CHAPTER 3

ASSESSMENT OF THE EFFECTS OF CHANGES IN WATER QUALITY ON RIVERINE ECOSYSTEMS

3.1. INTRODUCTION

The chemical constituents and physical attributes of a water body are commonly termed water quality. Quality however, implies some value or significance which would vary depending on what it was being related to. Water quality in terms of the aquatic biota may be different from water quality as perceived by a person requiring water for agriculture. In this thesis water quality is defined as the chemical constituents and physical attributes of a water body which render it suitable for habitation by aquatic biota. An alteration in water quality may reduce this suitability for habitation by aquatic biota with subsequent effects being manifested as alterations in biotic community structure and thus functioning of riverine ecosystems.

The assessment of the effects of changes in inherent water quality and its constituent variables in riverine ecosystems is complex. The high degree of interaction, both directly and indirectly, between the major chemical (such as pH and toxicants) and physical constituents (such as suspended solids and turbidity), and the influence of non-water-quality variables (such as substratum, current velocity, insolation) on these interactions is illustrated in Figure 3.1. Isolating the effect of a single constituent is often difficult because of interactions between other constituents. In general, the effect of a change in water quality can be assessed in terms of the physical/chemical and biological characteristics of the system under examination, either as separate entities or in combination with one another. Assessment of biological characteristics, e.g. a component of the biota such as benthic macroinvertebrates, results in a time- and constituent-integrated result. Physical and chemical assessments are generally periodic, i.e. the physical attributes and chemical

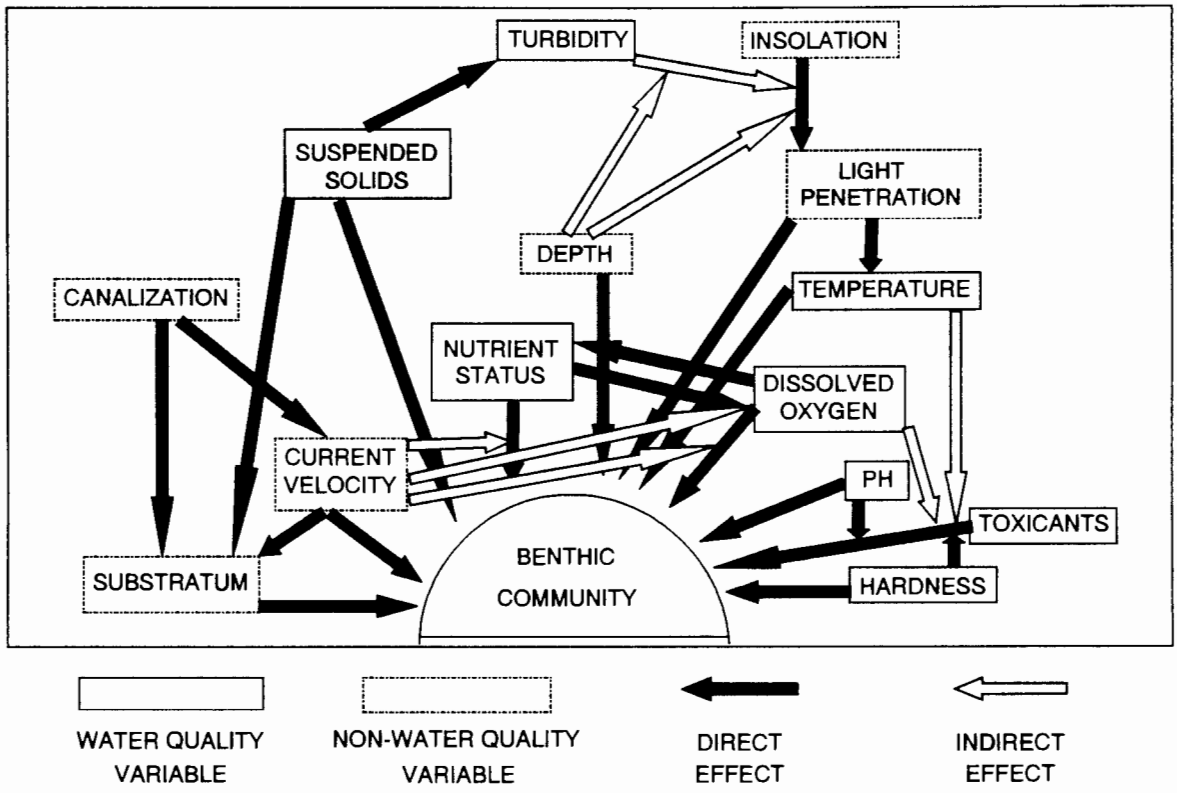


Figure 3.1. Major water-quality and non-water-quality variables potentially affecting riverine biotas, showing direct and indirect interactions between variables (from Dallas & Day 1993).

constituents of a water sample are measured sporadically. The frequency of these measurements depends on the nature of the study or monitoring programme. Because of differences arising from time-intervals and time-integration, it is often difficult to link changes in physical attributes and chemical constituents to changes in the biota. The following section outlines the advantages and disadvantages associated with the assessment of water quality from the physical attributes and chemical constituents of the water body, and from the biological components.

3.2. ASSESSMENT OF WATER QUALITY BY MEANS OF PHYSICAL AND CHEMICAL DATA

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. Unless these sample collections are continuous over time, pulsed releases of effluents that result in an alteration of water quality may not be recorded. The number of constituents that could be present is varied but can be vast, while routine analyses are usually limited to non-toxic determinants such as temperature, conductivity, total alkalinity and nutrients. The number and variety of potentially toxic compounds (e.g. trace metals, biocides, organic by-products of manufacturing processes) that could affect water quality is extremely large, and routine testing for all possible toxins is unrealistic given the time, cost and difficulty in identifying and quantifying many compounds, particularly organics. Further, the cost of such chemical analyses, in a country where socio-economic considerations are of increasing significance, should not be ignored. The sensitivity of chemical analytical methods when measuring very low concentrations of pollutants may be inadequate, particularly for substances that are characteristically present in these low concentrations but which are persistent and tend to accumulate in the environment.

Further complicating factors in the assessment of the effect of altered water quality by means of physical and/or chemical data, are synergism and antagonism. Although each 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, nickel and zinc are synergistic because they are five times more toxic in combination than either is individually (Haberer & Normann 1971, cited by Förstner & Wittmann 1981). Calcium and magnesium reduce the toxicity of copper and various other trace metals. This reduction in toxicity of some substances owing to the presence of other substances is known as antagonism. The toxicity of many compounds also varies considerably with pH. These subtle magnifying and reducing effects would not necessarily be revealed by routine physical and chemical monitoring.

Knowledge of the effect of changes in physical attributes or chemical constituents of a water body on the biota is limited. Extrapolation from information currently available provides a relatively simplistic solution to this problem.

3.3. BIOLOGICAL ASSESSMENT

The assessment of the biotic component of aquatic ecosystems may be broadly differentiated into two "categories". The examination of the biota such that the state of the biota may be assessed, or the examination of the biota such that inferences regarding other factors, such as water quality, may be made. The technique used in biological assessment will to a certain extent depend on which of these "categories" the respective study or monitoring programme is grouped with.

Biological assessment of water quality may be defined as the utilization of one or more components of the biota (e.g. diatoms, macroinvertebrates, fish) to assess the effect of a change in water quality. The assessment may be based on biological responses at the individual species level (e.g. growth rate, fecundity) or community level (e.g. species composition, alteration in key species). Many aquatic organisms are permanently present in a water body and assessment based on them therefore provides information on the integrated and cumulative effects of changes in the physical and chemical constituents of the water body. It also enables changes in non-water-quality parameters, such as current velocity and habitat degradation, to be assessed. According to Warren (1971), organisms living in water provide a more sensitive and reliable measure of the suitability of conditions than do physical and chemical measurements of water quality. In Ohio for example, an evaluation of instream biota indicated that 36% of impaired sites were not identified using chemical-based criteria (OHIO EPA 1987). It is important to recognise that biological data do not replace physical and chemical data any more than chemical data may adequately replace biological data. Rather, they provide converging lines of evidence that supplement each other but which are not mutually exclusive (Cairns & Dickson 1971).

3.3.1. Selection of organisms for use in biological assessment

A wide variety of organisms including bacteria, protozoans, algae, macrophytes, macroinvertebrates and fish, have been used in biological assessment of water quality . Hellawell (1986) conducted a survey of the most frequently used groups of organisms in biological assessment and noted that algae and macroinvertebrates were the two groups most

often recommended for use in assessing water quality. In South Africa macroinvertebrates and fish are the groups on which much biological assessment work has focused. Various methods of biological assessment have been developed in other countries using fish, e.g. Index of Biological Integrity (IBI: Karr 1981). Fish are long-lived, their life-histories are generally known and they are easy to identify. However, quantitative samples are difficult to obtain and fish are mobile and can thus move from polluted to unpolluted stretches. These factors may affect differences in fish species richness from site to site, thereby hindering comparative interpretation. There is general consensus that benthic macroinvertebrates are amongst the most sensitive components of aquatic ecosystems, in addition to being relatively non-mobile and readily sampled, and are thus useful for assessing biological integrity (Metcalfe-Smith 1991). Biological integrity is defined as the ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity and functional organisation comparable to that of the natural habitats within a region (Karr & Dudley 1981). The following sections outline the advantages and disadvantages associated with this group of organisms, and elaborate on the history of biological assessment using benthic macroinvertebrates.

Advantages of using benthic macroinvertebrates for assessing water quality

The advantages of using benthic macroinvertebrates for assessing water quality have been summarised by Rosenberg & Resh (1993). Essentially benthic macroinvertebrates are ubiquitous in rivers and can therefore be affected by environmental perturbations in many different types of aquatic systems and in most biotopes within these waters. Sensitivity to stress varies with species and the large number of species within a community offers a spectrum of responses to environmental stresses. Invertebrates in their aquatic phase are largely non-mobile and are thus representative of the location being sampled, which allows effective spatial analyses of disturbance and pollutants. One response of invertebrates to a perturbation, such as reduced pH, is to drift (Bernard *et al.* 1990). This may in itself be recorded as an effect. Invertebrates have relatively long life cycles compared to other groups (e.g. planktonic organisms), which allows elucidation of temporal changes caused by perturbations. The lifespan of many invertebrates is however still short enough to ensure observation of recolonization patterns following some form of perturbation. As a result of these factors, benthic macroinvertebrates act as continuous monitors of the water they inhabit

(Hawkes 1979), enabling long-term analysis of both regular and intermittent discharges, variable concentrations of pollutants, single and multiple pollutants, and synergistic or antagonistic effects.

Disadvantages of using benthic macroinvertebrates for assessing water quality

The heterogenous distribution (i.e. patchy or clumped and uneven spatial distribution) of many benthic macroinvertebrates in river beds makes quantitative sampling problematic (Hawkes 1979) and requires appropriate sample replication. The processing of quantitative samples is often labour intensive and time consuming although rapid biological assessment techniques alleviate this to a certain extent. The uncertainty of the taxonomic status of certain groups of invertebrates, particularly in South Africa, makes taxonomic resolution difficult and often requires consultation with specialists. Invertebrates may not be sensitive to all pollutants (e.g. low levels of herbicides were not detected using macroinvertebrates: Hawkes 1979) and their responses to toxic compounds have not been as well documented as has their response to organic pollutants. The distribution and abundance of benthic macroinvertebrates can be affected by factors other than water quality (e.g. natural conditions such as current velocity, habitat destruction and the nature of the substratum: Rosenberg & Resh 1993) and these should be taken into account when drawing conclusions regarding impairment of water quality. It is often difficult to decide when an observed change in some aspect of the biota represents a deviation caused by the presence of a pollutant, or whether such changes are part of the "natural" fluctuations inherent in the system (Miller 1984). This can be taken further when one considers temporal changes such as those responding to seasonal variations (Hellawell 1977), intrinsic spatial changes such as those along the longitudinal gradient of a river, and inter-annual variations such as flood/drought cycles, particularly in South Africa and other arid lands.

3.3.2. Approaches to biological assessment using benthic macroinvertebrates

Numerous attributes of individual species, biotic communities and natural processes can be

used to assess the biological integrity of an aquatic ecosystem. Each of these attributes may be considered a biological indicator. Historically the term "biological indicator" has been associated with a particular "indicator organism", but in the current context its application is much broader and incorporates structural and functional attributes of individual organisms within a community, and whole communities. Some of the major biological indicators are listed in Table 3.1 (Dallas & Day 1993).

Table 3.1. Some biotic indicators that can be used to estimate the effects of reduced water quality on ecosystems and their biotas (from Dallas & Day 1993).

ATTRIBUTES OF	BIOLOGICAL INDICATOR
Individuals of selected species	Behaviour Growth rate Metabolic rate Sensitivity to pathogens Condition Fecundity Age to maturity Survival rate Abundance Biomass Recruitment and turnover
Biotic communities	Species composition Biodiversity (e.g. number of species) Complexity of interrelationships Community succession Alteration in key species Resilience to change Sensitivity to change Rate of species colonization and emigration Rate of re-establishment of equilibrium densities
Natural processes	Rate of photosynthesis Rate of nutrient cycling Rate of decomposition

Individuals of selected species

The utilisation of individual species in the assessment of water quality is largely laboratory-based and in general is referred to as toxicity or tolerance testing. Numerous studies have

determined the effects of known toxic substances on specific organisms, either as mortality or as sublethal effects such as reproductive impairment, reduced growth rate, etc. Buikema & Voshell (1993) provide a comprehensive review of toxicity studies using freshwater benthic macroinvertebrates. Three basic types of toxicity tests are used: acute single-species tests; chronic single-species tests; and multi-species tests. The procedure involves the maintenance of a given number of organisms of a particular species (in a single-species test) or a suite of species (in a multi-species test) under standard conditions and then the introduction of different concentrations of the test pollutant to each batch of organisms. It is important to ensure that standard conditions, particularly in terms of water quality (e.g. temperature, pH, oxygen levels) be maintained since the toxic effects of many chemical compounds may be significantly affected by variations in water quality. The advantages of controlled toxicity testing include repeatability and thus reliability. The lack of suitable riverine organisms for testing, and the difficulties associated with extrapolation of laboratory information to field conditions, are however, two of the disadvantages. Biological communities often respond to pollution stresses in a different manner than laboratory criteria might predict or imply (OHIO EPA 1987). The extent to which these laboratory studies reflect environmental reality should therefore be taken into account.

Communities as "indicators"

The utilisation of biotic communities as a means of assessing water quality has become fairly well established in the aquatic sciences. The method selected for assessing water quality using biotic communities is largely dependent on the objective(s) of the particular study or monitoring system. Sampling may be quantitative (e.g. traditional box-sampling) or qualitative (e.g. a kick net method such as South African Scoring System, SASS). Many qualitative methods fall within the new terminology of rapid biological assessment (RBA). The rapidity of a RBA method is dependent on the particular sampling protocol followed and is affected by factors such as sampling design, percentage field- versus laboratory-based sorting and identification, and taxonomic resolution. Regardless of the sampling method employed, information gleaned by sampling of biotic communities can be summarised using indices. Three basic types of indices exist: diversity, biotic and comparison (similarity or dissimilarity) indices. The following section outlines the many approaches, both quantitative and qualitative, employed in utilising biotic communities to assess water quality.

The "Saprobien" system

The term "saprobia" refers to the dependence of an organism on decomposing substances as a food source (Persoone & De Pauw 1979). Kolkwitz & Marsson (1909, cited by Warren 1971) first used the presence and absence of different organisms to develop their "saprobien" system of zones in organically polluted rivers. In their study, emphasis was placed on the relative tolerance levels of individual species of organisms. Taxonomic effort was great (i.e. all specimens were identified to species) and all trophic levels were examined. The original system was geographically specific (Germany) and only appropriate for sewage pollution (Chutter 1972).

Diversity indices

Diversity indices are mathematical expressions of three components of community structure, namely richness (number of species present), evenness (uniformity in the distribution of individuals among species) and abundance (total number of organisms present), and can be used to reflect the response of a community to the quality of its environment (Metcalfe-Smith 1991). The underlying premise is that undisturbed environments will be characterized by high diversity or richness and an even distribution of individuals amongst species (Reynoldson & Metcalfe-Smith 1992).

The earliest forms of diversity indices (Margalef 1951, cited by Warren 1971) expressed the species richness of a community as the number of species relative to the total number of individuals. A modified version (the Shannon-Weiner index), which is now commonly used, also takes into account the number of individuals per species (Wilhm & Dorris 1968, cited by Hawkes 1979). Given that the calculation of diversity indices require quantitative data, traditional methods such as box-sampling need to be used.

Diversity indices are considered to have the following advantages: they are relatively independent of sample size (Pinder *et al.* 1987); they are quantitative, dimensionless, and lend themselves to statistical analysis (Cook 1976); and no assumptions, which may be very subjective, are made as to the relative tolerances of individual species (Pinder *et al.* 1987).

Many studies have assessed the effectiveness and sensitivity of the various diversity indices

in detecting alterations in community structure (e.g. Boyle *et al.* 1990, Barton & Metcalfe-Smith 1992, Camargo 1992). Godfrey (1978) questioned the accepted assumption that water pollution causes a depression in diversity, citing inconsistencies between diversity and other indices. Low diversity may in fact be caused by physical stresses, for instance in torrential mountain streams which nonetheless have excellent water quality (Wells 1992) and in cases where pollution may be masked by a change in species composition (from sensitive to tolerant species) that would not be reflected in a species diversity index. The response of a community to increasing pollution is also not necessarily linear (Metcalfe-Smith 1989). Based on a sensitivity analysis of nine diversity and seven similarity indices, Boyle *et al.* (1990) concluded that the response of community level indices are dependent on the initial structure of the community, and on the manner in which the community is changed. He suggests that diversity indices are not appropriate indicators of ecosystem integrity unless they are used in conjunction with other indices.

Biotic indices

Biotic indices are generally used to quantify the degree of pollution in an aquatic system by assigning scores based on organisms' sensitivity or tolerance to pollution (some indices also score abundance). These scores are summed to provide a value which is an indication of the pollution status of the particular site. Cook (1976) stated that the "ideal" biotic index should have the following attributes:

1. be sensitive to the effects of pollution,
2. have general application to different types of streams,
3. provide a continuous assessment from unpolluted to polluted sites,
4. be independent of sample size, and
5. have relatively simple data gathering and index calculations.

Sheehan (1984) recommended the inclusion of two additional properties. A biotic inded should:

6. have the ability to distinguish the cyclical and natural variability of the system, and
7. should be ecologically meaningful.

According to Herrick & Cairns (1982) the primary weakness in biotic indices is the subjective assessment which is often used to classify the tolerance of organisms. Winget & Mangum (1979, cited by Herrick & Cairns 1982) proposed a biotic condition index based on extensive correlations between species presence and water quality. The approach is data-intensive and requires complex analysis to establish local and regional species/tolerance relationships, but it does support continued use of biotic indices as an essential part of biological assessment of community structure and provides an objective framework for score allocation. Murphy (1978) examined the variability of biotic scores over time and summarised the potential sources of error which could affect the number of species present and hence the biotic score. They include altitude of the site, sampling method, sample size, prevailing conditions at the time of sampling, seasonal cycles and invertebrate drift. Certain biotic scores can be calculated from community data collected either quantitatively (e.g. box-sampling) or qualitatively (e.g. kick net), although certain indices require abundance values. It is important to acknowledge differences in sampling methods when using community data to calculate biotic scores.

Numerous biotic indices and score systems have been developed. The following section provides a brief overview of the biotic indices that have been developed (see Metcalfe-Smith 1991 and Resh & Jackson 1993 for details). Figure 3.2. is a relational diagram of the historic development of the biotic indices. It based on that of Metcalfe-Smith (1991) but has been modified to include the additional indices discussed in the following sections.

a. Trent Biotic Index (TBI)

This index was originally developed by the Trent River Authority (Woodiwiss 1964, cited by Johnson *et al.* 1993) in the United Kingdom for use in the Trent River area. It is based on the presence or absence of six key groups and their relative tolerance to pollution. The key groups are plecopteran nymphs, ephemeropteran nymphs, trichopteran larvae, *Gammarus* (a genus of Amphipoda), *Asellus* (a genus of Isopoda), and tubificid oligochaetes and/or red chironomid larvae (*Chironomus* spp.). Plecopteran and ephemeropteran nymphs need higher dissolved oxygen concentrations than do tubificid worms and red chironomid larvae. Each sample is given a score ranging from 0 (=polluted site where none of the above animal groups are present) to 10 (=clean water site where more than one species of Plecoptera and

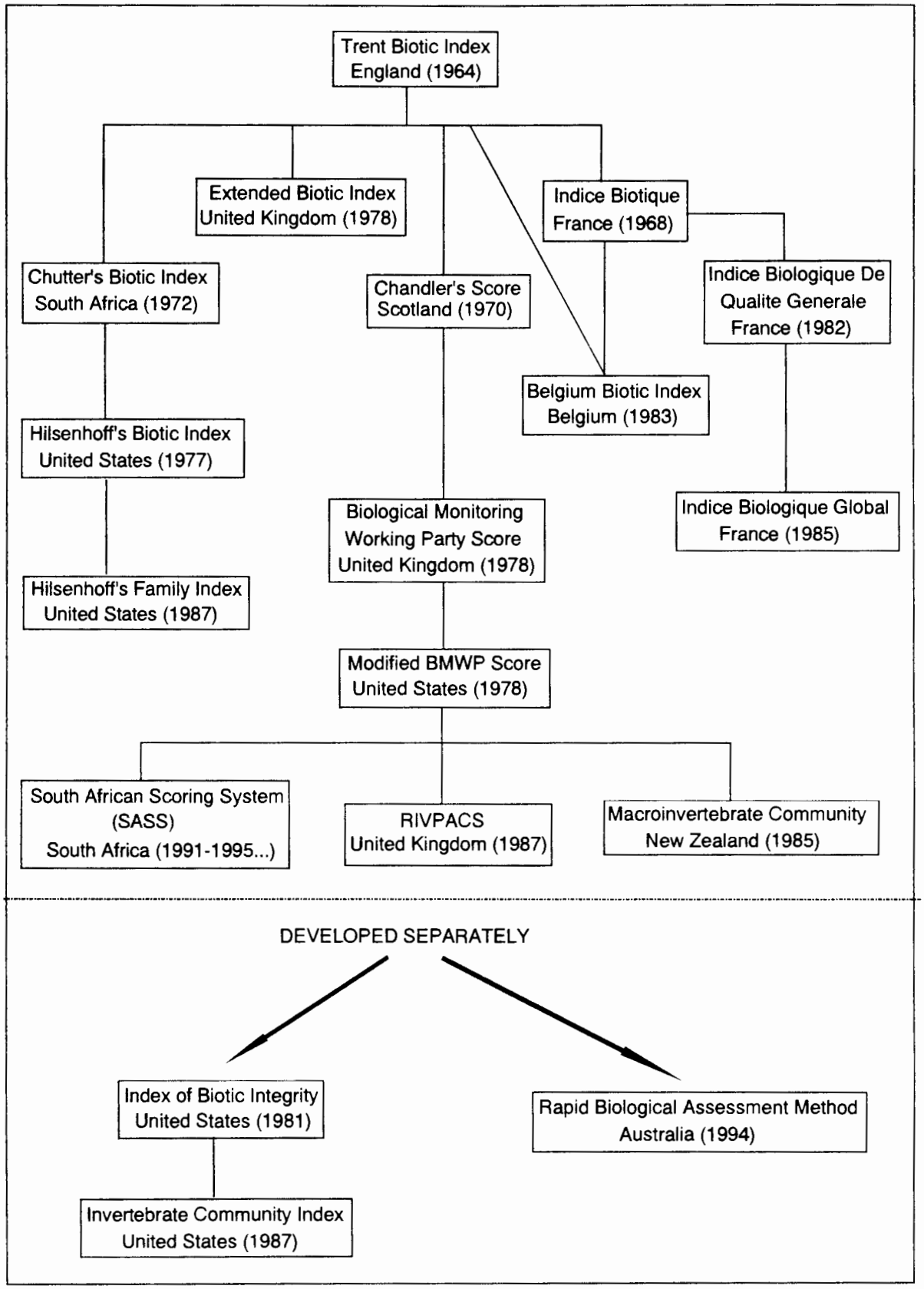


Figure 3.2. Diagram to illustrate the historical development of biotic indices (modified from Metcalfe-Smith 1991).

several taxonomic groups are present). The TBI has been criticized for being insensitive (Washington 1984, cited by Johnson *et al.* 1993) and providing erroneous results. Over a fifteen-year period this index was modified to form many subsequent indices such as Chandler's Score, the Biological Monitoring Working Party (BMWP) Score, the modified BMWP Score and the Belgian Biotic Index, which are discussed below.

b. Chandlers's Biotic Score (CBS)

This scoring system (Chandler 1970, cited by Johnson *et al.* 1993) is based on the TBI, particularly in terms of the relative sensitivity of key groups to pollution, but it also incorporates an abundance factor in the final calculation of index score. Because CBS's were low in unpolluted, headwater sites, Balloch *et al.* (1976, cited by Johnson *et al.* 1993) modified the CBS by normalising it for the number of groups present in a sample. The usefulness of this scoring system was questioned by Pinder & Farr (1987, cited by Johnson *et al.* 1993), who showed that the average CBS was insensitive to subtle changes in water quality in the River Frome, England. Other criticisms include the non-uniform nature of the taxonomic identification and the complicated nature of the scoring system (Metcalfe-Smith 1991).

c. Biological Monitoring Working Party (BMWP) Scoring System

This system is a simplification of Chandler's scoring system, where all organisms are identified to Family level for uniformity and families with similar pollution tolerances are grouped together (Armitage *et al.* 1983). The abundance factor of Chandler's scoring system was eliminated because it was time-consuming and had a small effect on score value (Metcalfe-Smith 1991). Pollution-intolerant families are given a high score (10), whereas pollution-tolerant families are given a low score (2). The sum of the scores of individual families present in a sample yields the Total Score for the site. This Total Score is then divided by the number of taxa to give an "Average Score per Taxon" (ASPT) values. Armitage *et al.* (1983) assessed the performance of the BMWP score and ASPT at 268 unpolluted running water sites in the United Kingdom. Changes in both Total Score and ASPT in relation to season were slight; Total Score increased with sampling effort, although ASPT remained relatively constant. Pinder *et al.* (1987) compared the BMWP Scores and ASPT on a chalk stream in southern England, and found that ASPT was relatively

independent of sample replication, sampling method, season and habitat.

More recently, Wright *et al.* (1989) have produced the computer-based River Invertebrate Prediction and Classification System (RIVPACS) program. RIVPACS uses the probability of capture of taxa as described by Moss *et al.* (1987), and predicts the occurrence of macroinvertebrates at a given site from a small number of environmental variables (Johnson *et al.* 1993). The comparison of the type of community observed with that predicted from RIVPACS, allows a quotient, the Ecological Quality Index (EQI), to be calculated. The development of a classification system which utilizes both physical and chemical constituents, in addition to a component of the biota, namely benthic macroinvertebrates, has proved to be usable and useful. RIVPACS provides a method for the classification of unpolluted aquatic habitats, which in turn provides information on the number of such sites within each class. The potential for selecting sites, reaches or whole rivers that warrant a "hands-off" conservation approach is clearly evident. The RIVPACS system may also be used to estimate the degree of impact at a site, reach or river by comparing predicted with observed communities; thereby facilitating the rehabilitation of impacted sites to a pre-determined state as established by target communities. The utility of developing a system similar to RIVPACS for the management of aquatic ecosystems in South Africa is stressed. The South African Scoring System (SASS), which is described later in this chapter, could be one tool to be used in the development of such a system.

d. Indice Biotique (IB) and the Indice Biologique Global (IBG)

The Indice Biotique (IB) was developed in France by Tuffery & Verneaux (1968, cited by Johnson *et al.* 1993) and the Indice Biologique de Qualite Generale and the Indice Biologique Global (IBG) are derived from it. The IBG method requires the sampling of eight different biotopes, which are defined on the basis of substrate and velocity conditions. Organisms are mostly identified to Family, but in some cases to class (e.g. Oligochaeta). Organisms are assigned to faunistic groups and ranked in order of increasing tolerance to pollution (rows). The total number of taxa present is used to give a measure of community diversity (columns). The intersection between the appropriate row and column gives the index value for the sample. Major criticisms directed at these methods are the lower scores associated with lowland rivers, primarily because of the absence of a variety of biotopes (substrate was

mostly muddy) and subsequent absence of high scoring Plecoptera, Trichoptera and Ephemeroptera, and the subjectivity of the taxonomic level of identification (Metcalfe-Smith 1989).

e. Belgian Biotic Index (BBI)

The Belgian Biotic Index index (De Pauw & Vanhooren 1983) combines the scoring procedure of the French Index Biotique and the sampling procedure of the Trent Biotic Index. Taxa are identified to predetermined levels (usually Family or Genus) and comprise "systematic units" for special faunistic groups (e.g. Plecoptera, cased Trichoptera). A biotic score is based on the total number of systematic units and the number of units in different faunal groups. Its major advantages are simplicity, speed, reliability, low cost, and practical utility (De Pauw & Vanhooren 1983). The surface water quality of Belgian rivers has been routinely surveyed using the BBI since 1978, and by 1985, 30 000 km of watercourses had been surveyed and mapped using BBI (Metcalfe-Smith 1991). Results were reproducible over long periods of time in areas where no changes in pollution status occurred, and seasonal changes were minor (Metcalfe-Smith 1989). The small size and relative uniformity of Belgium would aid in providing reproducible results. In larger countries, and ones which cover wider geographic areas, problems may arise. The BBI has been shown to be applicable in other countries including Spain, Algeria, Luxembourg, Portugal and Canada (De Pauw *et al.* 1986). De Pauw & Roels (1988, cited by Metcalfe-Smith 1991) found that correlations between chemical variables and the BBI were consistently positive (dissolved oxygen) or negative (BOD, COD, NH_4, PO_4), but that the slopes of the regression lines varied considerably among catchments. This indicated that the degree of stress associated with a particular chemical factor in one river was not necessarily of the same magnitude as that in another. They suggested that "....biological assessments should be used as an early warning system and be the precursor of extensive chemical analyses, identifying the causes of biological stress". Subsequent considerations and recommendations by the developers of this method include the need to establish reference sites or communities against which the BBI scores can be assessed.

The concept of reference sites against which biotic scores taken at a site can be compared has been commented on in various discussions on biotic indices (e.g. RIVPACS and BBI).

This is particularly important in countries that are spread over a wide range of geographic regions. Intrinsic biogeographic differences will result in differences in biotic scores. These differences need to be taken into account when using a method such as SASS to assess the impairment of water quality. There is a need to establish reference sites against which a SASS score can realistically be compared, which are in the same geographic region, river zone (e.g. upland versus lowland rivers) and water type (e.g. acid south-western Cape mountain Streams or lowveld middle-reach rivers). One of the potential applications of SASS is as a "goalpost", i.e. a value that can be strived towards during rehabilitative measures undertaken by a particular authority. This "goalpost" needs to be realistic, and it can only be such if it is comparable to the site undergoing rehabilitation, i.e. in the same biogeographic region, river zone and water type. The question of reference sites is discussed further in chapter 5 where SASS and potential problems associated with this rapid bioassessment technique are discussed.

f. Chutter's Biotic Index

Chutter's Biotic Index was developed in South Africa (Chutter 1972). For a given sample, the number of individuals in a taxon is determined, and then multiplied by the taxon's "Quality value" which is based on the occurrence of individual taxa in polluted waters. The products of these multiplications are summed for the sample. This sum is then divided by the total number of individuals in the whole sample to give the Biotic Index Value (Chutter 1972). The average of the tolerance values for all individuals therefore constitutes the Site Index Value. The index is restricted to fauna associated with the stones-in-current biotope and to the assessment of waters subject to organic pollution. This index has been fairly widely used within South Africa (e.g. Coetzer 1978, Fowles *et al.* 1979) and has been modified for use internationally (Hilsenhoff 1987).

g. Hilsenhoff's Biotic Index (BI)

Hilsenhoff's Biotic Index index (Hilsenhoff 1987) was modified from Chutter's Biotic Index by changing tolerance values for local fauna (Wisconsin, United States) and excluding selected invertebrate taxa. Approximately 400 species or genera have been assigned scores. Hilsenhoff (1988) also adapted his index for rapid biological assessment by providing tolerance values for families (Family-level Biotic Index: FBI). He found that the FBI tended

to be higher at unpolluted sites and lower at polluted ones, and that the FBI was more variable than the BI.

h. Invertebrate Community Index

The Invertebrate Community Index (ICI, Ohio EPA 1987) index is derived from the IBI (Index of Biotic Integrity), which is based on the attributes of fish communities and is described in detail in Karr (1991). The ICI consists of ten structural and functional community metrics: total number of taxa, total number of mayfly taxa, total number of caddisfly taxa, total number of dipteran taxa, percentage mayfly composition, percentage caddisfly composition, percentage midge (tribe Tanytarsini) composition, percentage other dipteran and non-insect composition, percentage tolerant organisms (oligochaetes and various dipterans and molluscs) and taxon richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT Taxa). The scores allocated to each of these metrics are summed to generate a site ICI score. Both Hilsenhoff's Biotic Index and the Invertebrate Community Index are frequently used in the United States (Reynoldson & Metcalfe-Smith 1992).

i. Australian Rapid Biological Assessment Method

The Australian Rapid Biological Assessment Method (Chessman 1994) involves the standardised collection of 100 animals from each of the defined habitats (riffles, stream edges or backwaters, rocks in pools and submerged wood) within a water body. Specimens are identified to Family level and two metrics are calculated: SIGNAL biotic index (similar to BMWP- ASPT score) is the average of pollution sensitivity values for each macroinvertebrate family in the sample, and SIMREF: a similarity measure derived by calculating the Bray-Curtis association measure between the site and a reference site. This procedure has and is continually being tested within New South Wales and the correlation of metrics SIGNAL and SIMREF to stream disturbance, in particular proximity of sewage treatment plants and catchment land use and cover, is under investigation.

j. Macroinvertebrate Community Index (MCI)

The Macroinvertebrate Community Index was developed for use in New Zealand's stony

streams and is based on the BMWP method, although scores are allocated at the generic level (Stark 1985, Stark 1993). The MCI has facilitated the production of a site ranking list and site groupings for stony-riffle sites from streams on the Taranaki ringplain. It is widely used throughout New Zealand (Stark 1993).

k. South African Scoring System (SASS)

The SASS method of rapid biological assessment is currently being developed for use in South African streams and rivers (Chutter 1992, 1994a, 1994b, Chutter & Geuppert 1993, Moore & McMillan 1993). It is based on benthic macroinvertebrates and is specifically aimed at assessing the impairment of water quality. The scoring system is derived from the BMWP system although scores have been modified for local taxa and the range of scores expanded so that a tolerant taxon is allocated a score of one whereas a sensitive taxon is allocated a score of 15. These scores have largely been based on expert opinion, in particular the opinions of researchers within the Rapid Biological Assessment (RBA) Forum who have, and are, utilizing the SASS method. The Total Score per site is calculated by summing the taxon scores and the Average Score per Taxon (ASPT) is calculated by dividing this Total Score by the number of taxa. Both scores are considered when determining water quality impairment. SASS is the rapid bioassessment method used in the present study. Details of its application are given in Chapter 4.

Community Comparison Indices

Even when frequent, complete sampling of macroinvertebrates or other organisms is undertaken, analysis of the data may be problematic. A wide range of computer-based similarity and dissimilarity indices, all intended to provide comparisons between sites or between sampling programmes, is currently in use. The indices all have different mathematical properties and thus analyze different properties of the ecosystems under consideration (Reynoldson & Metcalfe-Smith 1992). Because of these differences, each of the programs may generate different results when applied to the same data set. For this reason, the objectives of the analysis, and type of data available, must be carefully considered before community comparison indices are applied. The indices that appear to have been most successfully applied are the Percent Similarity Index (PSC), Pinkham and Pearson's B, and the Bray-Curtis Index (Reynoldson & Metcalfe-Smith 1992). Of these the

Bray-Curtis index has been identified by Hruby (1987, cited by Reynoldson & Metcalfe-Smith 1992) as the most valuable because of the following properties: "Species abundances are included; the index uses transformed data, which increases the importance of rare species; the index values are not clumped; and there is a linear response to changes in species numbers and abundances". Boyle *et al.* (1990) tested the sensitivity of this index, however, and found that it was relatively insensitive at low levels of perturbation. Essentially, similarity indices compare community assemblages (either at the species, generic or Family levels) at different sites and/or at different times. These methods are informative and less reliant on sampling intensity, but require realistically comparable sites (e.g. upper mountain streams), sampling methods and taxonomic effort (e.g. Family-level data cannot be compared with generic-level data).

Functional Feeding Groups (FFGs)

Invertebrates can be classified into Functional Feeding Groups according to their feeding mechanisms. Common FFGs include scrapers, grazers, deposit feeders, filter feeders, shredders and predators. In unperturbed systems, the number of invertebrates within each FFG changes such that FFGs are assumed to occur in predictable proportions along the length of a river. These changes have been attributed to natural longitudinal changes in food sources down the length of the river (Cummins 1988). It is assumed that any perturbation would disrupt this pattern. The method requires identification to species and a good knowledge of the feeding mechanism of each species. Metcalfe-Smith (1991) suggests that since this approach is based on nutrient dynamics, it can only be used to assess the effects of organic enrichment. The information available on the FFGs of South African riverine organisms is poor and there is also some doubt about the theoretical basis of studies of this kind. The topic is reviewed in Palmer (1991).

Reduced Assemblages

This technique assumes that useful information about the integrity of a biotic community can be gleaned by examining a single well known component of that community. In terms of invertebrates, commonly used groups include oligochaetes, chironomids and trichopterans. All three groups have been shown to possess species with a wide variety of tolerance levels. By limiting assessment to one group, taxonomic refinement within the group can be greater

and individual research workers are able to use their expert judgement in relation to the group with which they are most familiar.

3.4. CONCLUSIONS

The preceding sections provide an overview of the various biological assessment methods developed locally and internationally. Whilst various components of riverine biota have been used in the assessment of the impairment of water quality, the recent development of SASS in South Africa directed the focus of this study towards its potential application in the assessment of the impairment of water quality. In the present study two methods of biological assessment using benthic macroinvertebrates have been used, namely traditional quantitative box-sampling and rapid bioassessment, SASS. The following chapter describes the Berg River catchment, study-sites and sampling methodology.

CHAPTER 4

DESCRIPTION OF THE CATCHMENT, STUDY-SITES AND SAMPLING METHODOLOGY

4.1. INTRODUCTION

This study was initiated to investigate the reliability of the rapid bioassessment method, SASS. Research was restricted to one geographic region in order to minimise variability resulting from regional differences. The Berg River in the south-western Cape was selected for two reasons. Firstly, the Berg River catchment is one of the main sources of water for domestic, industrial and agricultural purposes in the Western Cape. The persistent and ever increasing demand for water within this region has resulted in ever greater pressure being applied to the river as a source of water. This pressure has focused the attention of various "users" on the Berg River. Secondly the Berg River was the first river in South Africa on which a detailed limnological study was conducted (Harrison & Elsworth 1958). The extensive historical database associated with the Berg River is valuable in determining the historical status of the river, both in terms of the biota and the physical and chemical constituents of the river water.

This chapter describes the location, topography, climate and geology of the Berg River catchment. The zonation of the river and the characteristics of each zone are given, in addition to a detailed description of each site. The methods used for the collection of biological samples are described and a detailed account of the South African Scoring System (SASS) is provided. The methods for the analyses of physical attributes and chemical constituents are given and the methods used for data analysis are outlined.

4.2. DESCRIPTION OF THE BERG RIVER CATCHMENT

Location and topography

The Berg River (Figure 4.1) rises in the Franschhoek and Drakenstein Mountains (1220 to 1800 m above sea level) approximately 60 km east of Cape Town, and flows northwards past the towns of Paarl and Wellington and the village of Hermon, before arcing westwards past Gouda and Piketberg. It discharges into the Atlantic Ocean at Velddrif (ca 130 km north of Cape Town). The river valley is approximately 160 kms long from headwaters to sea and the catchment covers an area of about 6 415 km^2. There are nine major and seven minor tributaries. Historically six of the tributaries were perennial (Franschhoek, Wemmers, Dwars, Klein Berg, Twenty-Four, Maatjies Rivers), although flow is often considerably reduced in summer in all but the Wemmers River, which has an upstream catchment area of 125 km^2. A dam completed in 1957 regulates the water flow along this tributary and inflow into the Berg River. The other tributaries are ephemeral or seasonal and normally dry up in summer. Voëlvlei Dam, constructed prior to 1951 by enlarging a natural lake basin and later by supplementing the inflow with tunnels tapping the Klein Berg and Twenty-Four rivers, maintains a minimum flow for agricultural purposes in the lower river during the dry season.

Climate

The catchment of the Berg River has a mediterranean climate and falls within the winter rainfall region of the Western Cape. This rainfall is not evenly distributed throughout the season and 80% falls as short winter downpours. Rainfall is high in wet years, reaching a maximum of 5000 mm y^{-1}, in the southern mountains but dropping to 400-500 mm y^{-1} in the middle and lower reaches.

Geology

Geologically the Berg is an old river, and the vertical drop from the mountain source to Paarl (12% of its length) is 900 m. The course can be traced back for at least 80 million years and it once shared a mouth with the Proto-Upper Orange River (Dingle & Hendey 1983). The peaks and most of the mountain catchment are composed of quartzitic Table Mountain

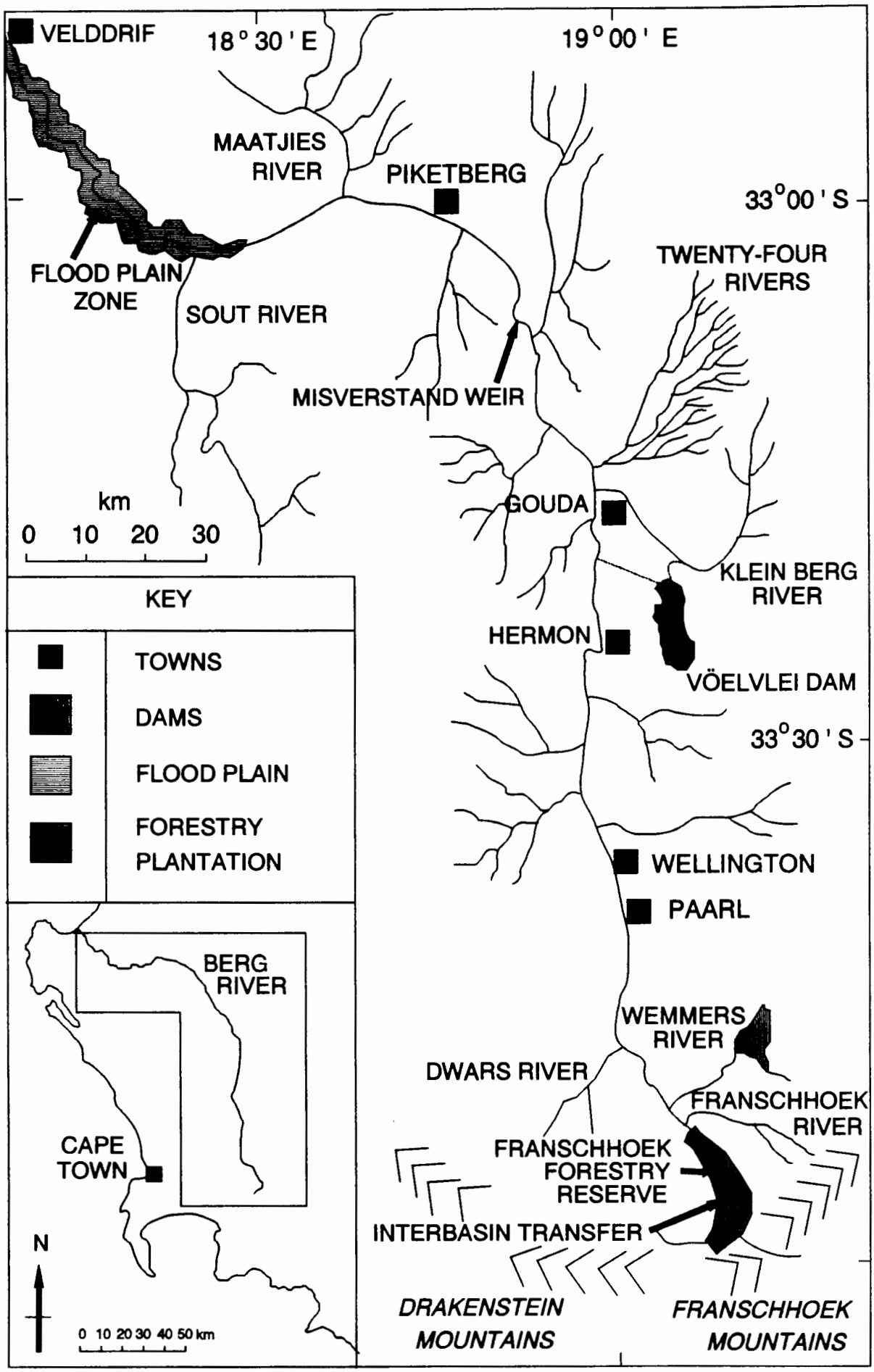


Figure 4.1. Map of the Berg River catchment showing major towns and tributaries.

Sandstone (TMS). The TMS, which was laid down largely between 440 and 380 my, forms part of the Table Mountain Group of the Cape Supergroup. TMS is ancient, leached and weathered so water running off these formations is generally very low in mineral content, acidic and poorly buffered. In the Paarl region a few small tributaries arise in granite hills and flow through clay-soils consisting of weathered granite material. Near Piketberg the erodible Malmesbury shales, which are much older (> 700 my) and are mostly mudstones and shales with high levels of salt, become the dominant underlying rock formation. Associated with these rock formations are large amounts of salts which leach out into the water as it flows over them, resulting in a high mineral content in the lower reaches of the Berg River. Tributaries with particularly high salt content include the Sout and Maatjies Rivers. Another characteristic of the lower reach is the meandering nature of the river. This feature is directly linked to the erodible nature of the Malmesbury shales. An extensive flood plain consist mainly of quartzitic sand and mud and the meandering nature of the river results in the formation of numerous flood plain pans.

Zonation and zone characteristics

The following section outlines the main characteristics, major impacts and water quality traits within each zone along the Berg River. The zones are the same as those described by Harrison & Elsworth (1958) in their study of the Berg River.

Source: The mountain streams, which form the Berg River, have two main types of source: cliff waterfalls and sponges. This zone is largely unimpacted since human access is restricted.

Mountain torrent zone: The uppermost reach in this zone is largely unimpacted. The river bed is unstable and stony and is broken by large rocks which create cascades, small waterfalls and pools. The riparian vegetation is natural fynbos (the sclerophyllous vegetation of the south-western Cape fynbos biome).

Foothill, stony run zone: The river bed in this zone is more stable and uniform and consists of cobbles. Impacts include forestry, trout farming, interbasin water transfer and agriculture (mostly vineyards). The trout farm is situated immediately below the Franschhoek Forestry

Reserve. The interbasin transfer connection conveys water from Theewaterskloof Dam (Riviersonderend catchment) to the Upper Berg in summer. The implications of this transfer in terms of water quality and the diversity of aquatic animals have not yet been determined. The trout farm and interbasin transfer scheme, together with increased agriculture in the Franschhoek area, most likely account for the changes in the aquatic fauna that have taken place since the first ecological survey in the 1950's (Dallas 1992). Several tributaries join the main river within this zone, namely the Franschhoek, Wemmers and Dwars rivers. Continuing within this zone, the river runs through the towns of Paarl and Wellington, where organic enrichment is prevalent.

Foothill, soft bottom zone: Between Wellington and Hermon the stony river beds are replaced by soft-bottomed runs and pools. The Vöelvlei Dam discharges into the Berg River between Hermon and Gouda. The Misverstand Dam is the current storage facility on the lower Berg and is earmarked for enlargement in 15 years time. Near Piketberg a small section of Malmesbury shales have been exposed, resulting in a short series of stony runs and riffles. Large quantities of salts are leached into the water as it flows over these shales. This, together with return-flow irrigation water from the adjacent agricultural (mostly grainlands) area, has lead to a dramatic increase in salinity of the lower reaches of the river.

Flood plain zone: The river in this zone becomes naturally canal-like as it meanders seawards. The river bed is mainly quartzitic sand topped by a layer of mud and there are numerous flood plain pans which provide good refuge for birds. This is the upper limit of tidal flow intrusion.

4.3. STUDY-SITE SELECTION AND DESCRIPTION

The main criteria for site selection were variation in water quality and the presence of stones-in-current biotope. The ability of biological assessment methods to demonstrate differences in water quality is one of the core objectives of this study. To facilitate realistic comparisons between sites, only sites where the stones-in-current biotope was available for sampling were selected. The stones-in-current biotope may be defined as an area where gravels, cobbles or boulders are present in a body of water and where flow velocity is sufficient to prevent

settling out of fine sediments or detritus, and includes such features as riffles, runs, flats, rapids, cascades and waterfalls (Wadesome 1993). The following section provides a description of each of the three study sites.

Site 1 (33°59'S, 19°04'E)

Site 1 (B1, Figure 4.2.) is situated on the upper Berg River in the Franschhoek Forestry Reserve at an altitude of 320 m. It is a relatively unimpacted, second-order mountain stream within the mountain torrent zone and is located above any impact points, in particular above any forestry operations. The river consists of a series of natural runs, riffles, pools and cascades and is lined with an open-canopy, natural fynbos riparian vegetation. The water runs off Table Mountain Sandstone and is therefore acidic, mineral-poor and poorly buffered. This site is the same as Site 1 of Harrison & Elsworth (1958) and Site 1 of Dallas (1992).

Site 2 (33°52'S, 19°02'E)

Site 2 (B6, Figure 4.2.) is situated immediately above the Jim Fouche Bridge, which crosses the Berg River on the route to Franschhoek, approximately 14 km from the source, at an altitude of 160 m. This fourth-order river is within the foothill, stony-run zone and has a uniform cobble bed consisting of a series of runs and riffles. The four main impacts upstream of this site [interbasin water transfer, a trout farm, agricultural and semi-urban run-off from the Franschhoek area, and forestry operations (*Pinus pinaster*) in the Franschhoek Forestry Reserve], are likely to contribute to the increased levels of organics and nutrients at this site. This site is the same as Site 5 of Harrison & Elsworth (1958) and Site 3 of Dallas (1992).

Site 3 (33°08'S, 18°52'E)

Site 3 (B12, Figure 4.2.) is approximately 116 km from the source at an altitude of 40 m. It is positioned below the Department of Water Affairs & Forestry gauging weir (G1M13; Drieheuwels) at which point the river is of fifth-order. The river consists of a series of cobble runs and riffles. It was selected because of the high conductivity. This site was approximately 4 km south-east (upstream) of Harrison & Elsworth's (1958) Site 16 and Site 13 of Dallas (1992).

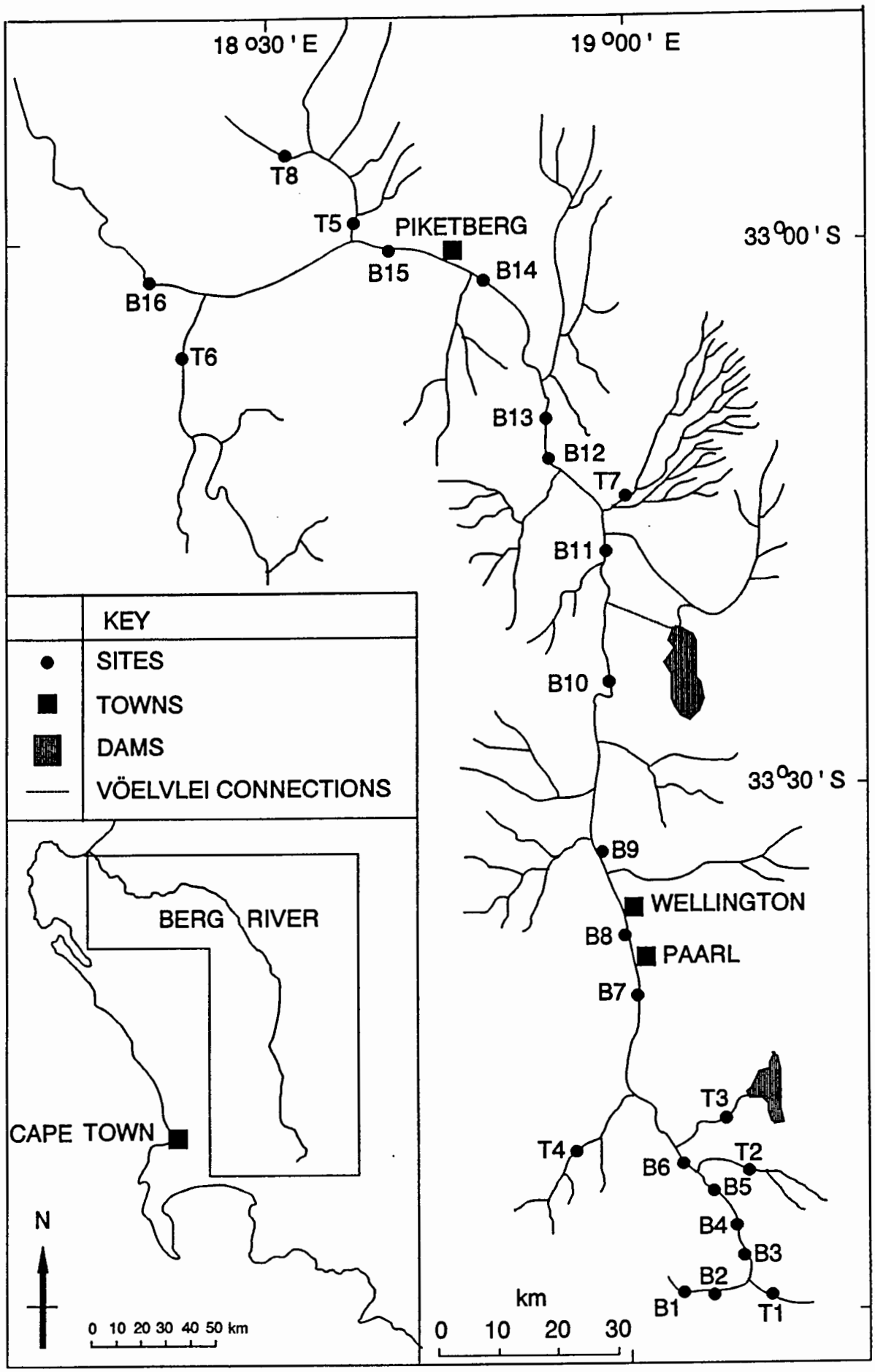


Figure 4.2. Map of the Berg River catchment showing the study-sites. Sites B1, B6 and B12 were sampled in February 1993, and Sites B1 to B16 and T1 to T8 were sampled in September 1993.

4.4. COLLECTION METHODS

Sampling was undertaken in February 1993, and data resulting from this sampling period were analyzed to address objectives one, two and three. These were: 1) to test the variability between samples taken within one biotope at a particular site using the two different methods, i.e. traditional quantitative box-sampling and qualitative rapid bioassessment using SASS (South African Scoring System); and thus 2) to ascertain the number of samples that should be taken using each method, in the case of quantitative sampling to allow adequate representation of the benthic macroinvertebrate community at that site, and in the case of SASS, to allow adequate representation of the SASS scores; and 3) to investigate the influence of mesh diameter of the sampling equipment on the adequate sampling of benthic macroinvertebrate communities. Objectives 2 and 3 are designed to determine the most labour- and time-effective method of benthic macroinvertebrate collection.

A second sampling session, conducted in September 1993, was designed to assess the "state of the river" in terms of SASS scores and to provide greater insight and preliminary data on potential problems associated with the method. Full SASS samples (i.e. incorporating all available biotopes) were taken at 24 sites within the Berg River catchment, sixteen sites of which were on the main river (B1 to B16) and eight on tributaries (T1 to T8) (Figure 4.2). The method used is as described in the following section, except sampling was not replicated and a single SASS score was attained for each site.

4.4.1. Benthic macroinvertebrates

In February 1993, benthic macroinvertebrates were collected using two methods: quantitative box-sampling and qualitative kick-sampling. The details of the respective techniques are described below.

Quantitative benthic sampling

Twenty samples were taken at random localities within the stones-in-current biotope at each site using a box-sampler (area=0.1 m^2, 250 μm-mesh). Site 1 covered a 200 m stretch of

Site 3 was approximately 50 m in length with a maximum width of 20 m. Fauna within the 0.1 m^2 area were displaced from the stones by brushing and then each stone was systematically brushed and removed from the sampling quadrat. Approximately 100 mm of substratum was vertically disturbed and all organisms were collected in the jar at the closed end of the box-net. Each sample was immediately fixed in 7% formalin and transferred to 70% alcohol in the laboratory. Each sample was separated into three size fractions, >950 μm, <950 μm but >500 μm, and <500 μm but >250 μm. Organisms were identified to Family level and counted. This taxonomic level was chosen since one of the primary objectives of the study was comparison with the rapid bioassessment method, which requires taxonomic resolution at Family level only.

Qualitative benthic sampling/rapid bioassessment

Twenty samples were taken randomly within the stones-in-current biotope at each site using the SASS (South African Scoring System) method (Moore & McMillan 1993). Although SASS is designed to incorporate all available biotopes at a given site (stones-in-current, stones-out-of-current, marginal vegetation, instream/aquatic vegetation, gravel, sand and mud) sampling during this study was restricted to the stones-in-current biotope to facilitate realistic comparison with the quantitative method, which cannot readily be done in other biotopes. A kick net (300x400 mm frame, 950 μm-mesh) was held immediately downstream of the area to be sampled. Stones 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 in the net. The contents of the net were tipped into a large sorting tray. Debris impeding the viewing of organisms was removed after checking that no organisms were clinging to it. Most organisms in the tray were identified to Family level, recorded and abundance estimated (A = 1 to 10, B = 11 to 100, C = 101 to 1000 and D >1000). The tray was searched for approximately 20 minutes or until five minutes had passed since an additional family was found. Data were recorded on a standardized datasheet (Table 4.1.) and representative samples were collected for preservation and curating.

Table 4.1. Field scoring sheet for the rapid bioassessment method, SASS (modified from Chutter 1994a).

SASS4

River.....Date.....Time.....

Sampling point.....

Temp °C.....pH.....Cond mS m^{-1}.....

Biotopes sampled:

SIC.....(Type/time.....)

Marg Veg.....Dom.sp.....

Aq Veg.....Spp.....

SOOC.....Sand.....Mud.....Gravel.....

Other.....

Procedure Protocols:

1. If stones-in-current (SIC) all kickable, sample for 2 min, otherwise for a maximum of 5 min.
2. Gravel 1/2 min.
3. Marg/Aq veg, back and forward sweep 2m.
4. Stones out of current (SOOC) kick +/- 1m^2.
4. Sand/mud stir with feet and sweep net over disturbed area for 1/2 minute.
5. Any other biotopes 1/2 min.
6. Complete top of form.
7. Tip net contents into tray. Remove leaves, twigs and trash.
8. Check taxa present on above list for the lesser of 15 minutes or 5 minutes since the last taxon was found.
9. Estimate abundances on scale:
   A: 1 to 10; B: 10 to 100;
   C: 100 to 1000; D: >1000
10. **Before leaving the sampling point check that this form has been fully completed.**

A*: score allocated to taxon.

TAXON	A*	
Porifera	5	
Coelenterata		
Hydra sp.	1	
Turbellaria		
Planaria	5	
Annelida		
Oligochaeta	1	
Hirudinea	3	
Crustacea		
Amphipoda	15	
Decapoda*	3	
shrimps	8	
Arachnida		
Hydrachnellae	8	
Plecoptera		
Notonemouridae	12	
Perlidae	12	
Ephemerellidae		
Polymitarcyidae	10	
Ephemeridae	15	
Baetidae 1 sp. or	4	
2 spp or	6	
> 2 spp	12	
Oligoneuridae	15	
Heptageniidae	10	
Leptophlebiidae	13	
Ephemerellidae	15	
Tricorythidae	9	
Prosopistomatidae	15	
Caenidae	6	
Odonata		
Chlorolestidae	8	
Lestidae	8	
Protoneuridae	8	
Platycnemidae	10	
Coenagriidae	4	
Calopterygidae	10	
Chlorocyphidae	10	
Zygoptera juvs.	6	
Gomphidae	6	
Aeshnidae	8	

Corduliidae	8	
Libellulidae	4	
Hemiptera		
Notonectidae*	3	
Pleidae*	4	
Naucoridae*	7	
Nepidae*	3	
Belastomatidae*	3	
Corixidae*	3	
Gerridae*	5	
Veliidae*	5	
Megaloptera		
Corydalidae	8	
Trichoptera		
Hydropsychidae		
1 sp or	4	
2 spp or	6	
> 2 spp	12	
Philopotamidae	10	
Polycentropodidae	12	
Psychomyiidae	8	
Ecnomidae	8	
Hydroptilidae	6	
Cased caddisfly larvae:		
Number of case types		
1	8	
2	15	
3 or more	20	
Lepidoptera		
Nymphulidae	15	
Coleoptera		
Dytiscidae (adults*)	5	
Elmidae/Dryopidae (adults*)	8	
Gyrinidae (adults*)	5	
Haliplidae (adults*)	5	
Helodidae	12	
Hydraenidae (adults*)	8	
Hydrophilidae (adults*)	5	
Limnichidae	8	
Psephenidae	10	

Diptera		
Blepharoceridae	15	
Tipulidae	5	
Psychodidae	1	
Culicidae*	1	
Dixidae*	13	
Simuliidae	5	
Chironomidae	2	
Ceratopogonidae	5	
Tabanidae	5	
Syrphidae*	1	
Athericidae	13	
Empididae	6	
Ephydridae	3	
Muscidae	1	
Gastropoda		
Lymnaeidae*	3	
Melaniidae*	3	
Planorbidae*	3	
Physidae*	3	
Ancylidae	6	
Hydrobiidae*	3	
Pelecypoda		
Sphaeriidae	3	
Unionidae	6	
Total Score		
Number of families		
ASPT		
Air breather families*		
Air breathers score*		

Other families present

South African Scoring System (SASS)

The SASS method, developed by Chutter for use in riverine ecosystems (1992, 1994a), is based on the Biological Monitoring Working Party (BMWP) method developed in the United Kingdom. Each macroinvertebrate taxon, mostly at Family level, is given a score based on its sensitivity/tolerance to water quality impairment. The SASS method has a number of additional and unique features which were incorporated into the scoring system as a result of discussion between members of the Rapid Biological Assessment (RBA) Forum. The first of these is the introduction of a sliding scale of scoring for two of the families, namely Baetidae (Ephemeroptera) and Hydropsychidae (Trichoptera). Both these families have one or two very tolerant species which are regularly present under impaired water quality conditions. However, there are other species within both these families which are extremely sensitive to pollution. By incorporating a sliding scale of scoring, the presence of more that one species ensures that sensitive ones are accounted for. The second development in the scoring system was the grouping together of the cased-caddisfly larvae (Trichoptera). Whilst it is possible to separate this group into their respective families given the correct level of taxonomic expertise and microscopic identification, the SASS method, which is designed to be a field-based and technician-driven monitoring system, does not permit this higher resolution. As a result of this, the cased-caddis families are grouped and scored on a sliding-scale which is graded on the basis of the number of cased-caddis types present. The number of air-breathing families and their contribution to the Total Score is also recorded.

The procedure outlined above is conducted on site and each family present is scored. These scores are summed to give a Total Score per site. The number of taxa is calculated and divided into the Total Score to provide an Average Score per Taxon (ASPT) value. Interpretation of the Total Score and the ASPT values provide a means of establishing the quality of the water at the site. It is essential that both values be used in assessing water quality. For example, upper mountain streams which have good water quality, often have low diversity and thus a low Total Score. The ASPT values for such streams are generally very high however, indicating that the macroinvertebrate fauna is comprised of a few sensitive, but high scoring taxa. The reverse situation also occurs, for example, in streams which have a high Total Score because of the high number of taxa present. The ASPT value however is low, indicating that whilst the Total Score is indicative of good or intermediate

water quality, the low ASPT value shows that the macroinvertebrate fauna comprises numerous low-scoring or tolerant taxa.

To date a single set of scores has been developed and is currently being applied and tested nationwide. SASS is now in the fourth version, each new version having slightly modified scores as more information is gained. One exception to the general scoring system is the family Leptophlebiidae (Ephemeroptera), whose distribution appears to be pH related (F.H. De Moor, Albany Museum, pers. comm.). In water with pH < 6.5, mostly in the southern and south-western Cape, it is given a higher score than in water with a pH > 6.5. The possibility of modifying scores on a regional basis, or establishing a series of reference sites to facilitate within-region comparisons, should be highlighted. This aspect will be discussed in chapter 5.

4.4.2. Physical and chemical methods and analyses

The primary objectives for measuring the physical attributes and chemical constituents of the water at each site were to determine the quality of the water, in addition to providing a means of calculating the ranges in which the various macroinvertebrate families are recorded. The difficulty in relating an integrated biological score to an instantaneous physical or chemical measurement is acknowledged. The potential for developing an objective means of assigning scores based on field-derived sensitivity/tolerance data indicates that these measurements are necessary and relevant. The following physical attributes and chemical constituents were measured. *In situ* measurements of:

1) Temperature: Mercury thermometer, accurate to $\pm$ 0.5 °C
2) Conductivity: Crison CDTM-523 conductivity meter, accurate to 0.01 mS cm^{-1} and with a built-in temperature compensation of 25 °C. Conductivity values were recorded as μm cm^{-1} and converted to mS m^{-1}.
3) pH: Crison pH/mv meter 506, accurate to 0.01 pH unit.
4) Dissolved Oxygen: YSI model 57 oxygen meter, accurate to $\pm$ 0.2 mg l^{-1}, calibrated for temperature.

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. All filtered water, except that for analysis of ammonia, 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 which had been pre-washed in HCl. The relevant analytical techniques are outlined below.

Total dissolved solids (TDS)

800 ml of filtered water was evaporated from pre-weighed pyrex glass beakers at 60°C. TDS was calculated by difference after evaporation and expressed in mg l^{-1}. Weighing was done on a Sartorius precision laboratory balance accurate to 1 mg.

Total suspended solids (TSS)

A measured volume of water was filtered through pre-weighed, precombusted Whatman GF/F filter papers, dried at 60°C for 48 hours and re-weighed. The organic fraction was calculated by difference after combustion at 450°C for 4.5 hours. Weighing was done on a Mettler AE 100 laboratory balance (readability and reproducibility of 0.1 mg). Measurements were expressed in mg l^{-1}.

Anions

Concentrations of 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 $\pm$ 0.005 mg l^{-1}.

Cations

Concentrations of potassium, sodium, calcium and magnesium were 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 $\pm$ 0.005 mg l^{-1}.

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) concentrations 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}.

Total alkalinity

This 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%.

4.4.3. Data analysis

Since many of the data gathered in the present study are community-based, multivariate procedures were selected for analyses of the data. In contrast to univariate analyses (e.g. ANOVA, regression), multivariate procedures consider each species/family to be a variable and the presence/absence or abundance of each species/family to be an attribute of a site or time (Norris & Georges 1993). Subtle changes in the species composition across sites or in abundance of particular species 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 benthic fauna. The multivariate procedure followed is given below.

Benthic macroinvertebrate abundances were calculated at the level of Family, primarily because this is the resolution to which rapid bioassessment (SASS) is taken. The data matrix consists of p rows (taxonomic groups) and *n* columns (samples), whose data entries are counts of individuals within each taxonomic group for each sample. The quantitative benthic macroinvertebrate data were transformed using the fourth root transformation as recommended in version (VER.4) of the computer package PRIMER (Plymouth Marine Laboratory, United Kingdom). The qualitative benthic macroinvertebrate data were transformed using the presence/absence transformation. A transformation is used to weight the contributions of the different taxonomic groups and the choice of transformation is a

biological not a statistical one. With no transformation, the two-thirds most common groups will dominate the similarity matrix; with moderate to severe transformations, (square root to fourth root), intermediate abundance groups tend to dominate the similarity matrix; and with the most severe transformation possible (presence/absence), rare species will tend to dominate the similarity matrix. The fourth root transformation is invariant to scale change. The Bray-Curtis coefficient has been recommended for biological data on community structure (PRIMER Ver.4) and Bray & Curtis (1957, cited by Field *et al.* 1982) was used on these transformed data. The Bray-Curtis measure has the form (Field *et al.* 1982, p.39):

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

where Y_{ij} = score for the ith species in the jth sample; Y_{ik} = score for the ith species in the kth sample; δ_{jk} = dissimilarity between the jth and kth 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}: $S_{jk} = 1 - \delta_{jk}$

This measure is not affected by joint absences (Field & McFarlane 1968, cited by Field *et al.* 1982) and it gives more weight to abundant species than to rare ones. 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 (1990) 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 location on a "map". A large dissimilarity indicates a greater distance.

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 1990).

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. According to Clarke & Warwick (1990) 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. 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. According to Clarke & Warwick (1990), a stress value of <0.05 gives an excellent representation with no prospect of misinterpretation. A stress value of <0.1 corresponds to a good ordination with no real 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 groups). 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.

Regression analysis and analysis of variance

One Way Analysis of Variance (ANOVA) was conducted on macroinvertebrate abundance and richness data. Data were $\log(X+1)$ transformed where appropriate. Non-parametric analysis of variance, i.e. Kruskal-Wallis, was conducted on diversity and biotic indice data. Regression analysis was used to determine the relationship between Total Score and Average Score per Taxon, and Chi-square to test for significant differences between monthly SASS scores. The results of all analyses were considered significant at $p<0.05$.

CHAPTER 5

VARIABILITY AND SAMPLE REPLICATION ASSOCIATED WITH TWO BENTHIC MACROINVERTEBRATE SAMPLING METHODS AND A COMPARISON BETWEEN THE QUANTITATIVE AND QUALITATIVE METHODS

5.1. INTRODUCTION

Benthic macroinvertebrate sampling is an integral aspect of any study which aims to determine the structure and functioning of riverine ecosystems. Historically most studies have adopted the quantitative box- or Surber-sampling methods. Quantitative sampling is critical if the objectives of the study require that data on community structure (such as species richness, diversity, density and age structure) or functioning (e.g. functional feeding guilds, decomposition rates) be collected. Recently, particularly in relation to biological monitoring, other methods have been developed. The time expenditure associated with quantitative sampling methods (approximately a half hour in the field and five to eight hours in the laboratory for sorting and identification per sample) has necessitated the exploration and development of alternative methods of biological assessment. In South Africa, the South African Scoring System (SASS) is already being used by agencies responsible for biological monitoring and impact assessment. Whilst this method has proved its usefulness in assessing water quality impairment (Chutter 1994b), its relationship to the traditional quantitative methods has not been ascertained. This chapter, in addition to examining the variability and thus sample replication within sites, aims to explore the relationship between the two methods.

5.2. RESULTS

The physical attributes and concentrations of the chemical constituents of water at three sites on the Berg River are given in Table 5.1. These are based on "spot" measurements and samples taken at the time of the benthic sampling. pH, conductivity, total dissolved solids, total suspended solids, organics, total alkalinity, cation and anion concentrations all increase from Site 1 to Site 3. Nutrient concentration, in particular nitrate and ammonium, was considerably higher at Site 2 than at the other two sites.

Table 5.1. Physical attributes and concentrations of the chemical constituents of water at three sites on the Berg River measured in February 1993.

Variable	Unit	Site 1	Site 2	Site 3
Temperature	°C	17.8	21.3	24.2
pH		4.96	6.26	6.87
Conductivity	mS m^{-1}	2.98	6.78	22.00
Total dissolved solids	mg l^{-1}	10.00	37.05	101.11
Total suspended solids	mg l^{-1}	<0.01	4.30	18.40
Organics	mg l^{-1}	<0.01	2.55	3.70
Dissolved oxygen	mg l^{-1}	9.3	8.4	6.4
Total alkalinity	meq l^{-1}	0.004	0.125	0.502
Sodium	mg l^{-1}	4.00	7.50	16.10
Calcium	mg l^{-1}	0.98	2.58	3.85
Potassium	mg l^{-1}	0.31	2.50	3.35
Magnesium	mg l^{-1}	0.48	2.04	9.12
Chloride	mg l^{-1}	4.21	11.60	33.80
Sulphate	mg l^{-1}	0.63	2.57	7.21
Nitrate	mg l^{-1}	0.004	8.340	1.530
Nitrite	mg l^{-1}	<0.001	0.165	0.140
Ammonium	mg l^{-1}	0.011	2.880	1.880
Phosphate	mg l^{-1}	0.001	0.030	0.030

5.2.1. QUANTITATIVE BENTHIC SAMPLING

Variability and sample replication

The mean ($\bar{X}$) abundance of benthic macroinvertebrates and mean number of taxa per sample (0.1 m^2; all size fractions combined) in the stones-in-current biotope at each site is given in Table 5.2.

Table 5.2. Mean ± standard deviation ($\bar{X}$ ± S.D.) of benthic macroinvertebrate abundance and mean ± standard deviation of number of taxa ($\bar{X}$ ± S.D.) in the stones-in-current biotope determined by the quantitative benthic sampling method at three sites on the Berg River.

		ABUNDANCE		NUMBER OF TAXA	
Site	***n***	**$\bar{X}$ ± S.D.**	**Range**	**$\bar{X}$ ± S.D.**	**Range**
1	20	518.4 ± 403	61 - 1533	15.7 ± 4.0	9 - 22
2	20	1284.8 ± 557	537 - 2748	17.0 ± 2.4	13 - 21
3	20	2627.5 ± 1290	941 - 4916	13.9 ± 2.1	10 - 20

One Way Analysis of Variance (ANOVA) was run on log (X+1) transformed data. All sites were significantly different in terms of abundance (F-ratio = 42.79, $p<0.05$), whilst Site 2 and 3 were significantly different in terms of the number of taxa (F-ratio = 5.16, $p<0.05$).

Variability, as a function of cumulative number of sampling units, was examined by calculating the coefficients of variation (CV) for abundance and number of taxa at each site Cv of abundance and CV of number of taxa are plotted as a function of the number of sample units (Figure 5.1). Samples 1 to 20 have been randomised. The plots enable the effects of each additional sample on variability, presented as the coefficients of variation, to be established. At Site 1 the CV of abundance and CV of number of taxa tracked one another closely, both peaking at sample unit six, and then decreasing steadily. At Site 2, both coefficients of variation were highest at sample unit two, and decreased steadily, although CV of abundance peaked again at sample unit 13. At Site 3, the CV of abundance

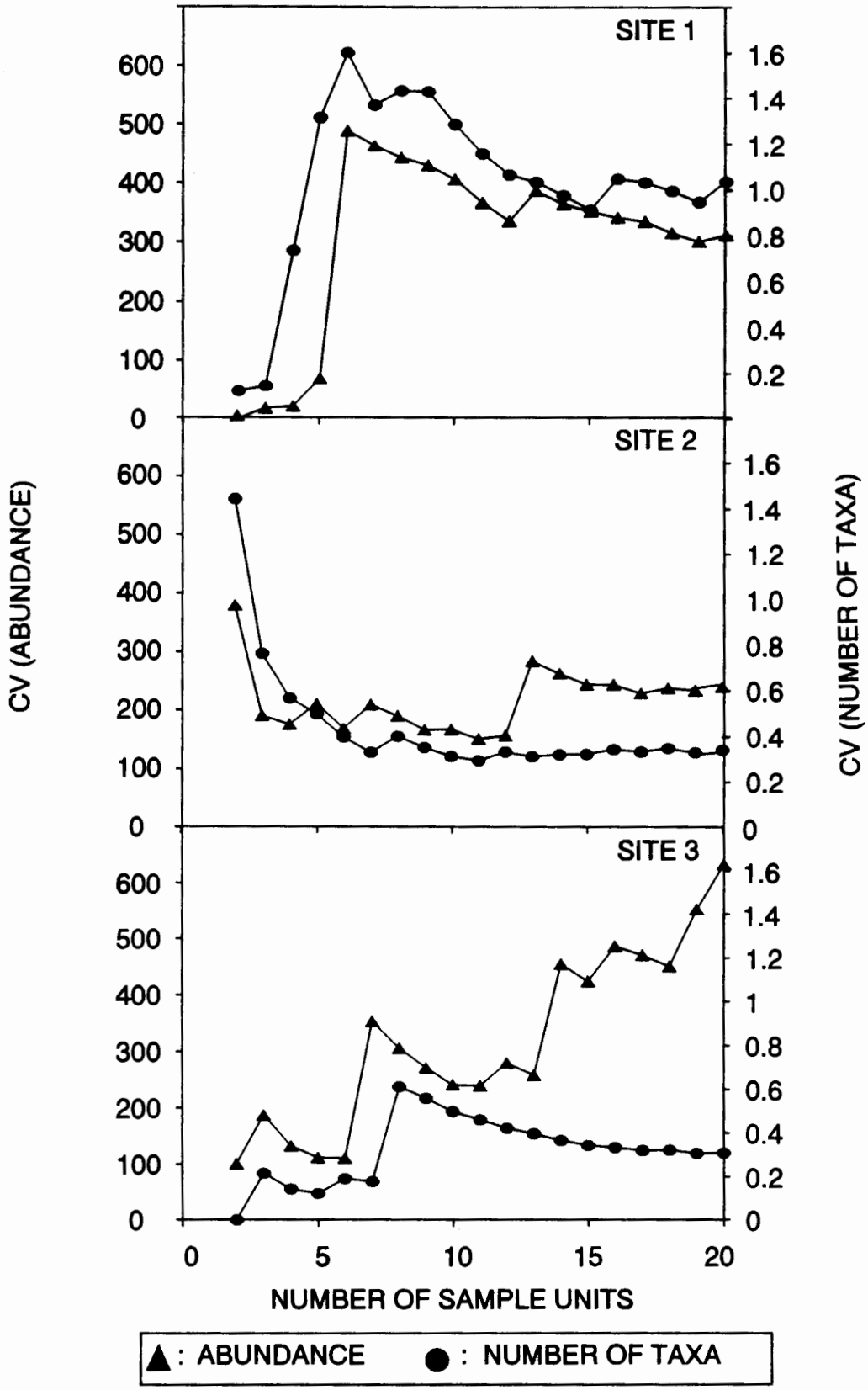


Figure 5.1. The coefficients of variation of abundance and number of taxa plotted against the number of sample units at three sites on the Berg River.

increased steadily and showed no indication of reaching a plateau. This may largely be attributed to the highly variable abundance of Hydropsychidae which range from 293 to 2865 individuals per sample, and constitute 25% to 74% of the total abundance per sample. The CV of number of taxa peaked at sample unit 8 and then steadily decreased.

The relationship between cumulative number of taxa (i.e. new or additional taxa found with each additional sample unit) and the number of sample units was investigated (Figure 5.2).

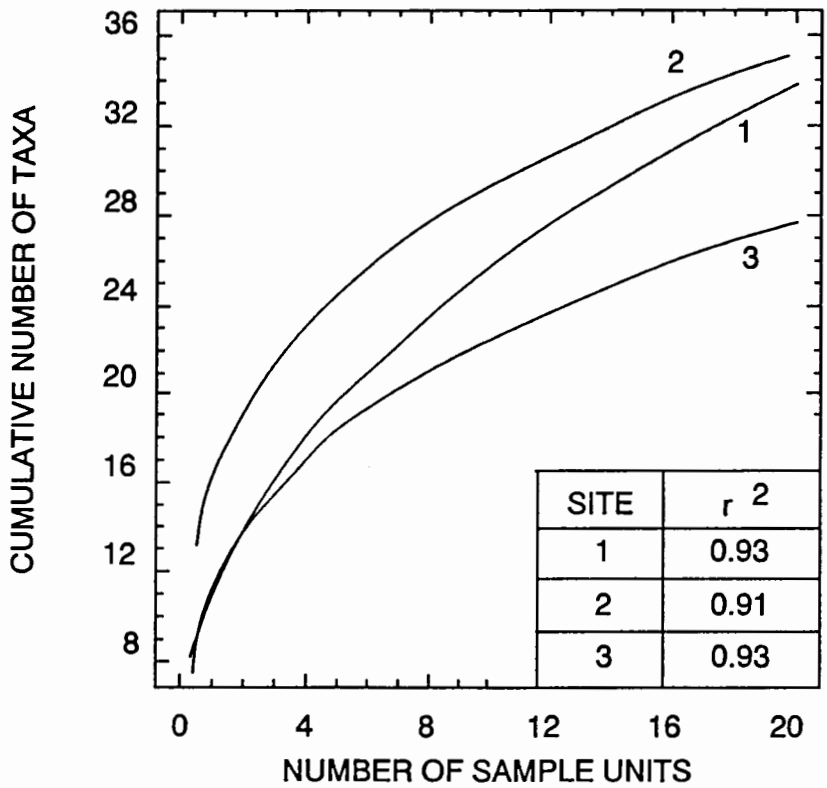


Figure 5.2. Cumulative number of taxa regressed (multiplicative model) against number of sample units collected using the quantitative benthic sampling technique at three sites on the Berg River. r^2 values are given for each site; $p<0.05$.

The number of taxa increased steadily with each additional sample unit. Cumulative number of taxa were significantly positively correlated (multiplicative model, Y=aX^b) with the number of sampling units at all sites. The rate of increase in cumulative number of taxa with increasing number of sample units was greatest at Site 1. Doubling the number of samples taken from five to ten, resulted in an increase by 6.0, 4.9 and 4.0 in the cumulative number of taxa at Sites 1, 3 and 2 respectively. A test for homogeneity of slopes was conducted on

log(X+1) transformed data. There was a significant difference in slopes ($p<0.05$). The number of samples needed to achieve adequate representation of benthic community composition at a site may also be established when no additional taxa are found within the last three samples (L. Underhill, Department of Mathematical Statistics, University of Cape Town, pers. comm.). Three new families found in replicate sample number 20 at Site 1, suggesting that 20 samples is not sufficient to achieve adequate representation of the benthic community at this site. At Site 2 no additional taxa were found after replicate sample number 17. At Site 3 the graph levelled out at sample number 17 but one additional taxon was found in the twentieth sample unit. The number of samples needed to ensure collection of 95% and 75% of the taxa was calculated and is given in Table 5.3.

Table 5.3. Number of replicate samples needed to ensure collection of 95% and 75% of the taxa collected in the stones-in-current biotope using the quantitative sampling technique at three sites on the Berg River.

Site	%	Number of sample units
1	95	17
	75	6
2	95	12
	75	4
3	95	13
	75	8

Based on data from all three sites a minimum of twelve samples is needed to ensure collection of 95% of the taxa (mostly Family-level) and a minimum of four samples for 75% of the taxa. Site 1 required the greatest number of replicate samples (in the 95% range) for adequate collection of taxa.

Calculations in Table 5.3. are based on samples collected within the stones-in-current biotope as defined in Chapter 4, section 4.3. Ecologists however, often select a particular component of such a biotope, for example a "riffle" or "run". In shallow streams, stratification into riffles, runs and pools and then by substrate size in riffles has been

undertaken to reduce inter-sample variation. The objective of stratification is to reduce variability of estimates, thereby reducing required sample sizes and increasing "sensitivity" of the respective study to detection of environmental change (Resh & McElvary 1993). The number of replicate samples needed if one distinguishes between these components is considerably reduced (Table 5.4).

Based on data from three sites a minimum of nine samples is needed to ensure collection of 95% and a minimum of three for 75% of the taxa in riffles. A minimum of six samples is needed to ensure collection of 95% and a minimum of two for 75% of the taxa in runs.

Table 5.4. Number of replicate samples needed to ensure collection of 95% and 75% of the taxa associated with the "riffle" or "run" components of the stones-in-current biotope collected using the quantitative sampling technique at three sites on the Berg River.

Site	n	%		Number of sample units
1	10	95	riffle	9
	10	75	riffle	5
2	13	95	riffle	11
	7	75	riffle	3
3	11	95	riffle	9
	9	75	riffle	4
1	10	95	run	7
	10	75	run	2
2	13	95	run	6
	7	75	run	2
3	11	95	run	7
	9	75	run	4

Site differentiation based on quantitative benthic samples

From the preceding sections it is apparent that variability within sites is a factor that needs to be taken into account when assessing benthic community structure. The number of replicate samples needed to accurately represent the community is relatively high, particularly at the least impacted site. This may be reduced by honing in on microhabitat characteristics such as differentiation into riffle and runs. Given this within-site variability, analyses were conducted to assess the ability of quantitative benthic sampling to differentiate between sites which differed in water quality.

a. Cluster analysis and Multi-dimensional scaling

Cluster and ordination analyses were conducted on faunal samples collected at three sites. Analyses were run on samples which had all size classes combined (hereafter referred to as the combined group), and on the >950 μm size fraction only. The latter was conducted with the intention of determining if the larger size fraction would reflect a similar pattern to that produced when all size fractions were combined. Combining all size classes together and analyzing the resultant 60 samples (20 replicates per site) showed a split into two groups at the 50% similarity level (Site 1 separated from the others) and four groups at 60% similarity (Figure 5.3). At this level each site formed a distinct group, with Site 1 splitting into two groups. These groupings are reflected in two-dimensional ordination (Figure 5.4). The stress values is 0.07. The >950 μm faunal samples (Figures 5.5 and 5.6) split identically into the four groups at the 50% similarity level (four groups) and the ordination shows the same grouping, although the stress value is higher at 0.12.

b. Identification of distinguishing taxa

The taxa principally responsible for differences in community structure between Sites 1, 2 and 3, as measured by the Bray-Curtis dissimilarity measure, are listed in Table 5.5. For the combined groups (i.e. all size classes combined) Sites 1 and 2 (average dissimilarity = 55.1%) differed in the abundance of Notonemouridae, Ephemerellidae and Leptophlebiidae

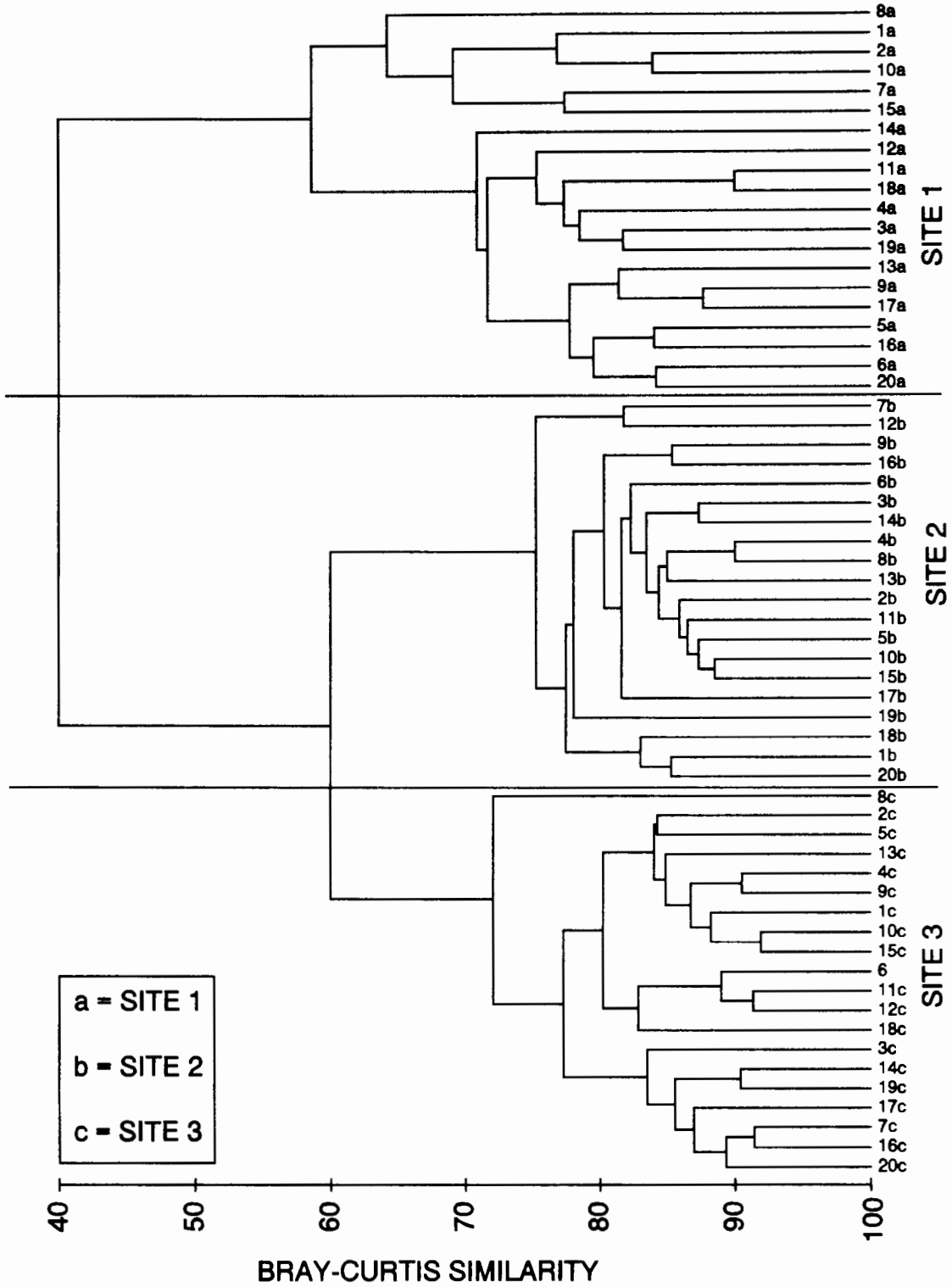


Figure 5.3. Dendrogram showing the classification of 60 quantitative stones-in-current faunal samples collected at three sites on the Berg River. The three size classes, 950, 500 and 250 μm were combined.

which were more numerous at Site 1, and the abundance of Hydropsychidae, Chironomidae, Oligochaeta, Caenidae and Hydracarina which were more abundant at Site 2. At Sites 1 and 3 (average dissimilarity = 64.9%) the greater abundance of Notonemouridae and Ephemerellidae at Site 1, and Hydropsychidae, Chironomidae, Simuliidae, Caenidae and Tricorythidae at Site 3, distinguished the two sites from one another. Sites 2 and 3 were the least dissimilar (average dissimilarity = 40.0%) and Leptoceridae, Hetageniidae and Libellulidae were of greater abundance at Site 2, whilst Hydropsychidae, Simuliidae, Tricorythidae and Hydroptilidae were of greater abundance at Site 3.

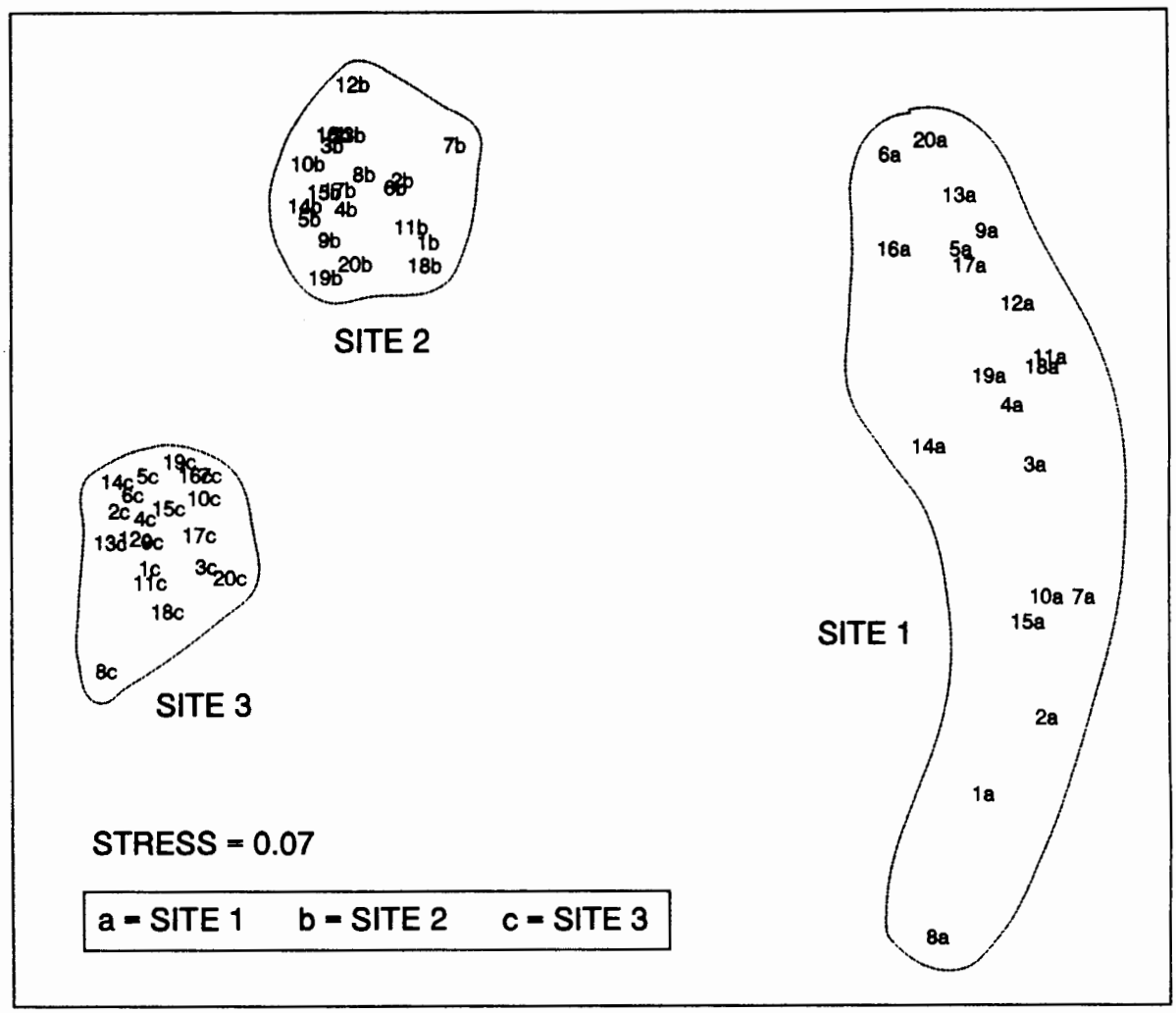


Figure 5.4. Ordination of 60 quantitative stones-in-current faunal samples collected at three sites on the Berg River. The three size classes, >950, <950->500 and <500->250 μm were combined.

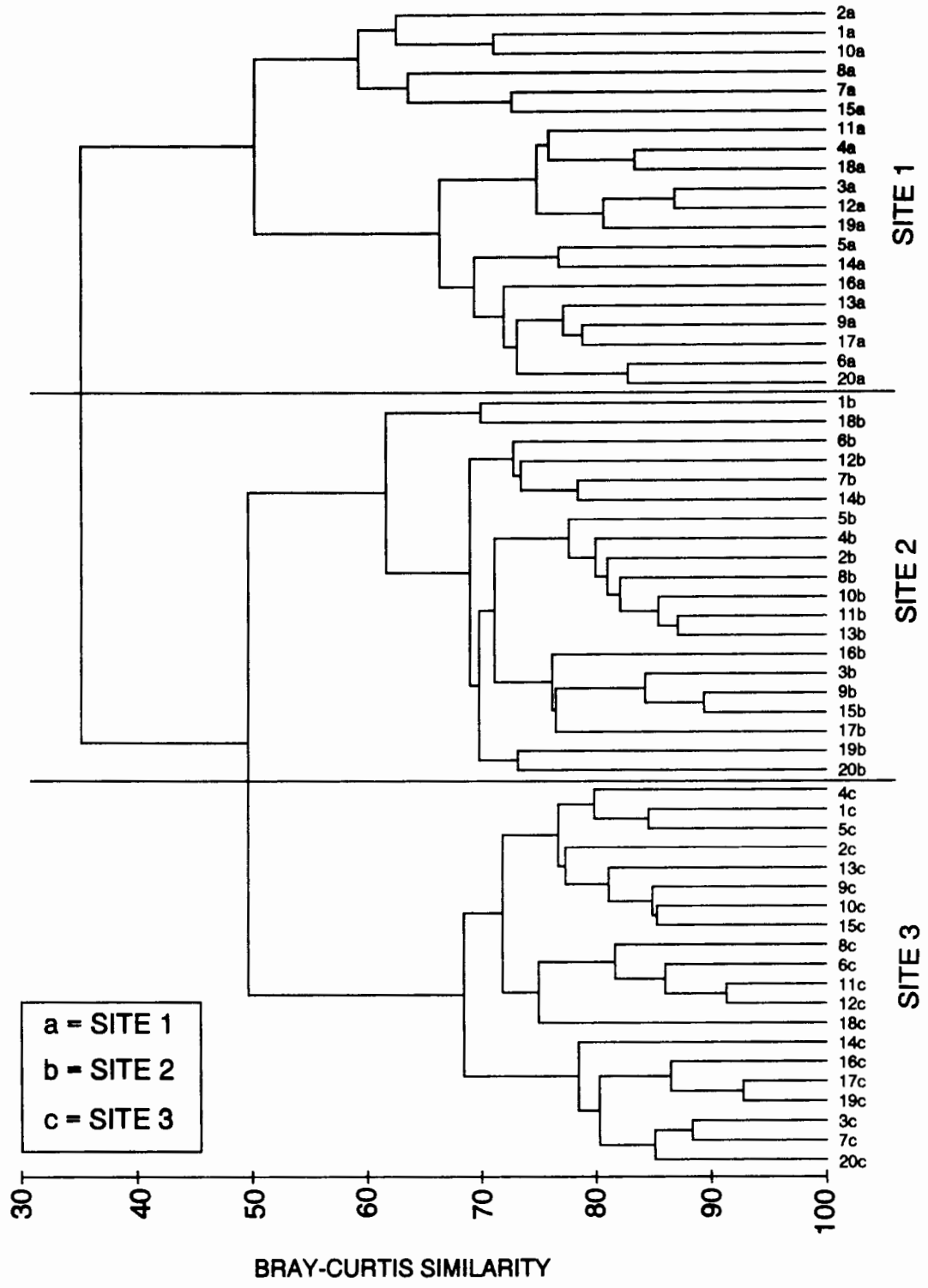


Figure 5.5. Dendrogram showing the classification of the >950 μm size fraction of 60 quantitative stones-in-current faunal samples collected at three sites on the Berg River.

When the >950 μm fraction was considered independently, the average dissimilarity increased between 5 and 10% (Table 5.5.). Sites 1 and 2 (average dissimilarity = 59.7%) differed in the abundance of Notonemouridae, Leptophlebiidae and Helodidae which were more numerous at Site 1, and the abundance of Hydropsychidae, Simuliidae, Caenidae, Leptoceridae and Libellulidae which were more abundant at Site 2. At Sites 1 and 3 (average dissimilarity = 69.8%) the greater abundance of Notonemouridae, Leptophlebiidae and Helodidae at Site 1, and Hydropsychidae, Chironomidae and Simuliidae at Site 2, distinguished the two sites from one another. Sites 2 and 3 were the least dissimilar (average dissimilarity = 50.3.%) and Leptoceridae, Libellulidae and Baetidae were of greater abundance at Site 2, whilst Hydropsychidae, Simuliidae, Chironimidae, Tricorythidae and Hydroptilidae were of greater abundance at Site 3.

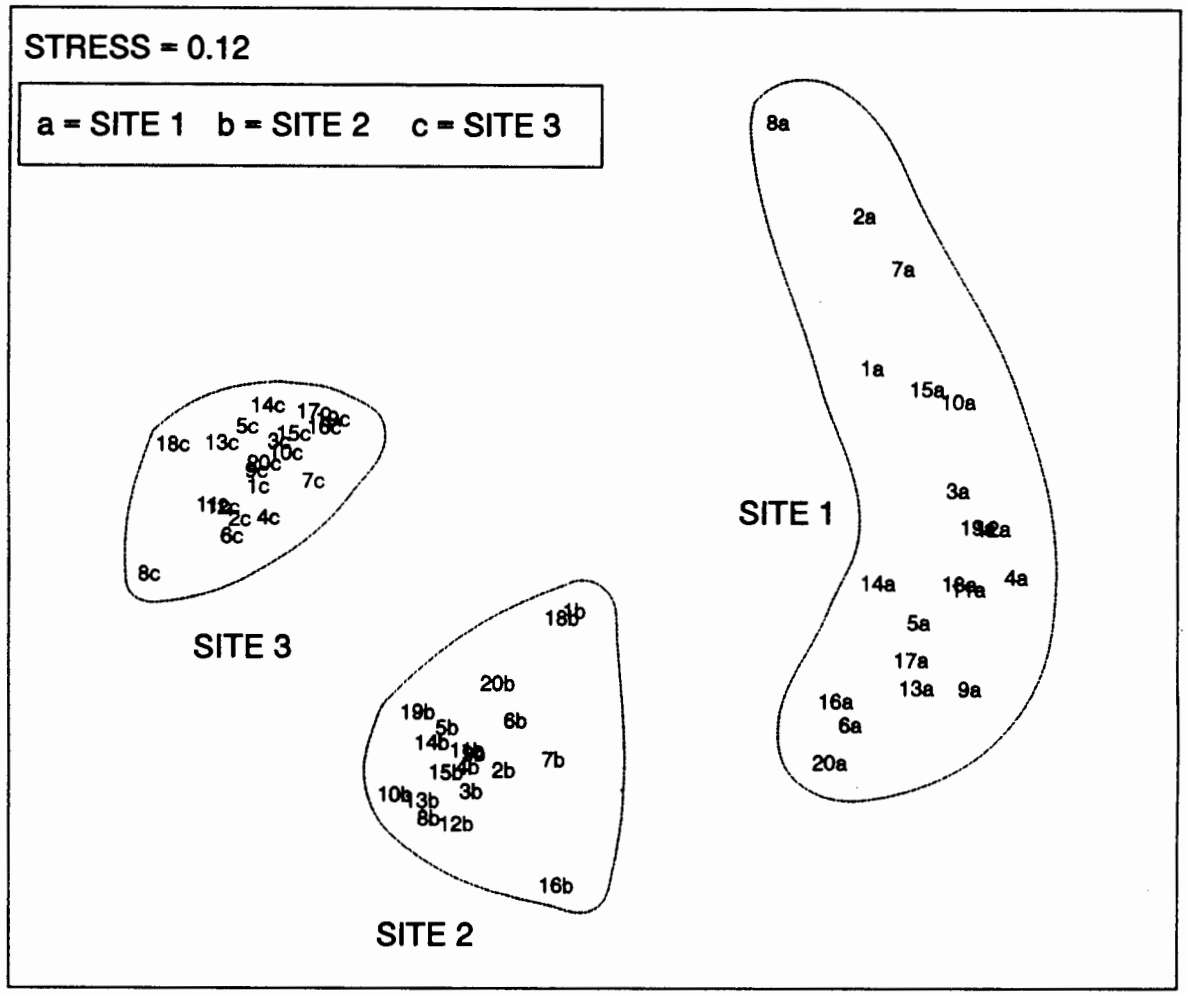


Figure 5.6. Ordination of the >950 μm size fraction of 60 quantitative stones-in-current faunal samples collected at three sites on the Berg River.

Table 5.5. SIMPER comparison in the mean abundance (0.1 m^2) between sites for combined data (i.e. all size fractions combined) and the >950 μm size fraction only. δi is the contribution of the ith taxon to the average Bray-Curtis dissimilarity, $\overline{\delta}$, between two sites, which is expressed as cumulative percentage ($\Sigma\ \delta i$ %). Taxa are listed in decreasing order of importance in contribution to $\overline{\delta}$, with a cutoff at $\leq$ 50% of $\overline{\delta}$. The higher abundance of each taxon is given in bold type.

Average dissimilarity between Site 1 and Site 2 = 55.1%				
Combined size fractions	Mean abundance		δi	$\Sigma\ \delta i$ %
Taxon	Site 1	Site 2		
Ephemerellidae	**134.7**	0	4.6	8.36
Hydropsychidae	1.6	**157.8**	4.3	16.15
Chironomidae	16.6	**428.9**	4.1	23.53
Notonemouridae	**66.2**	0.7	3.0	29.04
Caenidae	0	**16.9**	2.9	34.29
Leptophlebiidae	**31.3**	0.2	2.7	39.11
Oligochaeta	5.6	**43.5**	2.7	43.93
Hydracarina	22.1	**159.2**	2.5	48.49
Average dissimilarity between Site 1 and Site 3 = 64.9%				
Combined size fractions	Mean abundance		δi	$\Sigma\ \delta i$ %
Taxon	Site 1	Site 3		
Hydropsychidae	1.6	**1508.6**	8.7	13.33
Chironomidae	16.6	**490.1**	4.5	20.29
Ephemerellidae	**134.7**	0.5	4.4	27.05
Simuliidae	4.4	**248.6**	4.1	33.31
Notonemouridae	**66.2**	0	3.7	39.00
Caenidae	0	**53.8**	3.6	44.52
Tricorythidae	0	**25.9**	3.1	49.33
Average dissimilarity between Site 2 and Site 3 = 40.0%				
Combined size fractions	Mean abundance		δi	$\Sigma\ \delta i$ %
Taxon	Site 2	Site 3		
Hydropsychidae	157.8	**1508.6**	3.7	9.28
Leptoceridae	**23.5**	0.1	2.7	16.12
Tricorythidae	0	**25.9**	2.7	22.96
Heptageniidae	**17.7**	0	2.5	29.31
Simuliidae	76.9	**248.6**	2.2	34.81
Libellulidae	**10.8**	0	2.1	40.09
Hydroptilidae	0.1	**12.6**	2.1	45.3

Table 5.5 (cont.)

Average dissimilarity between Site 1 and Site 2 = 59.7%				
>950 μm size fraction only	Mean abundance		δi	$\Sigma\ \delta i$ %
	Site 1	Site 2		
Hydropsychidae	1.2	**64.2**	5.1	8.54
Leptophlebiidae	**15.0**	0.2	3.4	14.34
Simuliidae	2.6	**45.6**	3.4	20.10
Notonemouridae	**26.9**	0.4	3.4	25.85
Helodidae	**13.6**	0	3.4	31.56
Libellulidae	0	**10.3**	3.3	37.10
Leptoceridae	0.7	**9.9**	3.2	42.44
Caenidae	0	**5.3**	2.9	47.36
Average dissimilarity between Site 1 and Site 3 = 69.8%				
>950 μm size fraction only	Mean abundance		δi	$\Sigma\ \delta i$ %
Taxon	Site 1	Site 3		
Hydropsychidae	1.2	**666.2**	10.5	14.97
Simuliidae	2.6	**157.1**	5.7	23.12
Chironomidae	6.3	**179.3**	5.2	30.61
Notonemouridae	**26.9**	0	3.9	36.21
Leptophlebiidae	**15.0**	0	3.9	41.77
Helodidae	**13.6**	0	3.5	46.79
Average dissimilarity between Site 2 and Site 3 = 50.3%				
>950 μm size fraction only	Mean abundance		δi	$\Sigma\ \delta i$ %
Taxon	Site 2	Site 3		
Hydropsychidae	64.2	**666.20**	4.5	8.98
Leptoceridae	**9.9**	0.5	3.2	15.31
Libellulidae	**10.3**	0	2.9	21.13
Hydroptilidae	0	**8.9**	2.9	26.94
Tricorythidae	0	**11.1**	2.9	32.74
Simuliidae	45.6	**157.1**	2.9	38.50
Chironomidae	61.5	**179.3**	2.6	43.67
Baetidae	**147.4**	26.2	2.4	48.40

c. Diversity Indices

Differentiation of sites based on the Shannon-Wiener diversity index was investigated for the combined size groups. Norris & Georges (1993) recommended that nonparametric alternatives to ANOVA, such as Kruskal-Wallis procedure, be used on diversity and biotic indices since generally the sampling distribution of the index is unknown. Site 3 was significantly different ($p<0.05$) from both Site 1 and Site 2 which were not significantly different from one another (Figure 5.7).

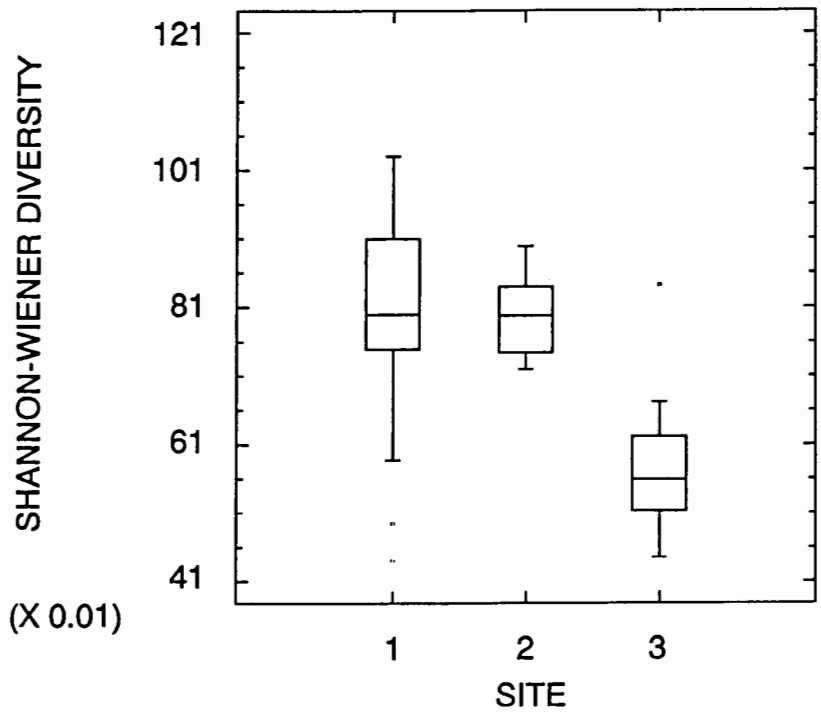


Figure 5.7. Box-and-whisker plot of Shannon-Wiener diversity indices for three sites on the Berg River (n=20 for each site).

d. Biotic Indices

The scores assigned to taxa by the South African Scoring System (SASS) were applied to intensive benthic samples and Total Score and Average Score per Taxon (ASPT) were calculated for each replicate sample at each site. Nonparametric analyses of variance indicated that all three sites were significantly different from one another (Kruskal-Wallis, $p<0.05$), both in terms of Total Score and ASPT values (Figure 5.8.).

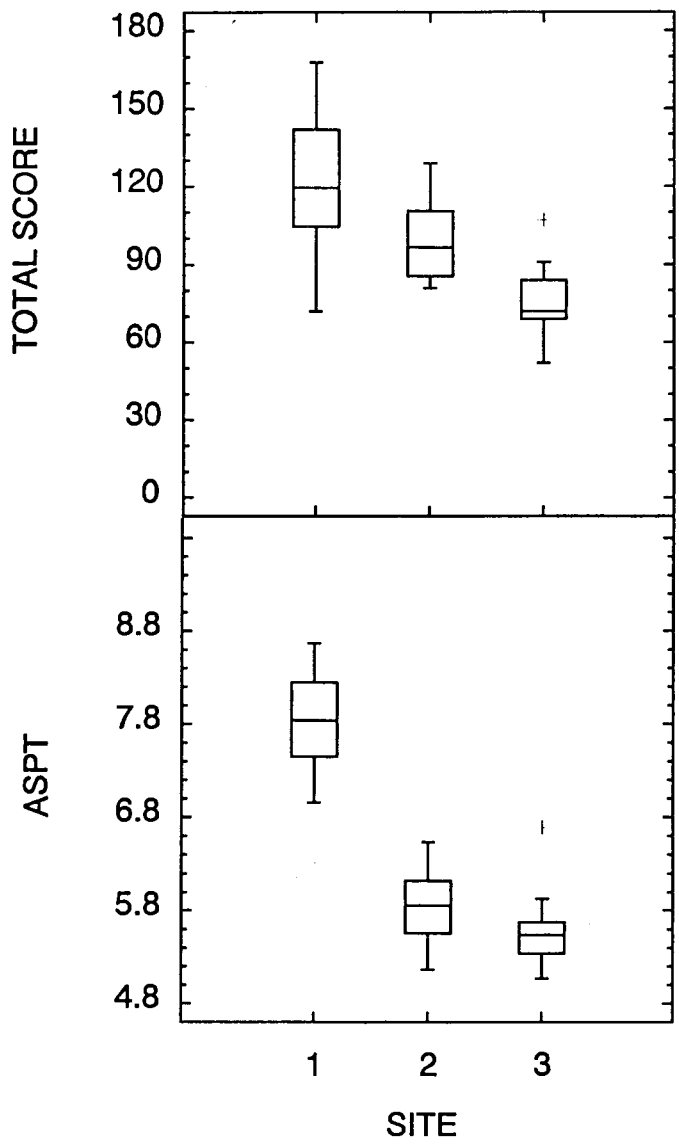


Figure 5.8. Box-and-whisker plots of Total Score and ASPT values calculated for the combined size data at each site (n=20 for each site and the "+" indicate outliers)

Sampling mesh diameter

Laboratory separation of quantitative samples into different size classes was conducted to provide an indication of the effect mesh size may have on total abundance and number of taxa. The relative contribution of each size fraction to the total abundance at each site, expressed as a percentage, is given in Table 5.6.

Table 5.6. Relative percentage contribution of each size fraction to total benthic macroinvertebrate abundance collected in the stones-in-current biotope using the quantitative sampling technique at three sites on the Berg River.

Site	>950 μm	<950 and >500 μm	<500 and >250 μm
1	38.3	49.9	11.7
2	31.2	31.7	37.2
3	42.1	36.1	21.7

Total number of taxa, number of taxa in >950 μm size class, and number of additional taxa represented in the (<950->500 μm) and (<500->250 μm) size classes is given in Table 5.7.

Table 5.7. Total number of taxa, number of taxa in the >950 μm size class, and number of additional taxa represented in the (<950 - >500 μm) and (<500 - >250 μm) size classes determined using the quantitative sampling technique at the three sites on the Berg River.

Site	Total number of taxa	>950 μm	<950 and >500 μm	<500 and >250 μm
1	33	31	1	1
2	33	29	4	0
3	28	24	3	1

Analysis of Variance (ANOVA) was conducted on log(X+1) transformed "abundance" data for each size fraction within each site and the homogeneous groups are indicated in Table 5.8. The (>950 μm) and (<950 μm - >500 μm) were not significantly different at any of the sites, the (<500 μm - >250 μm) fraction was significantly different at Sites 1 and 3, and the combined group (i.e. >250 μm) were significantly different at all sites. Analysis of Variance (ANOVA) was also conducted on the log(X+1) transformed "number of taxa" data for each size fraction within each site (Table 5.8). At Sites 1 and 3 the (>950 μm) and (<950 μm - >500 μm) were not significantly different, whilst the (<500 μm - >250 μm) fraction and combined group (i.e. >250 μm) were significantly different from each other

and the other size classes. At Site 2 the (>950 μm) and combined group (i.e. >250 μm) were not significantly different, whilst the (<950 μm - >500 μm) and (<500 μm - >250 μm) fractions were significantly different from each other and the other size classes.

Table 5.8. Homogeneous groups derived by performing Analysis of Variance (ANOVA) on log(X+1) transformed data of the abundance and number of taxa within each size class determined using the quantitative sampling technique at three sites on the Berg River. In each column the shaded blocks indicate homogeneous groups.

SITE	SIZE CLASS	HOMOGENEOUS GROUPS	
		ABUNDANCE	NUMBER OF TAXA
1	>950 μm		
	<950 μm - >500 μm		
	<500 μm - >250 μm		
	combined group (>250 μm)		
2	>950 μm		
	<950 μm - >500 μm		
	<500 μm - >250 μm		
	combined group (>250 μm)		
3	>950 μm		
	<950 μm - >500 μm		
	<500 μm - >250 μm		
	combined group (>250 μm)		

Analysis of data for abundance and number of taxa from each site which had been combined into the respective size classes revealed that the >950 μm and (<950->500 μm) size classes were not significantly different, whilst the (<500->250 μm) and combined group (>250 μm) were significantly different, both in terms of abundance and number of taxa ($p<0.05$). From this one can postulate that it is the <500 - >250 μm size fraction which distinguishes the >950 μm and <950 - >500 μm size fractions from the combine size group). The taxa

with smaller individuals (e.g. Chironomidae, Hydracarina) and the immature individuals in smaller size classes (e.g. Ephemerellidae) largely contributed to these differences.

Sample mesh diameter, replication and variability

The effect of mesh size on the number of replicate sample units needed to ensure collection of 95% and 75% of the taxa collected using the quantitative sampling technique at three sites on the Berg River was examined but found to be insignificant. The similarity of the different size classes within a site was investigated by conducting cluster and ordination analyses. Three size classes were generated, namely >950 μm, (>950+>500) μm, and (>950+>500+>250) μm, so that the effect of adding smaller size classes could be assessed. Analyses were run separately for each site. The different size classes did not emerge as distinct groups, and within each site faunal samples were at least 60% similar. A similar pattern emerged with ordination analysis. These figures are not provided.

5.2.2. RAPID BIOASSESSMENT (QUALITATIVE SAMPLING)

The South African Scoring System (SASS) method of biological assessment was used to assess the benthic community at three sites on the Berg River. In February 1993, SASS2 was used, although the scoring system has subsequently been modified to SASS4 as a result of discussions amongst members of the Rapid Biological Assessment (RBA) Forum. The main modification was the inclusion of a sliding-scale in scoring for three of the families (details given in Chapter 4). To enable SASS2 scores to be compared with SASS3 or SASS4 scores, Chutter (1994a) developed regression formulae for predicting sliding-scale scores [both Total Score and Average Score per Taxon (ASPT)] from SASS2 scores. These formulae are based on data collected at 25 unpolluted sites in the south-western Cape.
The formulae are as follows:

Total Score: $SASS_{(sliding)} = 0.96 + 1.14 \times SASS_{(fixed)}$ ($r^2 = 0.86$, n = 25)

ASPT: $ASPT_{(sliding)} = 1.13 \times ASPT_{(fixed)} - 0.12$ ($r^2 = 0.89$, n = 25)

where "(fixed)" refers to the Total Score or ASPT calculated after incorporating all other score modifications excepting for sliding scales. These formulae have been used to allow all scores presented in this study to incorporate the sliding-scale values. SASS samples were restricted to the stones-in-current biotope to facilitate comparison with the benthic samples collected using the quantitative method. The scores therefore are likely to be lower than those determined if full SASS sampling (i.e. incorporating all available biotopes) had been conducted at the same sites.

Variability and sample replication

The mean ($\bar{X}$) ± standard deviation (S.D) for Total Score, number of taxa and ASPT values for each site (based on 20 replicates per site) are given in Table 5.9.

Table 5.9. Mean ($\bar{X}$) ± standard deviation (S.D) for Total Score, number of taxa and ASPT values for benthic communities in the stones-in-current biotope determined using the SASS technique for each of the three sites on the Berg River. Homogeneous groups as determined by nonparametric analysis of variance are indicated. In each column the shaded blocks indicate homogeneous groups (H. Groups).

Site	n	Total Score	H. Groups	Number of Taxa	H. Groups	ASPT	H. groups
1	20	73.3 ± 23.8		7.9 ± 2.2		8.77 ± 1.28	
2	20	70.6 ± 13.4		10.4 ± 1.5		6.52 ± 0.52	
3	20	47.7 ± 10.3		7.9 ± 1.5		5.75 ± 0.45	

Nonparametric analysis of variance revealed that Site 1 and 2 differed significantly in terms of the number of taxa and the ASPT, Site 1 and 3 differed significantly in terms of Total Score and ASPT, and Site 2 and 3 differed significantly in terms of Total Score, number of taxa and ASPT (Table 5.9.). [Kruskal-Wallis; t-statistic (Total Score)=22.6; t-statistic (number of taxa)=19.8; t-statistic (ASPT)=39.6; all $p<0.05$].

The relationship between cumulative Total Score; cumulative number of taxa and "average"

ASPT; and the number of sample units was investigated (Figure 5.9).

The cumulative Total Score and number of taxa were significantly positively correlated (Multiplicative model) with number of sample units at all sites ($p<0.05$). The rate of increase in Total Score and number of taxa with increasing sample units was greatest at Site 1. Doubling the number of samples from five to ten, resulted in an increase of cumulative Total Score of 27, 19 and 13 at Sites 1, 2 and 3 respectively, and an increase in the cumulative number of taxa of 4.0, 1.9 and 2.9 at Sites 1, 2 and 3 respectively. ASPT values are calculated by dividing Total Score by number of taxa. The "average" ASPT values at Sites 1 and 3 fluctuated around a median score and were neither positively nor negatively correlated with number of sample units. At Site 2, ASPT was positively correlated with number of sample units ($p<0.05$), although if one plots the points it is clear that the majority of the ASPT values were approximately 6.5. The low ASPT values at low number of sample units and high ASPT values at higher number of sample units contributed to the significant positive correlation. All correlations were based on the multiplicative model. A point of interest is the high fluctuation of the ASPT within the first three samples at Site 1. Since the ASPT value is dependent on both Total Score and number of taxa, the addition of a low scoring taxon would actually result in a decrease in ASPT, even though the number of taxa has increased.

The number of samples needed to ensure collection of 95% and 75% of the taxa was calculated (Table 5.10).

Table 5.10. Number of replicate sample units needed to ensure collection of 95% and 75% of the taxa using the SASS technique at three sites on the Berg River.

Site	95%	75%
1	13	9
2	4	2
3	12	6

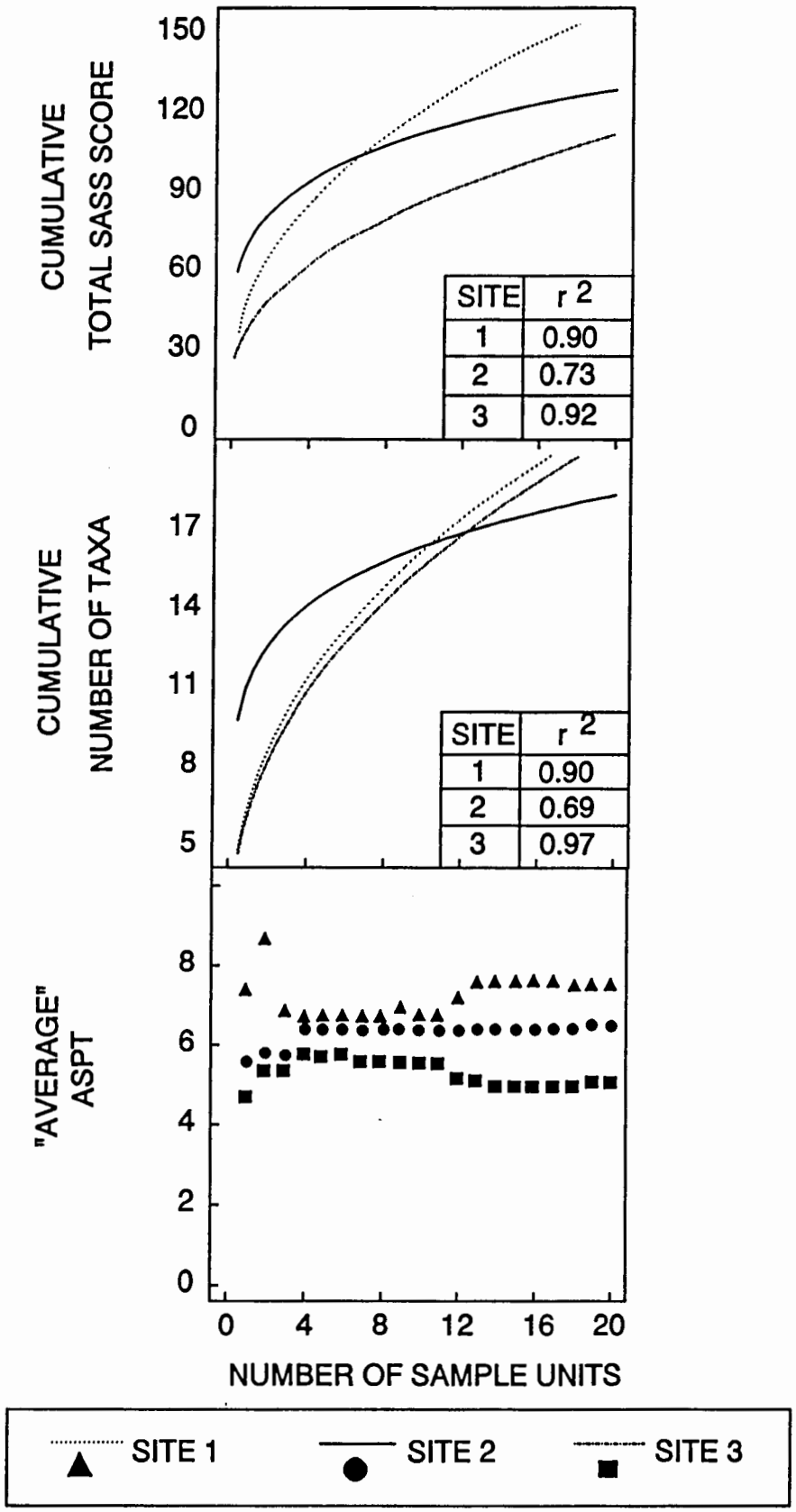


Figure 5.9. Cumulative Total Score and cumulative number of taxa regressed against number of sample units, and cumulative ASPT values plotted as a function of number of sample units, collected using the rapid bioassessment method SASS at three sites on the Berg River.

To enable the assessment of variability as a function of the number of sample units, the coefficients of variation were calculated for Total Score and ASPT (Figure 5.10). The coefficient of variation was highest and most variable at Site 1. Both Site 2 and 3 had low and relatively uniform coefficients of variation which generally decreased as the number of sample units increased.

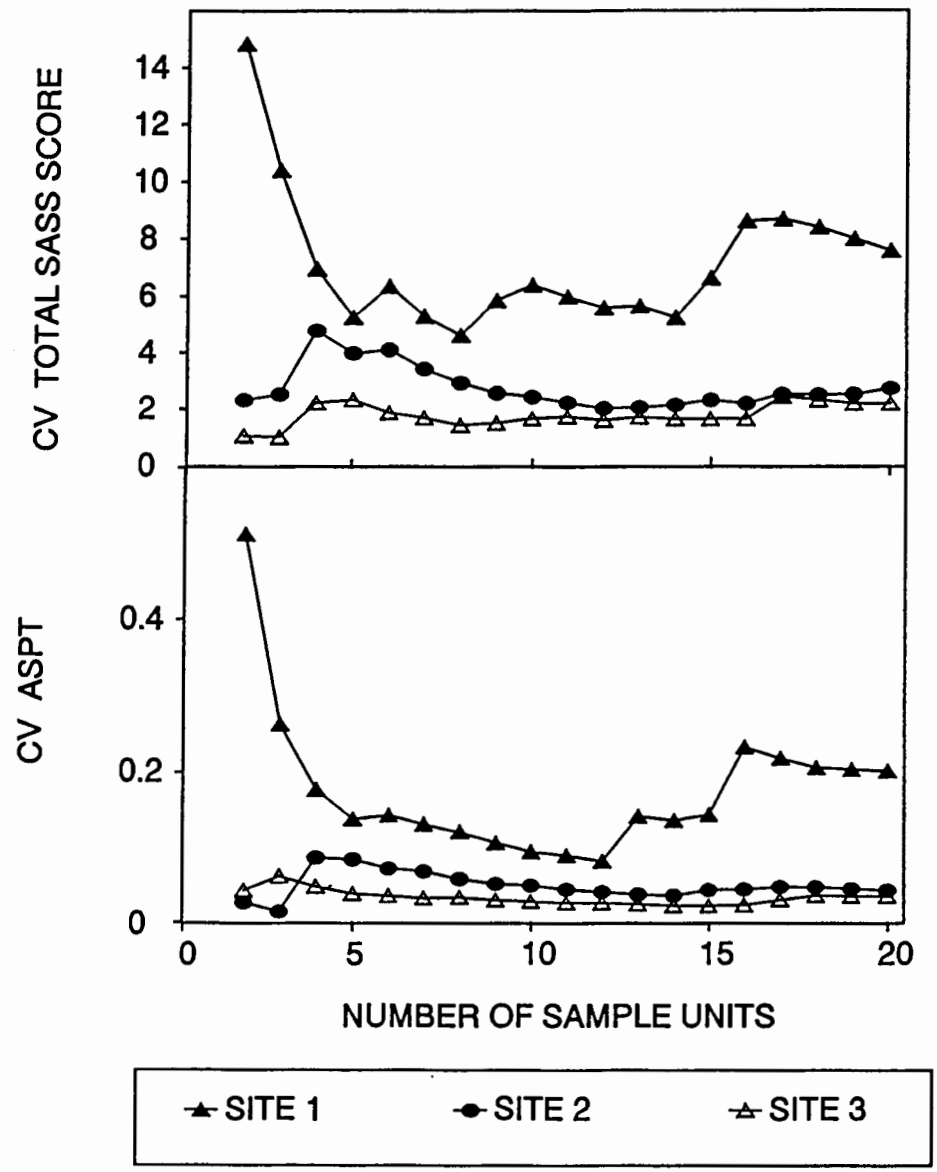


Figure 5.10. Coefficient of variation of Total Score and Average Score per Taxon (ASPT) at each site plotted as a function of number of sample units determined using SASS method at three sites on the Berg River.

Site differentiation based on the SASS method

From the preceding sections it is apparent that there is a certain degree of variability of scores within sites, particularly at the least impacted site. This is emphasised by the relatively high variability at Site 1. Given this within-site variability, the ability of SASS to distinguish between three sites which differed in water quality was investigated.

a. Cluster and Multi-dimensional scaling

Cluster and ordination analyses were conducted on the 60 samples (20 replicates per site). The data were transformed using the presence/absence transformation. Site 1 split from the other sites at 50% similarity level, with the exception of replicate sample six from Site 3 (Figure 5.11). Sites 2 and 3 split at the 55% similarity level. These groupings are reflected in the two-dimensional ordination (Figure 5.12). The stress value is 0.14.

b. Identification of distinguishing taxa

The taxa primarily responsible for the differences in community structure between Sites 1, 2 and 3 as measured by the Bray-Curtis dissimilarity measure are listed in Table 5.11. Site 1 and 2 (average dissimilarity = 52.0%) differed in the abundance of Notonemouridae and Leptophlebiidae which were more numerous at Site 1, and the abundance of Hydracarina, Aeshnidae, Libellulidae and cased-caddis larvae (Trichoptera) which were more abundant at Site 2. At Sites 1 and 3 (average dissimilarity = 57.1%) the greater abundance of Notonemouridae, Leptophlebiidae and Helodidae at Site 1, and Tricorythidae, Ecnomidae and Chironomidae at Site 3, distinguished the two sites from one another. Sites 2 and 3 were the least dissimilar (average dissimilarity = 45.4%) and Hydracarina, Libellulidae and cased-caddis larvae (Trichoptera) were of greater abundance at Site 2, whilst Tricorythidae and Ecnomidae were of greater abundance at Site 3.

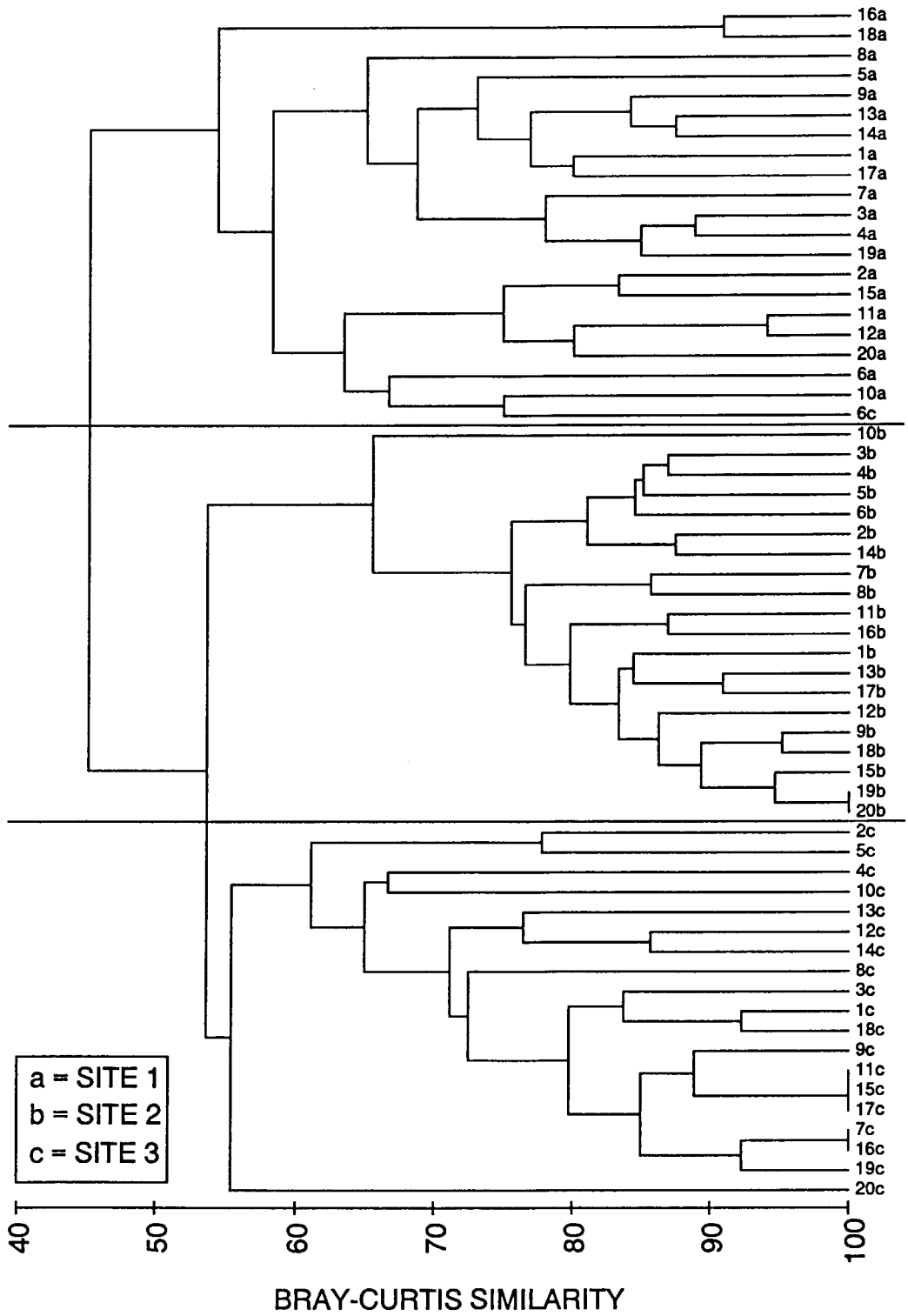


Figure 5.11. Dendrogram showing the classification of 60 stones-in-current SASS samples collected at three sites on the Berg River.

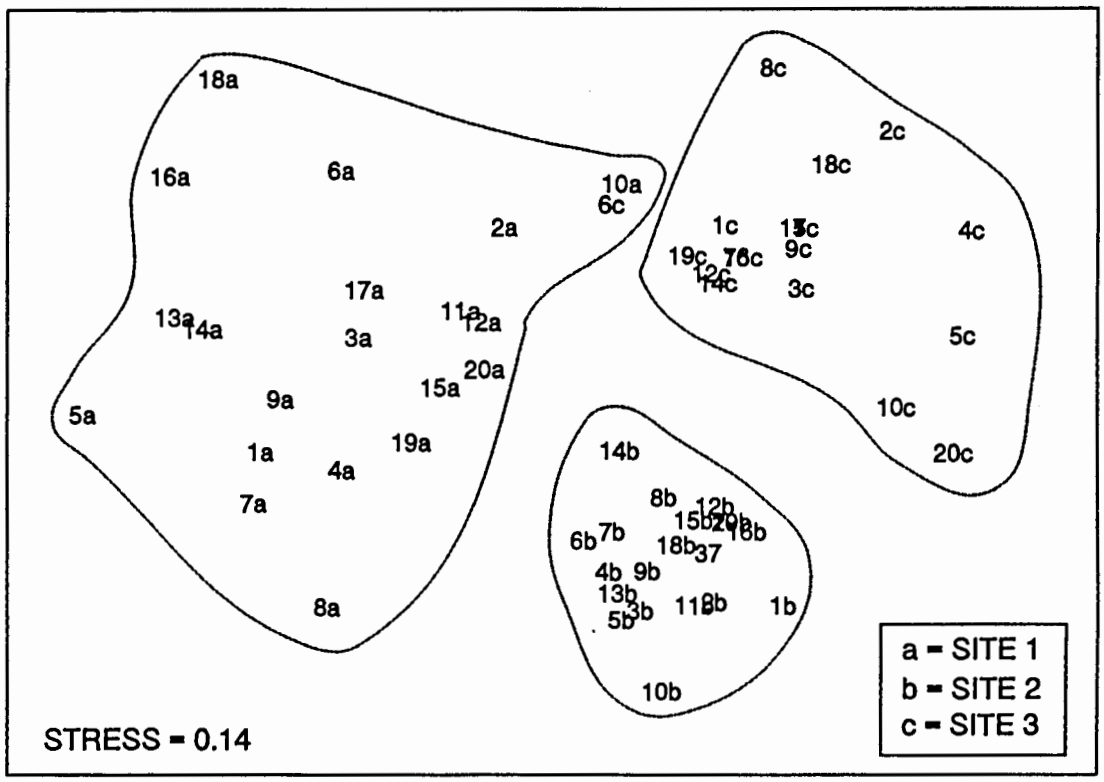


Figure 5.12. Ordination of 60 stones-in-current SASS samples collected at three sites on the Berg River.

Table 5.11. SIMPER comparison for presence/absence transformed taxa between sites for SASS data. δi is the contribution of the ith taxon to the average Bray-Curtis dissimilarity, $\overline{\delta}$, between two sites, which is expressed as cumulative percentage ($\Sigma\ \delta i\ \%$). Taxa are listed in decreasing order of importance in contribution to $\overline{\delta}$, with a cutoff at $\leq 50\%$ of $\overline{\delta}$. The higher abundance of each taxon is indicated with an asterix.

Average dissimilarity between Site 1 and Site 2 = 52.0				
SASS samples	Greater abundance		δi	$\Sigma\ \delta i\ \%$
Taxon	Site 1	Site 2		
Notonemouridae	*		5.4	10.34
Leptophlebiidae	*		5.0	20.01
Hydracarina		*	4.4	28.46
Aeshnidae		*	3.9	35.95
Libellulidae		*	3.5	42.72
Cased-caddis larvae		*	3.2	48.89
Average dissimilarity between Site 1 and Site 3 = 67.1				
SASS samples	Greater abundance		δi	$\Sigma\ \delta i\ \%$
Taxon	Site 1	Site 3		
Notonemouridae	*		5.7	9.95
Leptophlebiidae	*		5.1	18.79
Helodidae	*		4.6	26.90
Tricorythidae		*	3.9	33.70
Ecnomidae		*	3.4	39.62
Chironomidae		*	3.1	45.00
Average dissimilarity between Site 2 and Site 3 = 45.4				
SASS samples	Greater abundance		δi	$\Sigma\ \delta i\ \%$
Taxon	Site 2	Site 3		
Hydracarina	*		4.7	10.03
Libellulidae	*		4.4	19.97
Cased-caddis larvae	*		4.3	29.46
Tricorythidae		*	3.9	37.98
Ecnomidae		*	3.3	45.17

5.2.3. COMPARISON BETWEEN QUANTITATIVE BENTHIC SAMPLING AND RAPID BIOASSESSMENT

Variability and sample replication

SASS scores were allocated to taxa found in each quantitative benthic sample and Total Score, number of taxa and Average Score per Taxon (ASPT) values were calculated (20 replicates per site). Taxa not currently scored by the South African Scoring System (SASS), and which are often rare or not easily detectable in the field, have been excluded in the calculations. Means ± standard deviations (S.D) were calculated and compared to those calculated for SASS samples (SASS) (Table 5.12.).

Table 5.12. Mean ($\bar{X}$) ± standard deviation (S.D) for Total Score, number of taxa and ASPT values determined using the SASS technique and calculated from quantitative benthic samples for each of the three site on the Berg River.

Site		n	Mean ± S.D.	
			Quantitative Samples	SASS Samples
1	Total Score	20	103.3 ± 37.7	73.3 ± 23.8
	Number of taxa	20	11.2 ± 4.0	7.9 ± 2.2
	ASPT	20	8.83 ± 1.19	8.77 ± 1.28
2	Total Score	20	101.0 ± 23.9	70.6 ± 13.4
	Number of taxa	20	14.8 ± 3.4	10.4 ± 1.5
	ASPT	20	6.59 ± 0.47	6.52 ± 0.52
3	Total Score	20	69.9 ± 13.9	47.7 ± 10.3
	Number of taxa	20	11.2 ± 1.77	7.9 ± 1.5
	ASPT	20	5.95 ± 0.43	5.75 ± 0.45

Total Scores and number of taxa for the quantitative benthic samples were consistently higher than for the SASS samples at all three sites. ASPT values were however remarkably similar.

Those taxa commonly not recorded by each method were determined by calculating the percentage occurrence of each taxon within the 20 replicate samples at each site. At Site 1, eight taxa which were recorded in quantitative samples (percentage occurrence < 10%) were not recorded using SASS. These include Amphipoda, Heptageniidae, Veliidae, Hydroptilidae, Ecnomidae, Hydrophilidae, Limnichidae, Muscidae and Empididae. One taxon, Blephariceridae, was recorded in SASS samples (percentage occurrence < 5%), but not in quantitative ones. The summed Total Score was 67 points higher for quantitative samples, although the ASPT values were 8.27 and 8.30 for quantitative and SASS samples respectively. At Site 2, nine taxa which were recorded in quantitative samples were not recorded using SASS. These include Leptophlebiidae, Caenidae, Gomphidae, Corixidae, Ecnomidae, Limnichidae, Tipulidae, Empididae and Ancylidae. These taxa were recorded in less than 15% of the samples, with the exception of Leptophlebiidae and Caenidae which were recorded in 90% and 80% of the samples respectively. The summed Total Score was 70 points higher for quantitative samples, although the ASPT values were 7.58 and 7.60 for quantitative and SASS samples respectively.

At Site 3, five taxa recorded in quantitative samples were not recorded using SASS. These include cased-caddis (mostly Leptoceridae), Nymphulidae, Hydroptilidae, Hydraenidae and Ancylidae. In quantitative samples these taxa were recorded in less than 10% of the samples, with the exception of Ancylidae which was recorded in 45% of the samples. Three taxa, Libellulidae, Corixidae and Dytiscidae, were recorded in SASS samples (<15%), but not in quantitative ones. The summed Total Score was 34 points higher for quantitative samples, and the ASPT values were 6.66 and 5.64 for quantitative and SASS samples respectively.

Based on this more detailed examination of differences in the number and composition of taxa recorded using the two methods, it becomes clear that many taxa are not recorded using SASS. In general, the percentage of quantitative samples in which these taxa were found was <10%, suggesting that the quantitative sampling method, which includes laboratory sorting and identification with the aid of a microscope, is more likely to record rarer taxa. Three alarming exceptions to this were the high percentages of leptophlebiids, caenids and ancylids. As a result of these differences in the number of taxa recorded via the two different methods,

calculated Total Scores are consistently higher in quantitative samples (28%, 33% and 23% higher at Sites 1, 2 and 3 respectively). The ASPT values were less variable and at Sites 1 and 2 they were approximately equal, whilst at Site 3 the ASPT values for SASS samples was approximately one unit lower than that for quantitative samples.

SASS scores were applied to quantitative samples and the coefficient of variation as a function of the number of sample units was calculated for Total Score and ASPT for both quantitative and SASS samples (Figure 5.13). The coefficient of variation of Total Score was highest and most variable at Site 1 for both methods. The CV of number of taxa was higher in SASS samples than quantitative samples. Both Site 2 and 3 had low coefficients of variation for both Total Score and number of taxa which decreased as the number of sample units increased.

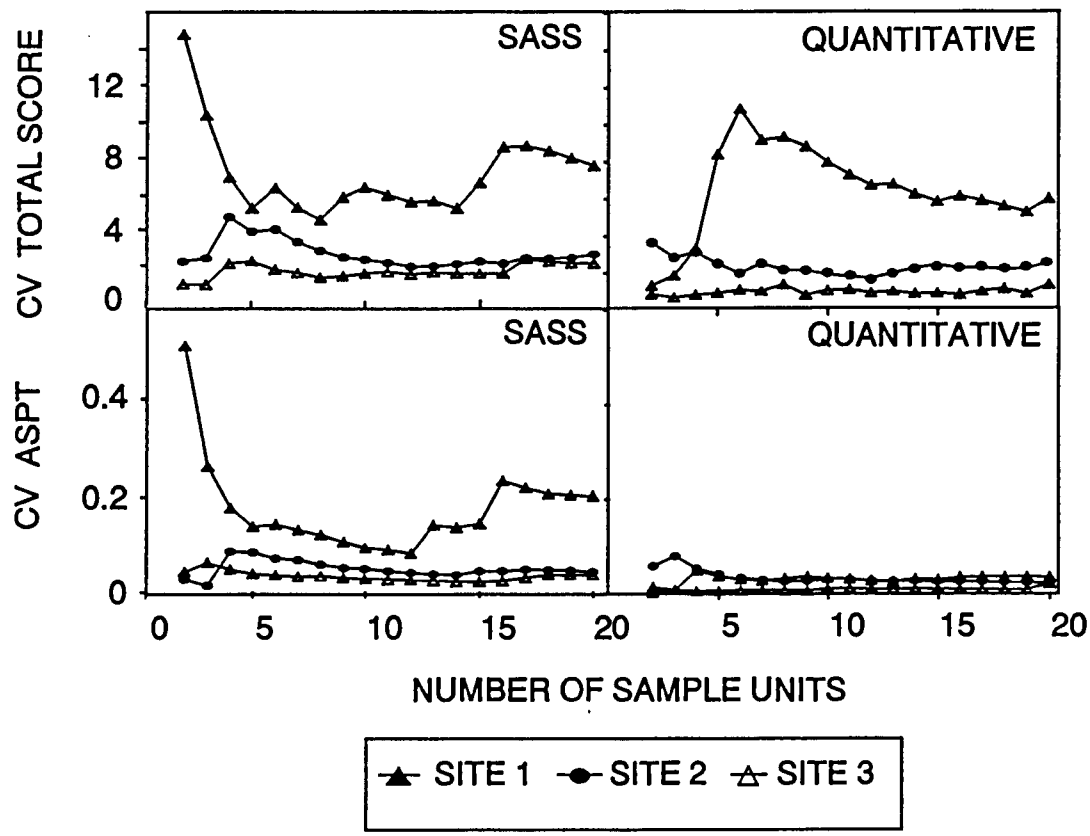


Figure 5.13. Coefficient of variation of Total Score and Average Score per Taxon (ASPT) calculated for quantitative and SASS samples at each site plotted as a function of number of sample units at three sites on the Berg River.

Comparing site differentiation: cluster analysis and multi-dimensional scaling

Cluster and ordination analyses were conducted on all samples together, i.e. those samples collected via both methods and at each of the three sites. Two sets of analyses were run, the first with all size fractions of the quantitative samples combined, and the second on the >950 μm fraction only. All data were transformed using the presence/absence transformation. Cluster analyses produced similar groupings, with quantitative and SASS samples from each site clustering together at approximately 50% similarity. The combined quantitative faunal samples and SASS samples were grouped separately within each site at approximately 75% similarity although there was a certain degree of intermixing between samples. This division was not apparent when the >950 μm size fraction faunal samples were analyzed. A dendrogram of the >950 μm size fraction groups and SASS groups is given (Figure 5.14.). The groupings are reflected in two-dimensional ordination for both the combined size group and >950 μm fraction analyses (Figure 5.15.). The stress values for the combined group analysis is higher (0.18) than the >950 μm analysis (0.16). Average dissimilarities between the >950 μm size fraction of the quantitative samples and the SASS samples as measured by the Bray-Curtis dissimilarity measure, were 39.8%, 32.0% and 32.9% at Sites 1, 2 and 3 respectively.

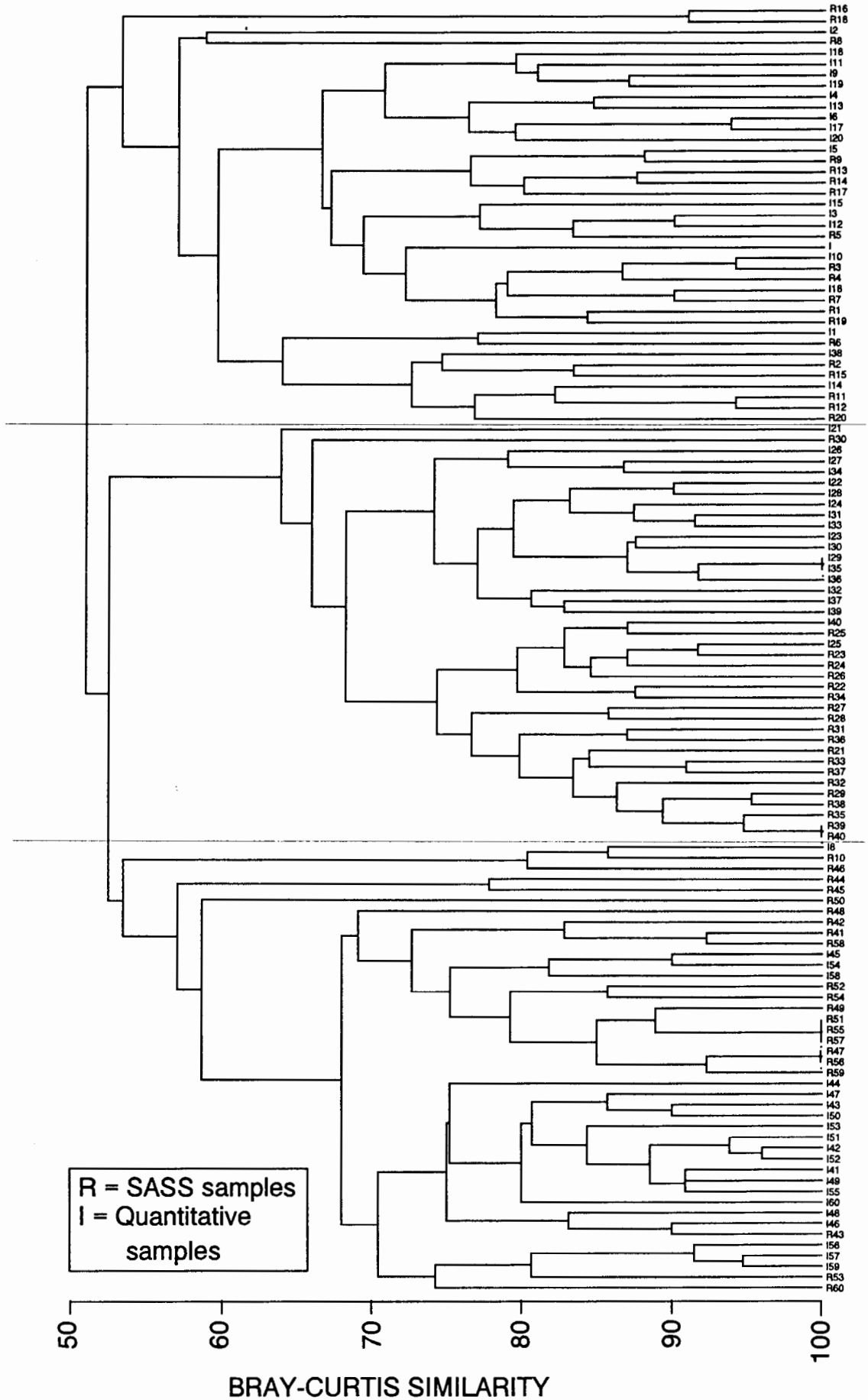


Figure 5.14. **Dendrogram showing the classification of 60 stones-in-current SASS samples (R) and 60 quantitative (I) benthic samples (>950 μm size fraction) collected at three sites on the Berg River.**

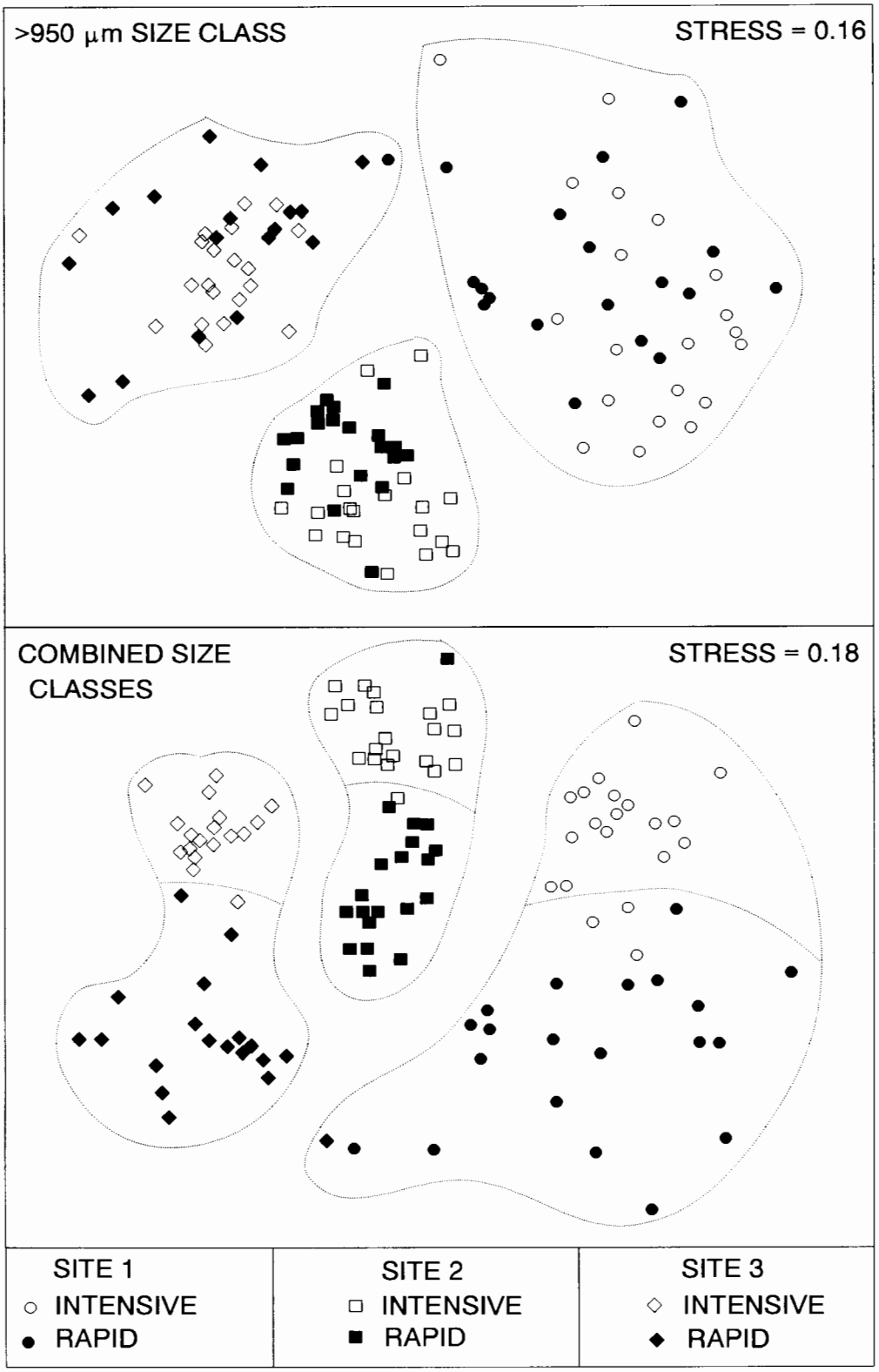


Figure 5.15. Ordination of 60 stones-in-current SASS samples (R) and 60 quantitative benthic samples (I) collected at three sites on the Berg River. Ordination plots of the >950 µm size fraction group and combined size group are given separately.

5.3. DISCUSSION

The inherent variability within most natural systems is well known and change is an intrinsic property of ecosystems (Hellawell 1977). The problem arises of how to decide when an observed change in some parameter represents a deviation caused by the presence of a pollutant, or whether such changes are part of the "natural" fluctuations inherent in the system (Sheehan 1984). It is imperative that this variability be taken into account when attempting to ascertain the potential effect of an impact or perturbation on a system. Measurement of natural variability is complicated and is often time-, labour- and cost-intensive, factors which often preclude the preliminary determination of natural variability within a system. The imminent implementation of SASS monitoring into a national water quality monitoring programme within South Africa, and the varied geographic nature of this country, clearly emphasise the need to gain insight into the inherent variability within riverine systems where SASS is applied. This chapter has attempted to provide some framework in which SASS can be applied within the south-western Cape. The discussion that follows is based on the first three objectives outlined in the introduction, namely: 1) to test the variability between samples taken within one biotope at a particular site using the two different methods, i.e. traditional quantitative box-sampling and qualitative rapid bioassessment using SASS (South African Scoring System), and thus; 2) to ascertain the number of samples that should be taken using each method, in the case of quantitative sampling to allow adequate representation of the benthic macroinvertebrate community at that site, and in the case of SASS, to allow adequate representation of the SASS scores; and 3) to investigate the influence of mesh diameter of the sampling equipment on the adequate sampling of benthic macroinvertebrate communities. Objectives 2 and 3 are designed to ascertain the most labour- and time-effective manner in which benthic macroinvertebrate collections can be undertaken.

5.3.1. Variability and sample replication

Error is defined as concluding that two or more sample means are from different populations (or groups) when in fact they are from the same population (Type I error), or concluding that all means are derived from the same population when in fact they are not (type II error)

(Resh & McElvary 1993). Sample replication is required to provide a measure of variability. There are two main objectives in replication: to estimate the value of a certain benthic measure, with a desired level of precision and risk of error, and to determine if a given degree of change in a particular benthic measure has occurred among several sites and times, again with a certain level of risk of error (Resh & McElvary 1993). Budget and time constraints often dictate the number of replicates that are taken. Examination of the data obtained by quantitative sampling at three sites on the Berg River indicate that the number of replicate sample units needed to adequately represent the benthic macroinvertebrate community is relatively high (n = 12 to 17 to ensure collection of 95% of the taxa), in comparison to the average number of replicates generally taken in benthic studies (n = 3 to 5, Resh & McElvary 1993).

The degree of replication is influenced by factors such as: the size of the mean, the degree of aggregation and the desired precision (Rosenberg 1979). The number of replicates needed increases with decreasing mean value, higher aggregation and desired precision. In the present study, Site 1 had the lowest mean abundance, and Sites 2 and 3 were 2.5 times and 5 times the mean abundance of Site 1 respectively (Table 5.2.). When the density of organisms is examined with reference to the area or volume of the substrate rather than the area actually available in terms of stone surface, mean density estimates will have a variance with two components: that due to the degree of population aggregation and that due to the fact that each sample may contain different amounts of stone surface (Rosenberg 1979). Aggregations may result from microhabitat preferences of substrate, current, food source etc. or behavioural interactions (Rosenberg 1979). The biological characteristics of the organisms under examination, such as lithophilic behaviour, hyporheic distribution, motility and catchability (Rosenberg 1979) will influence the resultant sample population. The non-random distribution patterns as indicated by differences in sample abundances and sampling variability observed in Hydropsychidae at Site 3 in the present study, may be the result of several biological features. Rosenberg (1979) elaborated on some of these features when studying factors responsible for the spatial distribution of *Cheumatospyche* spp. (Hydropsychidae). The factors included restriction of sampling to the hyporheic component thereby failing to consider the three dimensional aspect of distribution, spatial influences such as orientation by net-spinning caddisfly larvae to optimise current and feeding conditions, and

differences in the behaviour of different instars.

Based on these results and the coefficient of variation of both abundance and number of taxa (Figure 5.1.), it is clear that the least impacted site, Site 1, is the most variable and therefore requires the most replication to allow adequate representation of the benthic macroinvertebrate community at this site. Voshell *et al.* (1989, cited by Resh & McElvary 1993) concluded that six sample units generally will provide estimates of $\pm$ 40% of the mean total number of individuals (with 95% confidence intervals) in a community. Six sample units ensured collection of 62%, 74% and 68% of the total number of taxa at Sites 1, 2 and 3 respectively. Chutter & Noble (1966) found that heterogeneity influenced the variance of density of individual species. They calculated that to attain an estimate of population abundance within 20%, 50 sample replicates would need to be taken, although samples of three square feet (approximately 0.3 m^2) would ensure that at least one individual of the more common species would be found. This would be the equivalent to three 0.1 m^2 box samples. The effect of heterogeneity was demonstrated in the present study by dividing the stones-in-current biotope into "riffle" and "run" with the resultant decrease in the number of sample units required to record 95% (between 6 and 11) and 75% (between 2 and 5) of the total number of taxa present at each site. The objective of the particular study will, to a certain extent, dictate the degree of sample replication required. For example, in conservation studies precise estimates of the mean might be an appropriate goal, whilst in studies of disturbance, the difference between means over time or at different sites may be a more meaningful measure.

Rapid bioassessment using SASS is designed such that only one sample is taken per site. In the present study twenty replicate samples were taken in the stones-in-current at each site so that an indication of the representativeness of the SASS scores in a single SASS sample could be evaluated in terms of sample replication. The percentage of the Total Score and number of taxa that one sample would produce of the Total Scores and number of taxa determined in twenty sample units was therefore calculated. The percentage of Total Scores are 28%, 59% and 45% at Sites 1, 2 and 3 respectively and number of taxa 35%, 66% and 35% at Sites 1, 2 and 3 respectively. These percentages are relatively low, particularly at Site 1. The number of samples needed to ensure that 75% of the Total Score calculated after twenty

replicates are taken per site is achieved, are seven, four and eight for Sites 1, 2 and 3 respectively. Girton (1980, cited by Armitage *et al.* 1983) found that 75-85% of the BMWP score produced by ten samples was achieved by three and that three samples collected a large proportion of the taxa in ten samples (63-85%). In the present study, the number of sample units needed to collect 95% of the taxa was 13, 4 and 12 at Sites 1, 2 and 3 respectively. Pinder *et al.* (1987) found that five sample units taken in gravel recorded 95% of the total number of taxa, although this varied seasonally, and a greater number of sample units was needed in autumn as opposed to spring. Balloch *et al.* (1976, cited by Armitage *et al.* 1983) noted that 72.5% of the total number of taxa were recorded in five samples. Armitage *et al.* (1983) found that 97% of the Total Score calculated from six samples was achieved in five samples. Total Score increased with sampling effort, whilst ASPT changes were slight, suggesting that Total Scores were more dependent on sampling effort than ASPT and the fact that ASPT values increased at all was because the lower scoring families are more easily sampled. Pinder *et al.* (1987) applied the National Water Council (NWC) Biotic Index to their replicate data and found that Total Score increased with the number of sample units until the ninth sample after which no further increase was noted. ASPT changed very little with increasing sampling effort. Thus, whilst Total Score tended to increase with increasing number of sample units, the Average Score per Taxon (ASPT) values are less affected by number of sample units and the importance of the inclusion of this value in the interpretation of results is thus clearly demonstrated.

The SASS method generally required less sample replication than the quantitative method, although the coefficient of variation at Site 1 was high. The relatively heterogenous nature of the substratum at sites such as Site 1, an unimpacted mountain stream, even within a single SASS-defined stones-in-current biotope, suggests that either more than one SASS sample should be taken at such a site, or the total sampling time (currently set at two to five minutes) should be increased so that all possible components of the stones-in-current biotope can be adequately sampled and thus variability reduced. This is particularly important if SASS scores from such sites are used as reference sites against which SASS scores from sites with impaired water quality are compared. The sites that had impaired water quality had low coefficients of variation, suggesting that a single SASS sample would be adequate at such sites.

One difference that emerged between the two methods was the large difference in Total Score, with quantitative samples consistently achieving a higher score than SASS samples. This was largely the result of a greater number of taxa, particularly those taxa with a lower frequency of occurrence, collected in quantitative samples. Storey *et al.* (1991), in a comparison of Surber-sampling (similar to box-sampling) and kick-sampling, observed a similar pattern, and proposed that the Surber method is more suited to collecting cryptic and closely adherent taxa in sites with a highly heterogeneous substratum. The inability of SASS sampling (which uses the kick-method) to detect low-occurrence taxa is of concern in environmental impact assessments, particularly if such assessments are designed to determine the rare and potentially endangered taxa. Storey *et al.* (1991) advise that Surber-sampling be undertaken in previously unsampled areas so that rare taxa are detected, but that the more rapid kick-sampling method be used in biological monitoring programmes. ASPT values calculated using data from the two different methods at each site were similar, suggesting that the scores of additional rarer taxa were not necessarily high (sensitive) or low (tolerant) ones but that they ranged between the two extremes.

5.3.2. Site differentiation

Quantitative box-sampling and rapid bioassessment using SASS both resulted in benthic macroinvertebrate samples which ensured differentiation of the three sites with different water quality. Both cluster analysis and multi-dimensional scaling produced faunal groups that were consistent in distinguishing between the three sites. The fact that the same groups were delineated for quantitative sampling in which all size fractions were combined, for quantitative sampling in which only the >950 μm size fraction was used, and SASS sampling in which organisms are collected in the field at the >950 μm-mesh size, suggests that both quantitative sampling at >950 μm and SASS sampling would ensure site differentiation at the Family level. The higher stress values in both of these analyses however indicates that conclusions should be based on interpretation of both the ordination and cluster analyses.

The rapid field-orientated method, SASS, is therefore clearly able to distinguish the same site differences as were revealed under intensive (and labour and time costly) quantitative benthic sampling. The distinguishing taxa as determined by each set of analyses (i.e. quantitative,

all size classes combined, quantitative >950 μm size class only, and SASS) were Notonemouridae and Leptophlebiidae at Site 1. Ephemerellidae were important in the quantitative, combined group, whilst Helodidae were important in the others. Leptoceridae, Libellulidae and Caenidae commonly distinguished Site 2, whilst Heptageniidae were important in the quantitative, combined group, and Hydracarina and Aeschnidae in SASS. Tricorythidae and Chironomidae were the only two taxa commonly distinguishing Site 3. Hydropsychidae, Hydroptilidae and Simuliidae were distinguishing taxa in both quantitative analyses, whilst Ecnomidae was a distinguishing taxon in SASS. Based on these analyses, it appears that the smaller size fractions of the quantitative samples ensure collection of the younger individuals from taxa such as Ephemerellidae and Heptageniidae which were of less significance in either the >950 μm or SASS samples. Distinguishing taxa from the >950 μm size class groups and SASS samples overlap, with two or three distinguishing taxa common to both at each of the three sites.

5.3.3. Mesh diameter

Mesh diameter or mesh size is a component of the net used during sampling and screens used in subsequent sorting. A survey conducted by Resh & McElravy (1993) revealed that 50% of lotic studies used a mesh size of 301-500 μm. Sampling with fine meshes requires greater sampling, sorting and identification time. For studies using absolute data, Voshell *et al.* (1989, cited by Resh & McElravy 1993) suggested the following points in considering the trade-off between sorting time and accuracy: 1) use as fine a mesh as possible, 2) clearly state size of mesh in published results, and 3) interpret results knowing that some taxa and size classes probably passed through the net used.

While significant differences in abundance and number of taxa were determined between certain of the size fractions, and size fractions thus influence the total abundance of organisms present in a sample, the smaller size classes appear to have less effect on the number of additional taxa presence in a sample (Table 5.8.). If one calculates the contribution made by each of the size fractions to the total number of taxa within a sample, the >950 μm fraction generally constitutes between 86 and 94% of the taxa present. Mesh size did not significantly affect the number of sample units needed to ensure collection of

95% and 75% of the taxa at each site. Different size class groups from each site subjected to cluster and ordination analyses did not form distinct groups. Cluster and ordination analyses conducted to assess the ability of each method to differentiate sites and how this is affected by incorporation of smaller size fractions (<950 - >250 μm) in the quantitative samples, showed that similar groupings emerged at 50% similarity regardless of which size classes were analyzed. At 75% similarity the inclusion of this smaller size class resulted in additional differentiation into quantitative and SASS samples. This suggests that smaller size fractions do affect the groupings but at a low dissimilarity level which does not affect site differentiation. These factors suggest that faunal difference resulting from mesh diameter are minimal, and that larger size fractions can be used with a certain degree of confidence at the Family level for assessment using a taxon-dependent method such as SASS, with the stipulation that mesh diameter be clearly stated and data interpreted accordingly.

5.3.4. Diversity

The abundance of literature (e.g. Winterbourn 1980, Pinder *et al.* 1987, Barton & Metcalfe-Smith 1992) examining the potential use of diversity indices in the assessment of water quality and the conflicting opinions regarding their usefulness and validity, prompted a brief examination of data in the present study. Shannon-Wiener diversity was calculated for each site based on twenty replicates per site. Shannon-Wiener diversity was significantly lower at Site 3 (n=20). There was no significant difference in diversity between Sites 1 and 2. Diversity indices summarise richness, evenness and abundance of taxa in a collection, so lower values are thought to reflect a general response to stress cause by any type of pollution (Washington 1984, cited by Barton & Metcalfe-Smith 1992). On this basis Site 1, an unimpacted mountain stream with good water quality, should have a significantly higher diversity than Site 2, a nutrient- and organically-enriched site. This is however not the case. Other factors such as habitat availability and physical stresses may affect diversity. Physical stresses, such as those present in mountain streams, may override water quality factors, resulting in relatively low diversity at sites that have excellent water quality (Wells 1992). Hilsenhoff (1977) provides further evidence that many small streams have natural low diversity and therefore a low index value does not necessarily indicate reduction in water quality. Barton & Metcalfe-Smith (1992) observed that diversity indices were not useful in

drainage regions subject to numerous impacts, such as sewage effluent, agricultural runoff and industrial discharges. Diversity therefore should only be used in conjunction with other indicators or methods of water quality assessment to ensure that appropriate conclusions are reached.

5.4. CONCLUSIONS

Sample variability and replication associated with two methods of biological assessment were investigated. A minimum of twelve and four quantitative samples is needed to ensure collection of 95% or 75% of benthic macroinvertebrate taxa respectively. Sampling within a single biotope component, such as a "riffle" or "run" would reduce the number of samples needed. A minimum of four and two rapid bioassessment samples (SASS) is needed to ensure collection of 95% or 75% of benthic macroinvertebrate taxa respectively. The SASS technique is however, designed such that only one sample is taken per site. The percentage of Total Scores that one sample would produce relative to a total of 20 samples, were 28%, 59% and 45% for Sites 1, 2 and 3 respectively. Clearly, Total Score increases with increasing sampling effort. Average Score per Taxon however, changed very little with sampling effort. The importance of using this value in interpretation of scores is thus clearly demonstrated.

Variability, as determined by both quantitative sampling and rapid bioassessment, was greatest at the least impacted site. The recommendation that such sites are more intensively sampled, either by increasing the number of box-samples taken, or by increasing the time period for SASS sampling, is proposed. This is particularly important if SASS scores from such sites are used as reference sites against which SASS scores fom sites with impaired water quality are compared. Faunal samples from sites with impaired water quality were less variable and since these are the sites often focused on by SASS users, the technique is clearly adequate.

The ability of two biological assessment methods to differentiate between sites which differed in water quality was tested. Both quantitative box-sampling (including all size fractions combined and the >950 μm fraction only) and SASS sampling resulted in Family-level

faunal samples that were grouped by site. The rapid field-orientated method, SASS, is able to distinguish the same site differences as were revealed under intensive quantitative benthic sampling. Distinguishing taxa from the >950 μm size class groups and SASS samples show a good degree of overlap, with two or three distinguishing taxa common to both biological assessment methods at each of the three sites.

The effect of mesh size on total abundance of benthic macroinvertebrates and on number of taxa was investigated. While significant differences in abundance and number of taxa were determined between certain of the size fractions, the smaller size classes appeared to have less effect on the number of additional taxa present in a sample. The >950 μm fraction generally includes between 86 and 94% of the taxa at each site. Mesh size did not affect sample replication or site differentiation.

The advantages of a bioassessment method such as SASS, including factors such as rapidity, cost-effectiveness, ease-of-use and relatively low-intensity technician training, indicate its usefulness as a tool in routine monitoring of water quality. The following chapter focuses on the reliability of SASS in distinguishing between sites of different water quality. Potential problems such as biotope availability, temporal variability and longitudinal changes down a river course are investigated. The importance of establishing reference sites to be used within a water quality monitoring framework and a method for objective tolerance/sensitivity score allocation are discussed.

CHAPTER 6

SASS AND WATER QUALITY: POTENTIAL PROBLEMS ASSOCIATED WITH THIS RAPID BIOASSESSMENT TECHNIQUE

6.1. INTRODUCTION

This chapter aims to ascertain if the South African Scoring System, SASS, is reliable in distinguishing between sites in the south-western Cape that differ in water quality. Potential problems associated with this technique are investigated. The development of SASS was primarily aimed at facilitating the detection of an impairment of water quality in river systems within South Africa. The method was not designed to enable the exact nature of the impairment to be determined. The intention was that once an impairment of water quality had been established, it would be further assessed via intensive chemical studies. Chutter (1994a) and Chutter & Geuppert (1993) conducted a number of assessments at sites of known water quality in the Eastern Transvaal using SASS, and showed that SASS differentiates between sites that differ in water quality. Utilization of the SASS method in the south-western Cape began in February 1993, when the SASS2 version was in use. Information gained in these initial and subsequent stages has ensured that the SASS scoring system takes account of the benthic macroinvertebrate families that are largely endemic to the acid, brown-water streams of the Southern and Western Cape region. In the south-western Cape, a number of sites that vary in water quality have been sampled using SASS. In this chapter these data are assembled and analyzed to evaluate the SASS technique for applicability and reliability with regard to water quality conditions. The relatively large dataset that has been accrued during this study also makes it possible to undertake some preliminary investigations into potential problems associated with SASS.

Data used in these investigations include those from SASS sampling undertaken at sites within the Berg River catchment (24 sites) in September 1993, and September 1994. Various other rivers within the south-western Cape (Figure 6.1.), including the Palmiet, Molenaars, Eerste, Kraalstroom and Olifants, were sampled on behalf of the environmental consulting company, Afridev, and permission to use some of these data is gratefully acknowledged. Site codes and descriptions are given in Appendix A.

The following potential problems associated with SASS are investigated:

1) The influence of biotope availability and how the variety of biotopes accessible to the aquatic biota affects the SASS scores, in particular the Total Score and the Average Score Per Taxon (ASPT).

2) The influence of temporal differences in benthic macroinvertebrate abundances and community composition on SASS scores.

3) The effect and influence of longitudinal changes along a river course on SASS scores,

4) and in relation to this, the potential for using reference sites within-regions against which SASS scores within the same regions can be evaluated.

According to Herrick & Cairns (1982) a primary weakness in biotic indices is the subjective assessment which is often used to classify the tolerance/sensitivity of organisms. Winget & Mangum (1979, cited by Herrick & Cairns 1982) proposed a biotic condition index based on extensive correlations between species presence and water quality. The approach is data-intensive and requires complex analysis to establish local and regional species/tolerance relationships, but it would support continued use of biotic indices as an essential part of biological assessment of community structure and provides an objective framework for score allocation. In relation to this the following was also investigated:

5) the potential for developing an objective means of score allocation, and how the objective scores relate to the subjective ones derived from expert opinion, are explored.

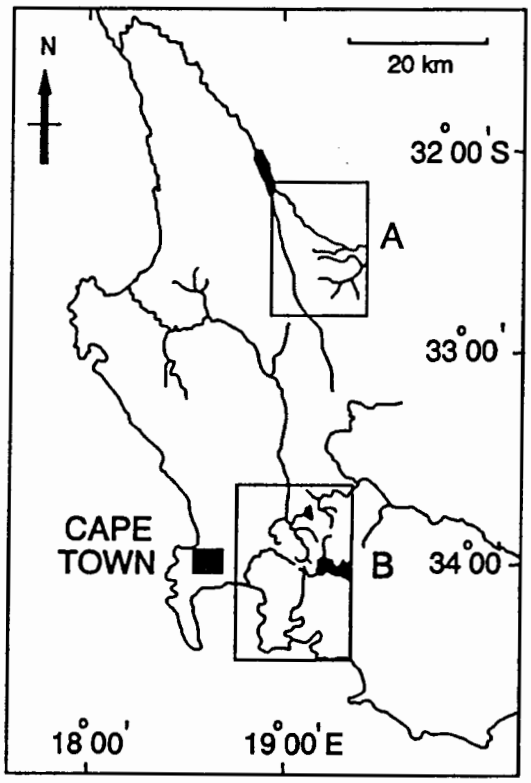
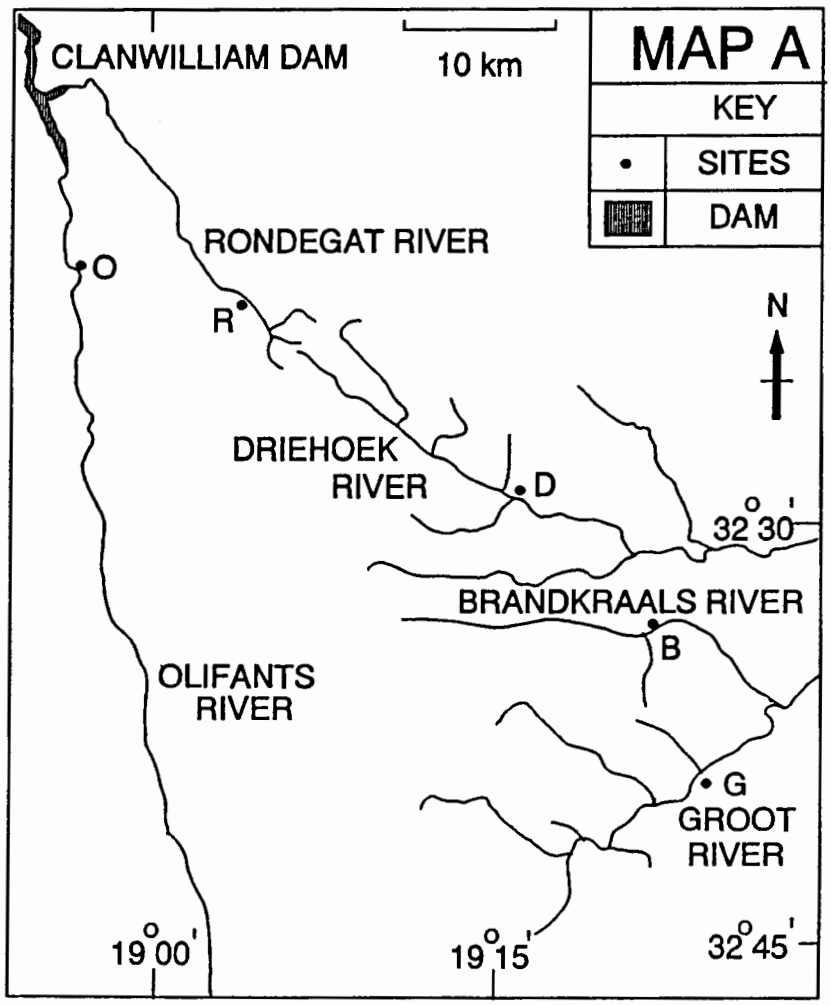
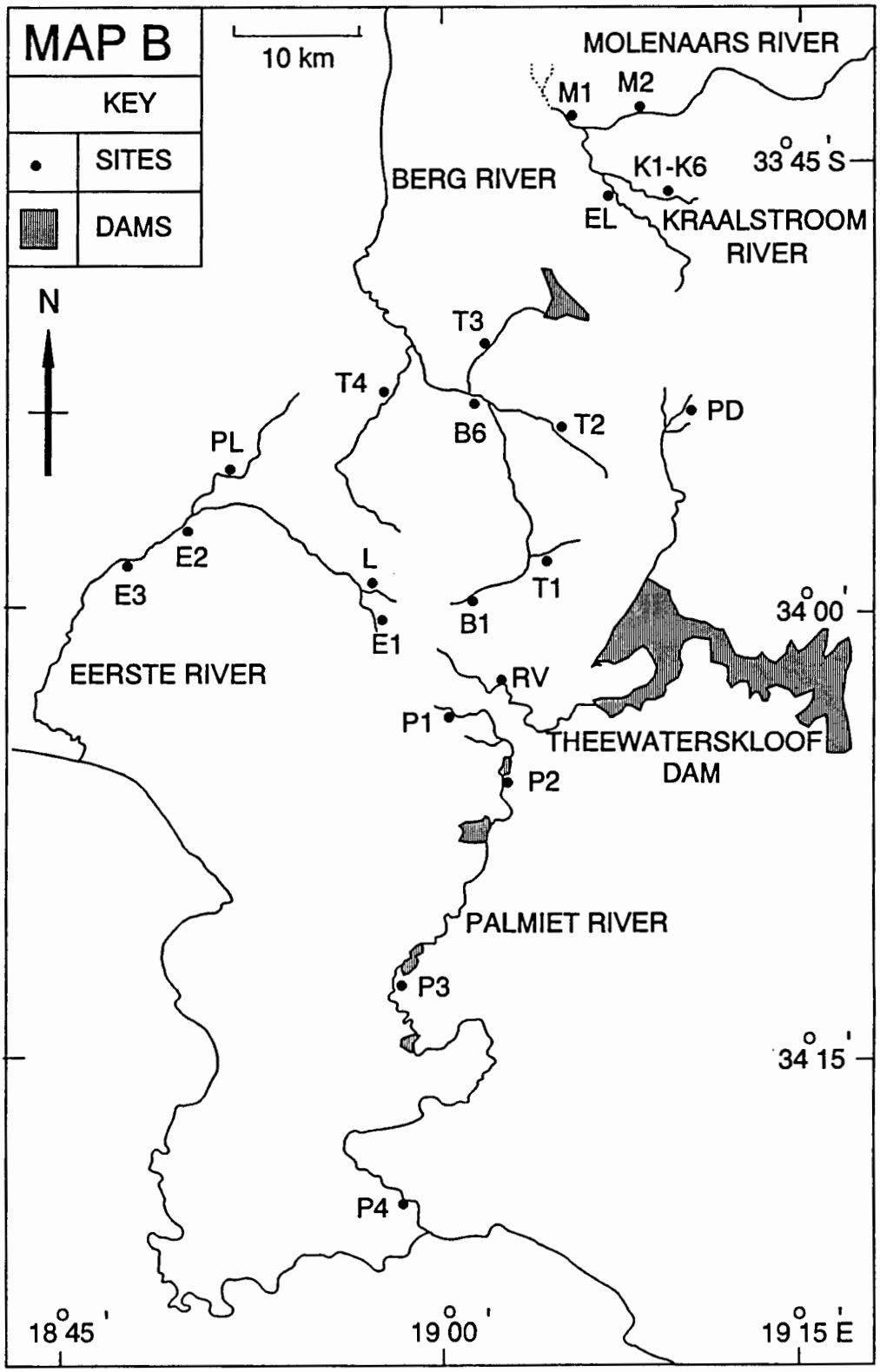


Figure 6.1. Map of the south-western Cape showing rivers on which SASS sampling was undertaken. Two insets are enlarged to detail sites in the Olifants River region and sites in the Palmiet, Eerste, Molenaars River region. Sites on the Berg River are as in Figure 4.2. All site codes and associated site details are given in Appendix A.



Map A: Sites in the Olifants River region.
O=Olifants River,
R=Rondegat River,
D=Driehoek River,
G=Groot River,
B=Brandkraals River.

Map B: Sites in the Palmiet, Eerste, Molenaars River region. Sites are coded according to each river as follows: B=Berg River, T=Berg River Tributary, E=Eerste River, L=Lang River, PL=Plankenbrug Stream, P=Palmiet River, K=Kraalstroom River, EL=Elands River, M=Molenaars River, PD=Perdekloof River, RV=Riviersonderend River.



6.1.1. History of score allocation

The South African Scoring System (**SASS1**) is based on the British Monitoring Working Party (BMWP) score system, an account of which is given in Armitage *et al.* (1983). Scores from the BMWP system formed the foundation of the tolerance/sensitivity scores (1 to 10) which were then modified (Chutter 1992) to take account of those families that are found in South Africa but not Britain. The SASS system is currently based on scores allocated to each taxon on the basis of expert opinion. Initial testing of this scoring system in South Africa revealed that the Average Score per Taxon (ASPT) values were not adequately indicating differences in water quality, and so the scores for taxa were modified by lowering the scores for tolerant taxa and increasing the scores for intolerant taxa, with the most sensitive taxa each being allocated a score of 15. This modified **SASS2** scoring system was used extensively in a number of geographic regions within South Africa, including the Eastern Transvaal and Natal, Eastern Cape, Southern Cape and Western Cape. The recording of a number of taxa which were not included in the SASS2 scoring system, and additional information on tolerance/sensitivity ratings of individual taxa, prompted the modification of the scoring system to **SASS3**. SASS3 had a number of additional features which were incorporated into the scoring system as a result of discussion between members of the Rapid Biological Assessment (RBA) Forum. The first of these was the introduction of a sliding scale of scoring for two of the families, namely the Baetidae (Ephemeroptera) and Hydropsychidae (Trichoptera). Both these families have one or two very tolerant species which are regularly present under conditions of impaired water quality. There are other species within both these families which are extremely sensitive to pollution however. By incorporating a sliding scale of scoring, the presence of more that one species ensures that sites with sensitive ones are not under-scored. The second development in the scoring system was the grouping together of the cased-caddisfly larvae (Trichoptera). Whilst it is possible to separate this group into their respective families given the correct level of taxonomic expertise and microscopic identification, the SASS method, which is designed to be a field-based and technician-driven monitoring system, does not permit this higher resolution. As a result of this, the cased-caddis families are grouped and scored on a sliding-scale which is graded on the basis of the number of cased-caddis types present. On the basis of expert opinion, one mayfly family, the Leptophlebiidae, has been allocated two different

scores depending on the pH of the system in question.

6.2. SASS AND WATER QUALITY

6.2.1. Interpretation of scores

The usefulness of the SASS technique is dependent on its ability to distinguish between waters of different quality. The influence on SASS scores of non-water-quality variables, such as biotope availability, flow velocity and insolation has not been established. The need to differentiate between changes in SASS scores that result from water-quality as opposed to non-water-quality effects clearly needs to be examined. Three of these non-water-quality factors, namely biotope availability, temporal variation and longitudinal changes, are examined later in this chapter.

Chutter (1994b, Chutter & Geuppert 1993) selected a number of sites over a wide geographic area, including the Eastern Transvaal and Natal, and the Eastern, Southern and Western Cape. Sites ranged from unimpacted, undisturbed sites in nature reserves to sites exposed to varying degrees of pollution, such as treated sewage outflows, mining effluents and agricultural runoff. Some physical attributes and chemical constituents at each site were determined. On the basis of exposure to pollution and water quality characteristics the sites were grouped into one of three water quality categories. "Good" water quality sites were those where there was no reason to suspect water quality impairment, "poor" water quality sites were those below point sources of pollution, where there was good reason to conclude that water quality was impaired. Most of the "intermediate" water quality sites were in recovery zones of polluted rivers (Chutter 1994b). This form of categorization is necessary because of the difficulties in interpreting a wide range of physical and chemical data in any more detail. SASS sampling was conducted at each of these sites and range of Total Scores and Average Score per Taxon (ASPT) values within each water quality category was calculated (Table 6.1).

At sites with poor water quality, Total Scores were low and ASPT values were low but more variable. At sites with good water quality, Total Scores were generally high but more

variable than the ASPT values which tended to be high and relatively constant. Chutter (1994a) emphasised the importance of using both Total Score and ASPT in interpreting results and recommended the following: When Total Scores are low (<35) more weight should be placed on SASS than on ASPT values, in assessing the probable quality of the water. When ASPT scores are high (>6.0), more weight should be given to them than to Total Scores. At intermediate Total Scores, equal weight should be given to Total Scores and ASPT values.

Table 6.1. Ranges of SASS3 scores and ASPT values associated with water of differing quality (Chutter 1994b).

Water Quality	Total Scores	ASPT
Poor	< 35	< 4.0
Intermediate	36 - 79	3.0 - 5.0
Good	> 80	> 4.5

Table 6.1. was developed by incorporating SASS data collected over a wide geographic area, including the Eastern Transvaal and Natal, and the Eastern, Southern and Western Cape. The distinct aquatic fauna associated with acid, brown-water streams in the south-western Cape prompted further examination of Total Scores and ASPT ranges at sites within this geographic region. Forty of the 49 sites (distributed on 23 rivers, within six catchments: Berg, Molenaars, Kraalstroom, Eerste, Palmiet and Olifants) sampled in the south-western Cape were used to facilitate calculation of Total Score and ASPT ranges. Each of these sites was grouped within one of three predicted water quality categories on the basis of proximity to a pollution source or point of impact. Unimpacted sites were normally in mountain catchments, situated above any major impact. Moderately impacted sites were sites sufficiently downstream of an impact such that restoration towards pre-impact conditions was evident, or were in areas of diffuse low intensity agriculture. Severely impacted sites were situated below point source pollution (such as fishfarming operations, sewage treatment works, industrial effluent), below dams, or in areas of intensive agriculture or with elevated TDS concentrations. Fourteen water quality variables, including total suspended solids, total dissolved solids, conductivity, pH, total alkalinity, major anions (chloride and sulphate),

major cations (sodium, calcium, magnesium and potassium) and nutrients (NH_4^+-N, NO_3^--N, NO_2^--N) were measured at 33 of these sites. Data were square-root transformed and analyzed using a multivariate clustering method for environmental variables (PRIMER VER. 4). The analysis was aimed at demonstrating differences in physical and chemical variables between sites and the resultant dendrogram is given in Figure 6.2. Both proximity to pollution or impact, and cluster analysis of water quality, were used to categorize sites into groups of similar water quality, namely good, intermediate and poor water quality. SASS assessments were then conducted at each of these sites and the ranges in Total Scores and ASPT values calculated. Discriminant function analysis was undertaken to validate the groupings of sites into these three water quality categories. 93% of the sites, on the basis of Total Score and ASPT values, were grouped in the predicted water quality categories ($P<0.05$). All unimpacted sites were correctly classified, two moderately impacted sites were incorrectly classified and one severely impacted site was incorrectly classified. Total Score and ASPT values within each water quality category (Table 6.2) are higher than those given by Chutter (1994b). Box-and-whisker plots of Total Score and ASPT values for each of these water quality categories is given in Figure 6.3. Non-parametric analysis of variance indicated that the water quality categories were significantly different in terms of both Total Score (Kruskal-Wallis Test-statistic=24.15, $p<0.05$) and ASPT (Kruskal-Wallis, Test-statistic=29.97, $p<0.05$). The establishment of ranges of SASS scores and ASPT values associated with water of different quality, enables water quality at new sites to be determined using SASS. Sites which have a lower than expected SASS score can then be examined further and potential points of pollution and impact determined. Measures to eliminate or control these deleterious effects can then be undertaken.

Table 6.2. Ranges of SASS4 scores and ASPT values as determined for sites in the south-western Cape which have poor, intermediate and good water quality.

Water Quality	Total Scores	ASPT
Poor	< 85	< 6.0
Intermediate	86 - 140	6.0 - 7.5
Good	> 140	> 7.5

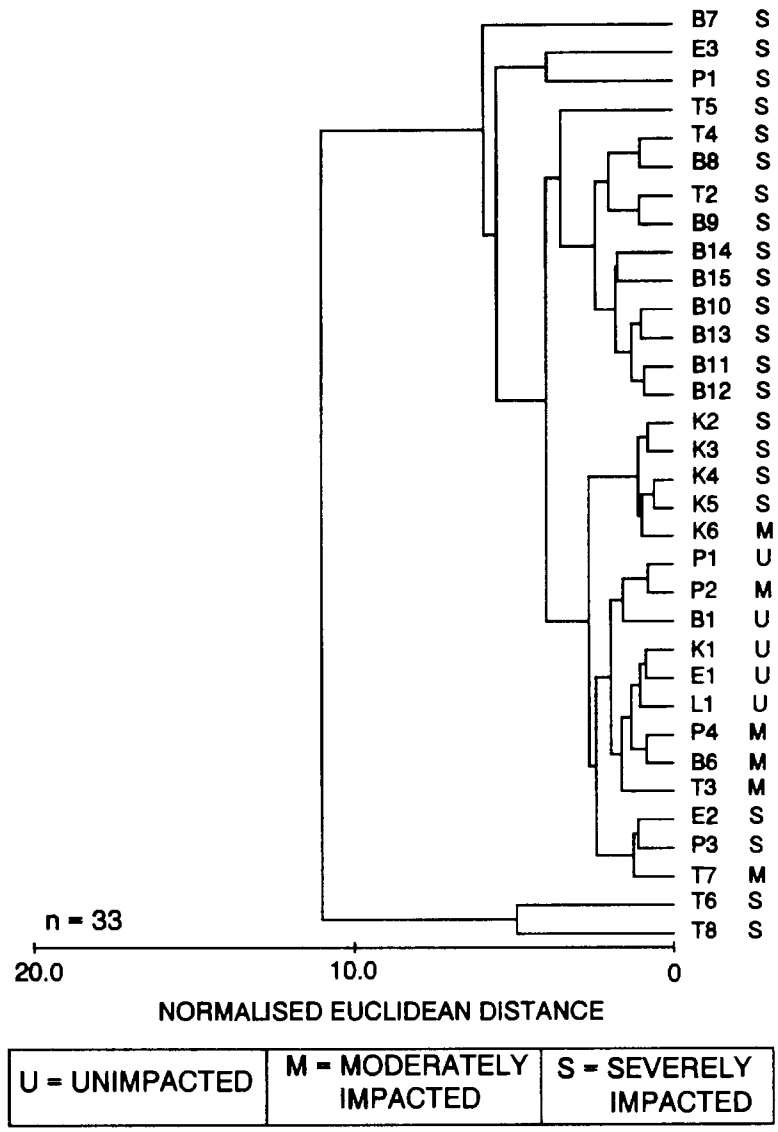


Figure 6.2. Dendrogram for hierarchical clustering of 33 sites in the south-western Cape on the basis of fourteen water quality variables (square-root transformed). The proximity to pollution or point of impact is indicated after each Site Code: U=unimpacted, M=moderately impacted, S=severely impacted.

6.2.2. Total Score and ASPT values

The relationship between Total Score and ASPT values was investigated using data from the same forty south-western Cape sites, which were grouped as unimpacted (1), moderately impacted (2) and severely impacted (3). Each observation is plotted as such on Figure 6.4. Total Score and ASPT values were significantly positively correlated ($r^2=0.77$, $p<0.05$).

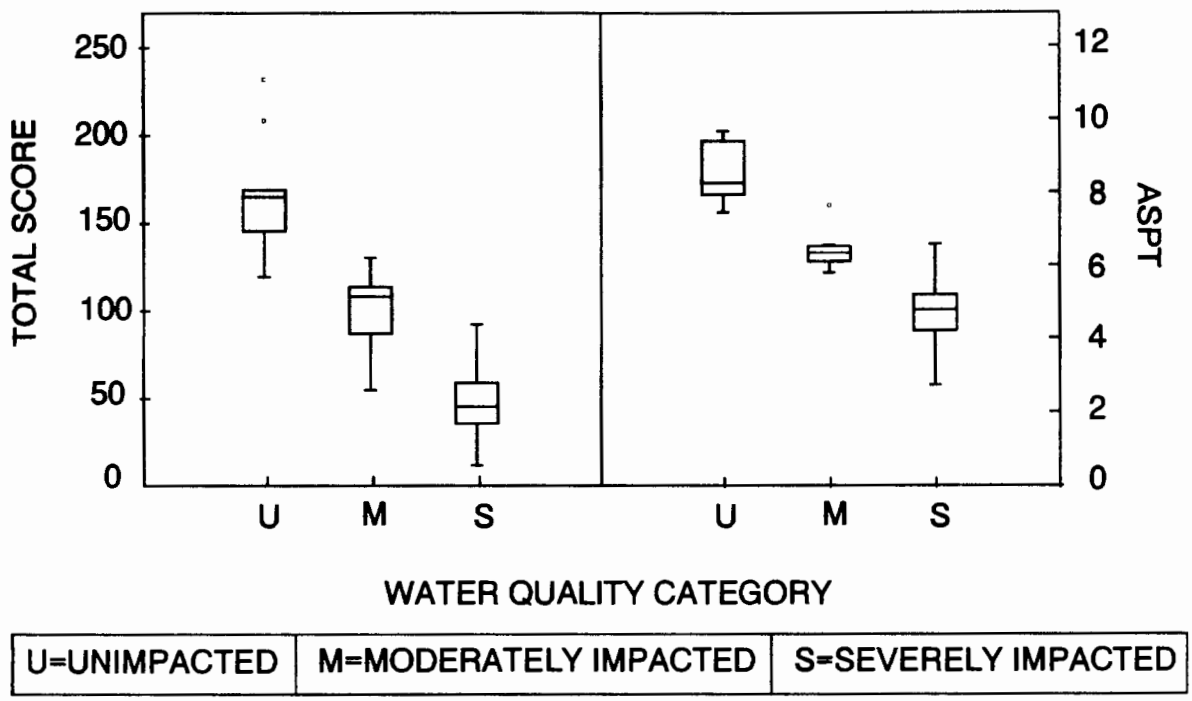


Figure 6.3. Box-and-whisker plots of Total Score and ASPT values in each water quality category calculated for 33 sites in the south-western Cape. U=unimpacted site (good water quality), M=moderately impacted site (intermediate water quality) and S=severely impacted site (poor water quality).

The severely impacted sites generally had a low Total Score and ASPT value, moderately impacted sites had intermediate Total Scores and ASPT values, whilst unimpacted sites had high Total Score and ASPT values. The scatter was greatest at unimpacted sites, and less in moderately and severely impacted sites. Three sites did not group with the water quality category that one would expect. The category-3 site (indicated with a circle) was on the Dwars River, a tributary of the Berg River. The high ASPT values is the result of a single mayfly of Family Ephemerellidae, which is given a score of 15. It is likely that this single individual drifted in from upstream. One category-2 sites (indicated with a square) was below impoundments in the Kogelberg Nature Reserve near the estuary. Gale (1992) suggests that the 34 km of undisturbed environment through which the river flows, before entering the estuary, will probably "reset" the system close to its original state. The high

Total Score and ASPT value indicates good water quality which supports this view. A second category-2 site (indicated with a circle) grouped with category 3, probably because of limited biotope availability, namely the absence of stones-in-current.

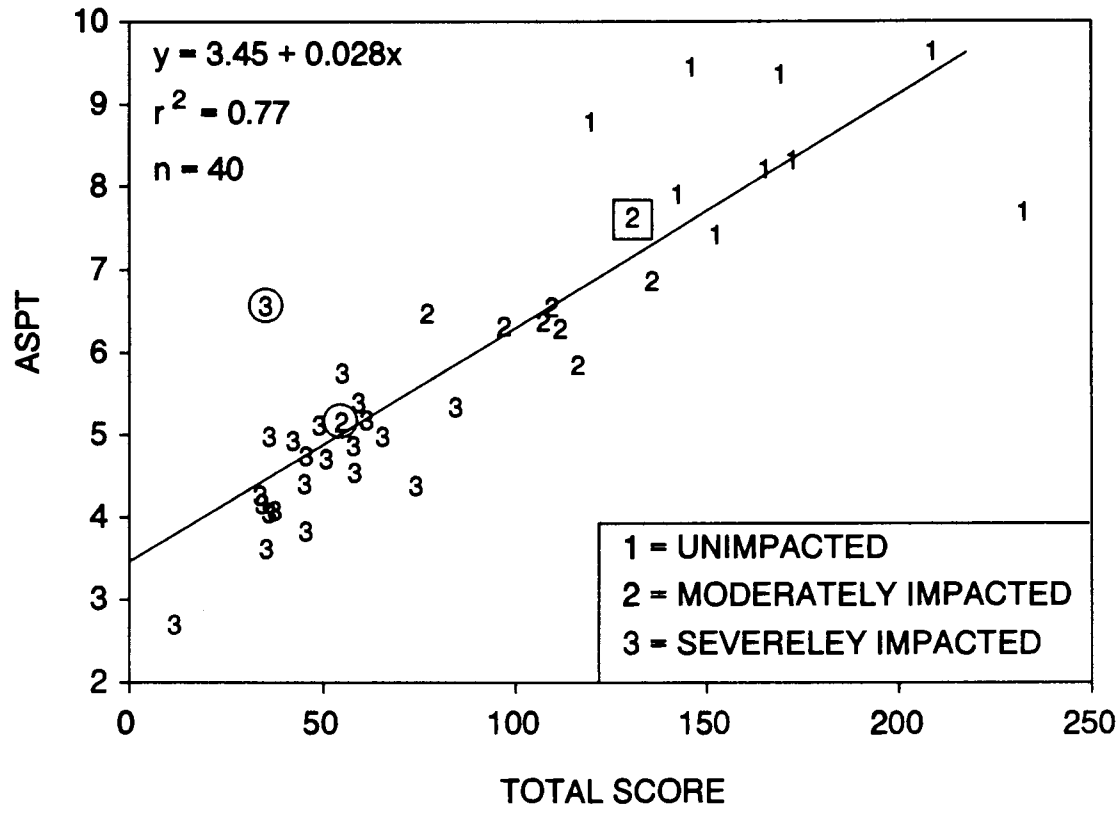


Figure 6.4. ASPT values plotted as a function of Total Score for unimpacted (1), moderately impacted (2) and severely impacted (3) sites in the south-western Cape.

6.3. POTENTIAL PROBLEMS ASSOCIATED WITH SASS

SASS has been developed to facilitate the assessment of an impairment of water quality. To ensure that an observed effect as determined by SASS, either expressed as Total Score or ASPT, is actually the result of an alteration in water quality, other factors which could potentially affect these scores need to be eliminated. Three of these factors include biotope availability, natural or inherent temporal variability and longitudinal variation down a river course. Each of these is explored. Other factors such as those related to water quantity (e.g.

flow velocity) and biological factors (e.g. food availability) may also affect SASS results but are not considered here.

6.3.1. Biotope availability

Chutter's Biotic Index (Chutter 1972) was developed for use in a single biotope, namely stones-in-current. This obviously imposed restrictions on its usefulness, particularly for assessing water quality at sites where this biotope is absent. SASS was designed to incorporate all available biotopes. Given that certain taxa are commonly associated with a particular biotope, it seems likely that the number and type of biotopes available for habitation by aquatic biota, and which are thus sampled by SASS, may have an effect on the resultant Total Scores. For example, there are stream types typified by certain biotope combinations. Mountain streams often only have stones-in-current and stones-out-of-current biotopes, whilst physically degraded streams, in over-exploited catchments, may have only sand or mud or sparse marginal vegetation biotopes. Many lowland river sites only have marginal vegetation and sand as available biotopes. Whilst physical degradation and impairment of water quality are not mutually exclusive, it is important that the physical degradation or general sampling limitations that result from biotope restrictions, be accounted for if water quality impairment is to be established.

To establish the relative importance of each biotope in determining the Total Score, number of taxa and ASPT value at a site, in this study biotopes were sampled separately and the taxa found within each biotope were recorded. The percentage contribution of taxa within each biotope to the Total Score, number of taxa and ASPT value for a site is given in Figure 6.5. Because certain taxa are found in more than one biotope the summed percentages from the biotopes does not equal 100%. Instead the percentage given for each biotope is that percentage relative to the total calculated for the site. For example taxa present in the stones-in-current biotope constituted 70% of the Total Score for the site. These percentages are calculated from SASS data collected at 27 sites, on 15 rivers, in six catchments within the south-western Cape. Six of the sites were sampled on two occasions and the total number of observations is therefore 33.

Based on these data, taxa present in the stones-in-current biotope (note that this includes both runs and riffles) constituted 70%, 69% and 103% of the Total Score, number of taxa and ASPT values for the site. Because ASPT values are calculated by dividing the Total Score

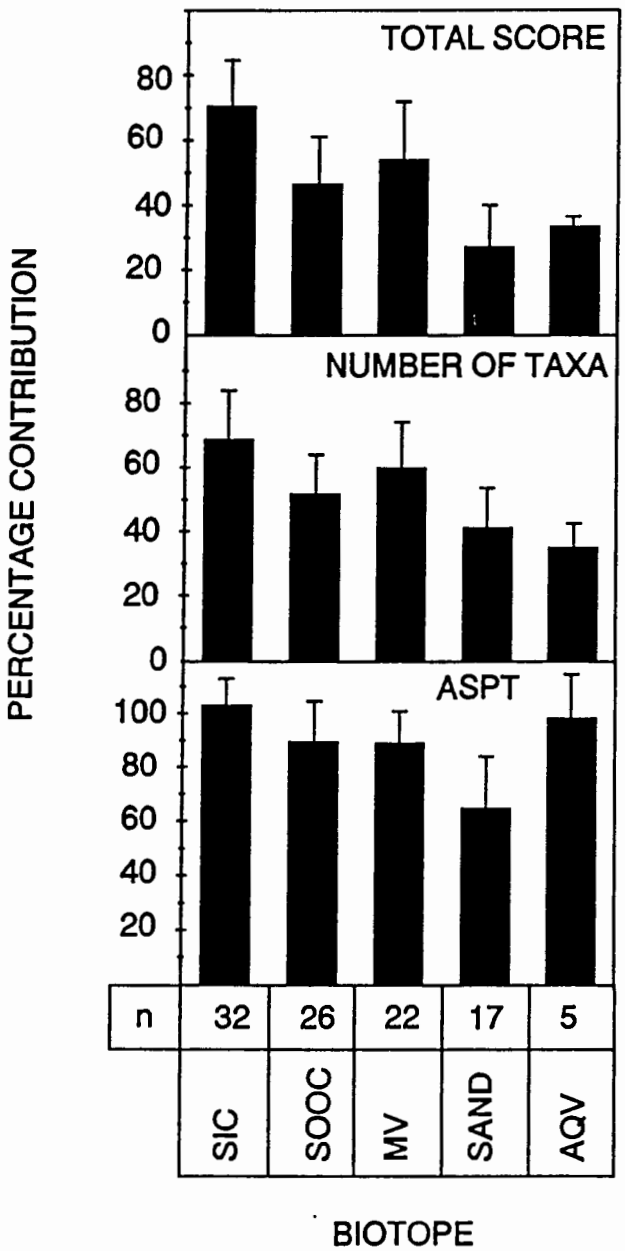


Figure 6.5. Mean + Standard Deviation of percentage contribution of Total Score, number of taxa and ASPT values for SASS samples (n=33, south-western Cape) collected separately in five biotopes (SIC=Stones-in-current, SOOC=stones-out-of-current, MV=marginal vegetation, SAND=sand, AQV=aquatic/ instream vegetation) to Total Scores, number of taxa and ASPT value calculated for the site.

by the number of taxa, subsequent calculation of the percentage contribution of the ASPT values within this biotope to the ASPT value for a site, resulted in an ASPT value greater than 100%. This indicates that more of the sensitive and high scoring taxa are present in this biotope. Stones-in-current (SIC) was the most common biotope sampled (n=32). Stones-out-of-current (SOOC) contributed 46%, 52% and 89% of the Total Score, number of taxa and ASPT value for the site respectively and was the second most common biotope to be sampled (n=26). Marginal vegetation (MV) contributed 54%, 60% and 89% of the Total Score, number of taxa and ASPT value for the site respectively and was the third most common biotope to be sampled (n=22). Although the sand biotope was present at 50% of the sites (n=17) only 41% of the taxa at the site were found in sand, and they contributed 27% and 41% to the Total Score and ASPT value at the site respectively. Aquatic or instream vegetation, often *Scirpus* spp. in the south-western Cape, was uncommon (n=5), but when present taxa in this biotope contributed 33% and 98% to the Total Score and ASPT value respectively.

Of the variation in Total Score, number of taxa and ASPT between biotopes, the range is greatest in Total Score. ASPT values, with the exception of the sand biotope, has a relatively consistent value across biotopes. In the United Kingdom, Pinder *et al.* (1987) allocated National Water Council (NWC) scores to their data and found that when scores from each biotope were combined, Total Score increased greatly, but ASPT remained relatively constant. Balloch *et al.* (1976, cited by Armitage *et al.* 1983) also found consistently higher biotic indices in riffle samples (SIC) and lowest values were recorded from marginal vegetation macrophytes. From these data it is clear that certain biotopes have a greater number of taxa associated with them, and this is reflected in the variations in Total Score.

Chutter (1994b) developed a biotope index (HABS1) in an attempt to ensure that differences in biotope availability were accounted for when SASS sampling was undertaken. Each biotope would be expected to support some taxa classified as pollution sensitive (high-scoring taxa) and some classified as pollution tolerant (low-scoring taxa). On this basis Chutter & Geuppert (1993) argue that whilst SASS scores may be affected by biotope availability, with Total Score increasing as a function of the number of available biotopes, ASPT values will

remain relatively constant since the absence (or presence) of a biotope would result in the loss of both tolerant and sensitive taxa. This is supported by the calculation of the percentage contributions of taxa in each biotope to the Total Score, number of taxa and ASPT values for a site. Chutter (1994b) hypothesised that there is a linear relationship between rapid biological assessment and biotope assessment in unimpacted sites. He developed a biotope assessment method (HABS1) which scores all possible combinations and number of biotopes (total number of biotopes = 7) on a scale of 0 to 100. The seven biotopes are as follows: stones-in-current, stones-out-of-current, fringing or marginal vegetation, aquatic vegetation (submerged or floating weeds), gravel, sand and mud. Chutter (1994b) plotted Total Scores against biotope assessment score (HABS1) for good, intermediate and poor water quality sites. At good water quality sites he noted a trend for Total Scores and ASPT values to increase with HABS1 scores. At intermediate and poor quality sites there was no tendency for Total Scores or ASPT values to increase with the number of biotopes available. This suggests that where water quality is impaired, biotope availability has less effect on SASS scores than at unimpacted sites.

The fact that ASPT values appear to be consistent between biotopes, suggests that sites that have different biotopes available for habitation by aquatic biota, can be compared on the basis of ASPT values so that an impairment of water quality can be established. It should be noted however that the location of the sites being compared, both in terms of their broader geographic location and in terms of their longitudinal position on a river, i.e. upland versus lowland sites, should be taken into account before undertaking comparisons. These factors are discussed in detail in sections 6.3.3. and 6.4.

6.3.2. Temporal variability

Temporal differences in macroinvertebrate communities need to be considered, in particular those related to seasonal changes. Temporal variation occurs as a result of life history features such as emergence, feeding and growth; and environmental perturbations. Resh & Jackson (1993) assessed the effect of season on the accuracy of rapid bioassessment measures and found that there were seasonal differences in almost all of the measures. In the present study, the influence of temporal variability on Total Score and ASPT values was examined

in three ways. Monthly differences were explored using data collected in three or four months at sites on three rivers, namely the Kraalstroom, the Eerste and the Palmiet rivers. Each of these rivers had an unimpacted site, moderately impacted sites (with the exception of the Eerste River) and severely impacted sites. Site details are given in the sections discussing each river and are tabulated in Appendix A. Seasonal differences were examined by applying SASS scores to historical data of seasonal benthic macroinvertebrate abundances collected by Harrison & Elsworth (1958) on the Berg River during 1951, 1952 and 1953. Annual differences were examined using data collected in the Berg River catchment (eight sites on the main river and one site each on seven tributaries) in September 1993 and September 1994.

Monthly differences

Data used in this section were collected on behalf of the environmental consulting company, Afridev and permission to present them is gratefully acknowledged. Six sites (K1 to K6; Figure 6.1. Map B) on the Kraalstroom River were sampled during December 1993, February 1994 and March 1994 (Figure 6.6.). Site K1 was an unimpacted mountain stream, Sites K2, K3, K4 and K5 (all severely impacted) were 0, 50, 250 and 500 metres below the input point of a fishfarm effluent and Site K6 was 1 km below the input point (moderately impacted). All sites were within the mountain stream zone of the river. Both Total Score and ASPT values clearly detected differences in water quality. Monthly Total Score and ASPT were significantly different only at Site K2 ($\chi^2=14.13$, $p<0.05$), which was immediately below the input point. Examination of the associated chemical data for December 1993 and February 1994 indicated that concentrations of total dissolved solids, nitrate-nitrogen and chloride increased from 8.375 to 54.5 mg l^{-1}, 0.003 to 0.103 mg l^{-1} and 0.26 to 4.75 mg l^{-1} respectively. The monthly differences in Total Score and ASPT values were not of a magnitude to mask the differences resulting from the effects of water quality impairment.

One site was sampled on the Jonkershoek River (E1), two sites (E2 and E3) on the Eerste River and one on the Plankenbrug Stream (PL) (Figure 6.1. Map B; Figure 6.7.). Site E1 was an unimpacted mountain stream, Site E2 was within the town of Stellenbosch above the

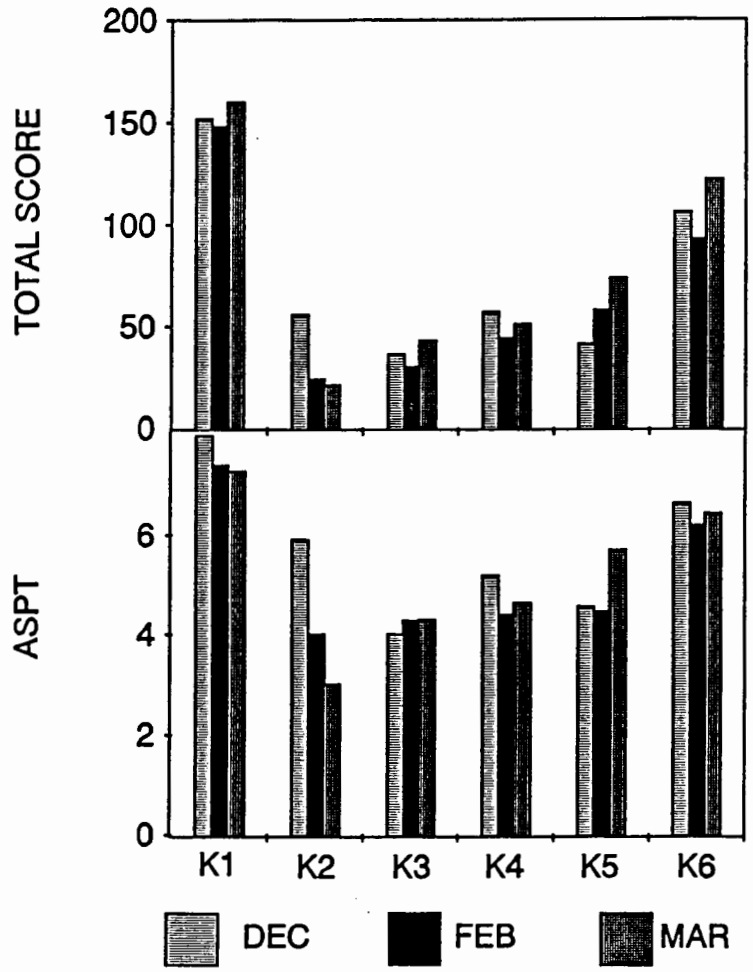


Figure 6.6. Monthly differences in Total Score and ASPT values at six sites (K1 to K6) on the Kraalstroom River, which is subject to input of fishfarm effluent.

sewage treatment works and Site E3 below the sewage treatment works. Both E2 and E3 were severely impacted. Site PL was in a stream below Stellenbosch's industrial area and was heavily polluted by industrial effluents. The sites were sampled in November and December 1993, and February and March 1994. Sites E2 and E3 were in the foothill zone of the river. Both Total Score and ASPT values demonstrated the differences in water quality between the sites. Monthly Total Score and ASPT varied the most at Site E1, the unimpacted mountain stream, and Total Score was significantly different ($\chi^2=17.23$, $p<0.05$). This monthly variation in Total Score and ASPT values at the unimpacted site did not mask the difference resulting from water quality impairment. The severely impacted sites (E2, E3 and PL) clearly had lower Total Scores and ASPT values than the unimpacted site.

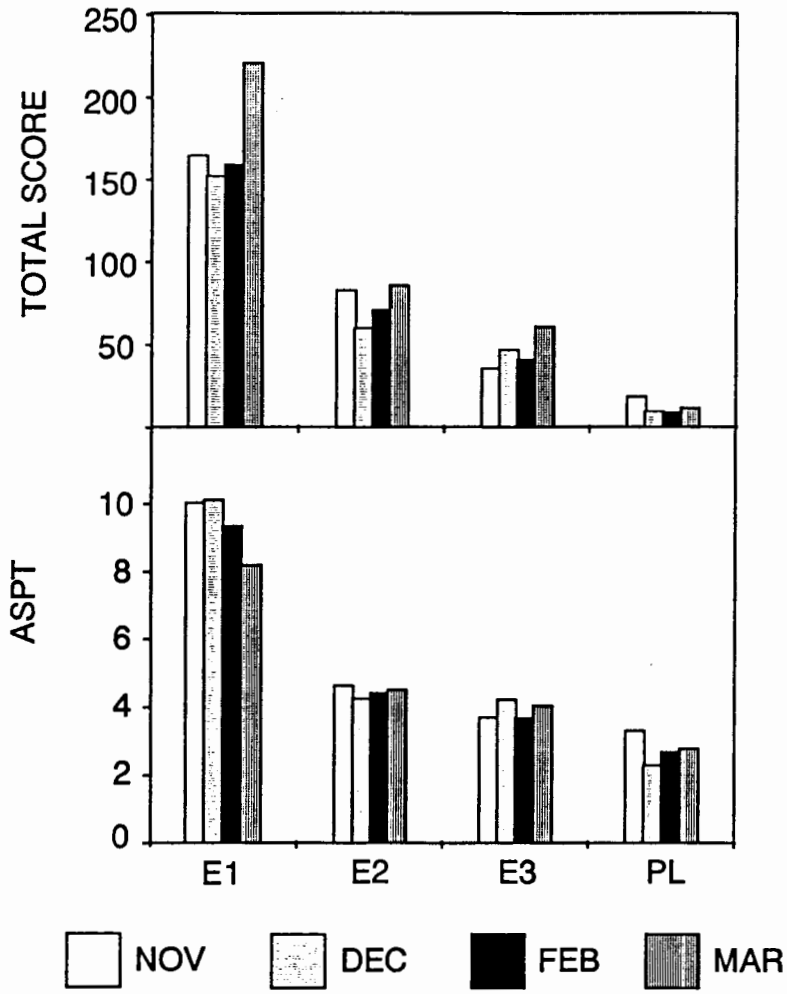


Figure 6.7. Monthly differences in Total Score and ASPT values at three sites on the Eerste River (E1, E2 and E3) and one site on the Plankenbrug Stream (PL), which are subject to treated sewage effluent and industrial effluent respectively.

Four sites (Figure 6.1. Map B) were sampled on the Palmiet River (Figure 6.8.) in November 1993 and December 1993, and February 1994 and March 1994. Site P1 was an unimpacted mountain stream, Site P2 was below the Nuweberg Dam but was classed as moderately impacted on the basis of hierachical clustering of water quality variables at this site (see Figure 6.2.). Site P3 was below the Kogelberg Dam and was classed as severely impacted. Site P4 was in the Kogelberg Nature Reserve, some distance below the dam sites and approximately 1 km upstream of the estuary. It was classed as moderately impacted. The Palmiet River is relatively short and is therefore not divided into zones. Both Total Score and ASPT values demonstrated the differences in water quality between the sites.

Monthly Total Score varied the most at Sites P1 and P4, the unimpacted and moderately impacted sites respectively, although this variability was considerably less in monthly ASPT values. Total Score was significantly different at Site P4 ($\chi^2=30.29$, $p<0.05$) only. ASPT dropped considerably at Site P2 from December 1993 to February 1994. Examination of the chemical data for Site P2 indicated an increase in the concentration of NO_3^--N from 0.004 to 0.524 mg l^{-1} during this period. The monthly differences in Total Score and ASPT values were not of a magnitude to mask the differences resulting from the effects of water quality impairment.

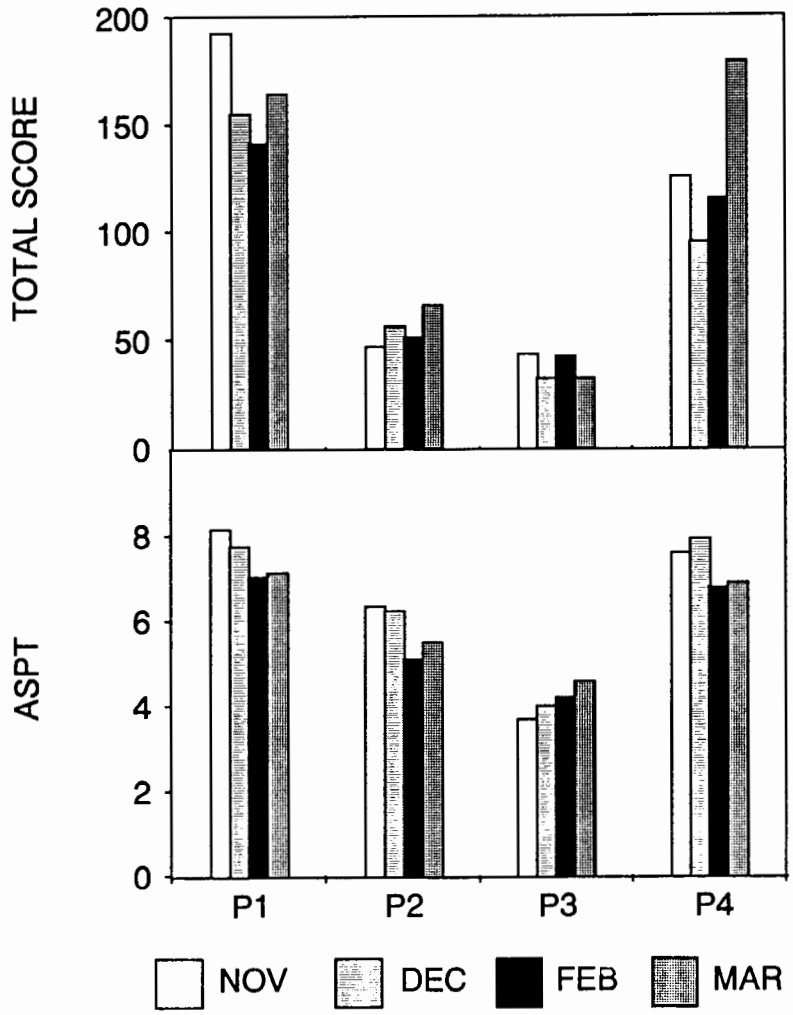


Figure 6.8. Monthly differences in Total Score and ASPT values at four sites (P1 to P4) on the Palmiet River, two of which are below impoundments (P2 and P3).

Seasonal differences

Investigation of seasonal differences in SASS scores is not possible from the data collected in this study. In order to gain some insight into the potential importance of this factor, SASS scores were applied to historical data (Harrison & Elsworth 1958) and ASPT values were calculated for six sites on the Berg River. The geographic location of each site is given in Chapter 4, Figure 4.2. It should be noted that these values are not comparable with other SASS scores because the method of collection was different. Each of the sites correspond to a sampling site in the present study and they are labelled accordingly. The general trend in ASPT values down the river was similar between seasons and when all data from seasons were combined (Figure 6.9.). ASPT values were approximately one unit higher at Sites B3 and B7 in spring than autumn, winter or summer, and 1.5 units higher in winter and spring than summer or autumn at Site B9. These preliminary results suggest that inherent seasonal differences in benthic macroinvertebrate communities may affect SASS scores and the highest ASPT values appear to be in spring. If financial or technical constraints limited monitoring to a single annual assessment, then spring would be a favourable period in which to sample.

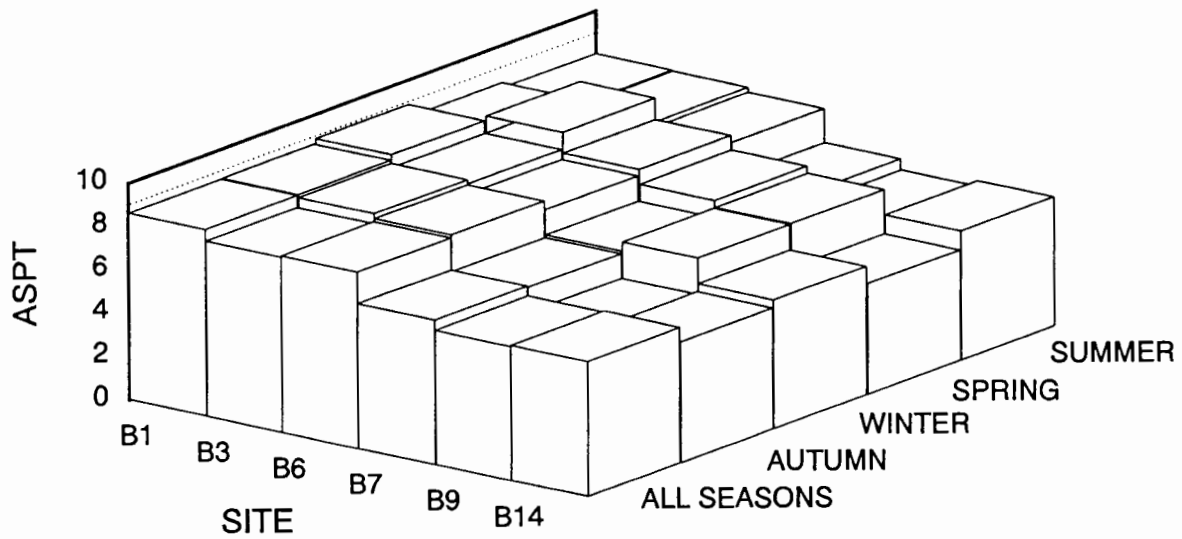


Figure 6.9. ASPT values calculated by applying SASS scores to historical data collected in the 1950's by Harrison & Elsworth (1958) at six sites on the Berg River.

Annual differences

The eight sites on the Berg River are numbered according to their longitudinal position on the rivers as ones moves from source to sea (Chapter 4, Figure 4.2). Of the eight sites, Site B1 is an unimpacted mountain stream, Sites B2 and B3 are in the Franschhoek Forestry Reserve forestry plantation. Site B4 is below the Theewaterskloof interbasin water transfer tunnel and therefore receives water from an adjacent catchment. Site B5 is below a fishfarm and Site B6 is at a point which receives Berg River water in addition to water from the Franschhoek Tributary, which runs through an intensive agricultural area. Sites B2 to B6 are all within the stony-foothill zone as described by Harrison & Elsworth (1958). Annual differences were evident in Total Score at all of the sites and differences in ASPT values at four of the sites (Figure 6.10). Additional data are needed however to establish if these differences reflect changes in water quality or if they reflect natural inter-annual differences within the river system.

Of the seven tributaries of the Berg River (Chapter 4, Figure 4.2), Site T1 is an unimpacted mountain stream (Assegaaibosch Stream), Site T2 (Franschhoek River) and T4 (Dwars River) are within intensive agricultural areas and are subject to organic and nutrient enrichment and Site T3 (Wemmers River) is below a spillage weir. All of these tributaries feed into the Berg River within the stony-foothill zone. Sites T5 (Maatjies Tributary) and T6 (Sout Tributary) have elevated levels of total dissolved solids and Site T7, on the Twenty-four Tributary, is subject to nutrient enrichment and is ephemeral. The last three sites feed into the lower part of the Berg River. Total Score varied considerably in three of the tributaries (Sites T1, T3 and T7) and ASPT values at Sites T1, T4 and T7 varied, but not to the same degree as Total Score (Figure 6.11). Additional data are needed however to establish if these differences reflect changes in water quality or if they reflect natural inter-annual differences within the river system.

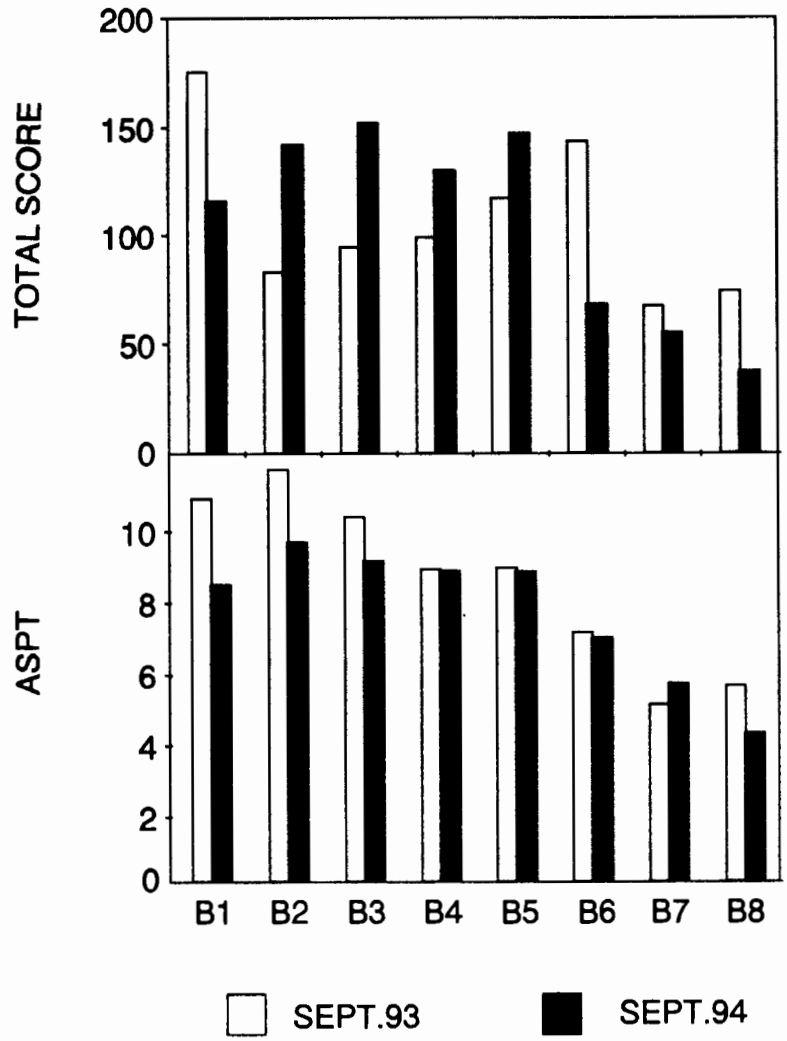


Figure 6.10. Total Score and ASPT values at eight sites on the Berg River sampled in September 1993 and September 1994.

Examination of these preliminary data on the effect of temporal variability on SASS scores suggests the following. Temporal variability from month to month, whilst resulting in minor variations in Total Score and ASPT, mostly at unimpacted sites, did not mask the effect of impaired water quality. Some seasonal effects were noted by applying SASS scores to historical data, suggesting that a detailed program designed to assess seasonal variation would be advantageous. Such seasonal differences should be taken into account when incorporating SASS assessments into a monitoring programme. If sampling frequency is conducted annually, sampling should be in the same season to ensure comparable results. Variation on

an annual basis was more apparent, but further assessments are needed to ascertain if the effect is the result of a change in water quality or part of the intrinsic variability within the system. Armitage *et al.* (1983) found that within the BMWP scoring system of Britain, Total Scores were higher in spring and autumn than in summer, and ASPT values higher in spring than in summer or autumn. The differences between maximum and minimum Total Scores or ASPT values between seasons at each site was relatively small when compared with differences between sites however.

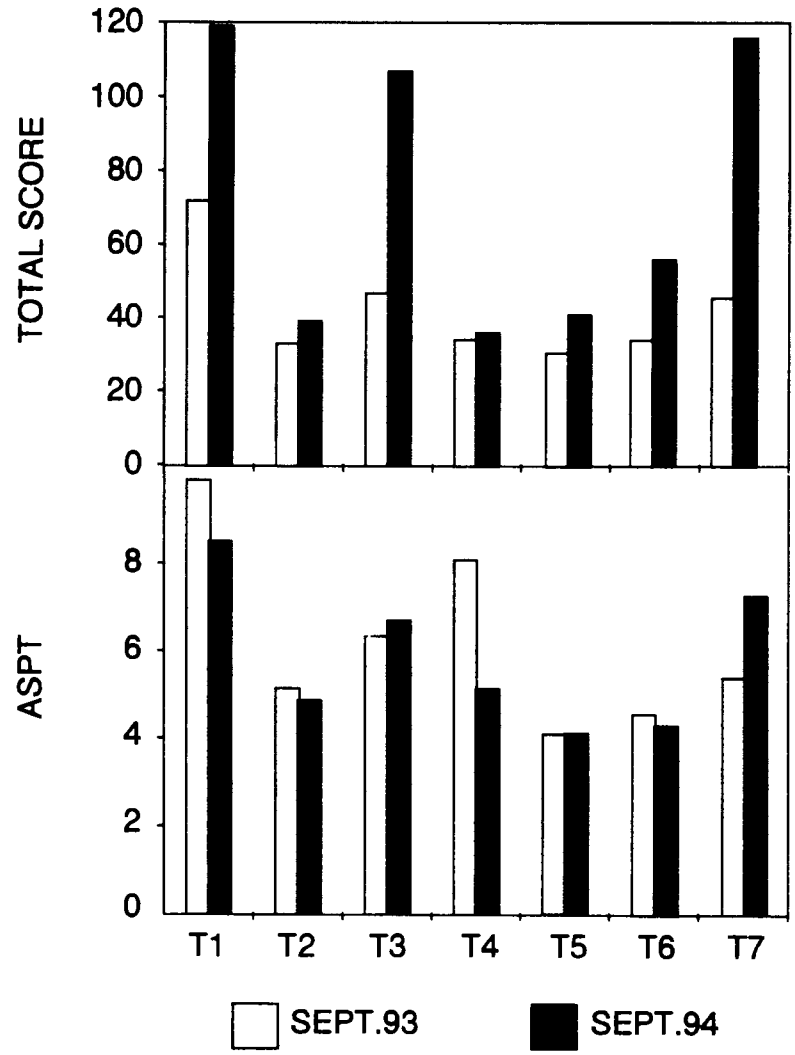


Figure 6.11. Total Score and ASPT values on eight tributaries of the Berg River ampled in September 1993 and September 1994.

6.3.3. Longitudinal changes

Natural changes in environmental factors (e.g. flow, water temperature, dissolved oxygen and food sources) down the longitudinal profile of river systems exert a direct control on the population dynamics of aquatic organisms, resulting in characteristic biological communities. Given these changes, an investigation into the variation in SASS scores down the length of the Berg River was undertaken. Total Scores and ASPT values were calculated for sixteen sites positioned longitudinally down the Berg River (Figure 6.12). At least one, and normally more than one site, was located within each of the five river zones as described in Chapter 4. No clear trend was evident from Total Scores. The highest Total Score was in the unimpacted mountain stream at the source of the river (Site B1), downstream of which Total Score dropped dramatically, followed by a gradual increase to peak again at Site B6, in the stony-foothill zone. Armitage *et al.* (1983) noted that BMWP scores were normally highest in middle reaches of rivers. Downstream of this Site B6, Total Scores fluctuated considerably and no trend was evident. The ASPT value increased from Site B1 to Site B2 downstream of which it steadily decreased at sites downstream until Site B7 when it reached a plateau.

Armitage *et al.* (1983) noted that ASPT values were highest in upper reaches of upland rivers and progressively decreased from upland to lowland reaches (5.6 to 6.24). It is clear that the lowest Total Scores and ASPT values are found at the lower sites of the river; these are also the most impacted ones, however. The problem of how to distinguish between low scores that result from longitudinal position, and those that result from an impact, becomes apparent. This is of particular importance if sites which vary in water quality are compared. Storey *et al.* (1990) classified the macroinvertebrate fauna of two river systems in south-western Australia in relation to physical and chemical parameters. There was a major separation of upland and lowland rivers and most of the spatial variation was mostly explained by physical characteristics as opposed to chemical ones. The differentiation of rivers into upland and lowland ones would enable more realistic comparisons and goals to be made in terms of SASS scores. The size, expressed as stream order, may also contribute to variation in scores prior to the additive effects of any form of perturbation. Crunkilton & Duchrow (1991) examined the interrelationship between stream order and number of taxa,

diversity and density and observed that the lowest number of invertebrates were in the lowest- and highest-order streams. Many of these problems could be alleviated by the introduction of a system that involved the use of reference sites against which SASS scores could be compared. This is discussed in the following section.

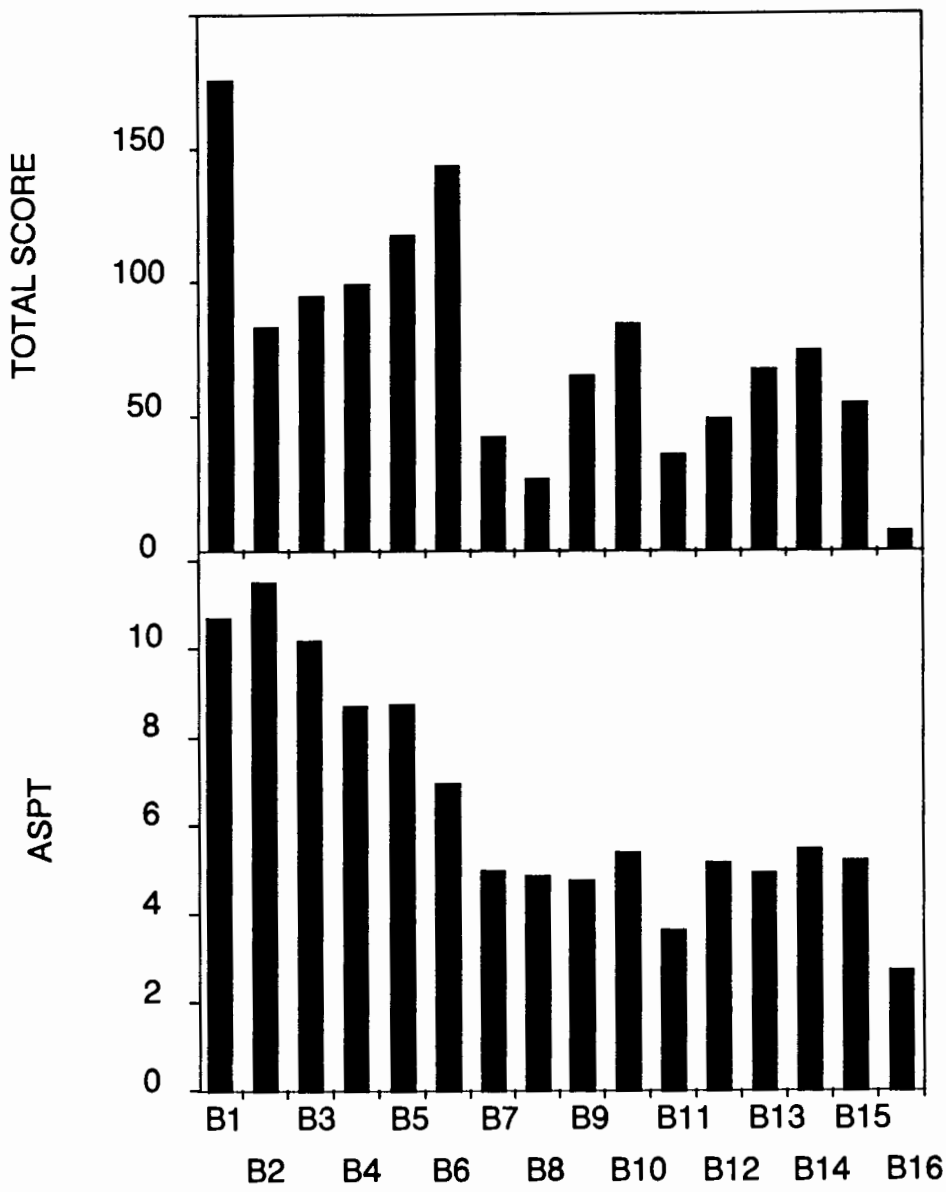


Figure 6.12. Total Scores and ASPT values calculated for sixteen sites positioned longitudinally on the Berg River.

6.4. REGIONAL DIFFERENCES AND REFERENCE SITES

The importance of establishing reference sites to facilitate the comparison of biotic scores from sites with impaired water quality with biotic scores from reference sites has been recognised (e.g. RIVPACS: Wright *et al.* 1989; BBI: De Pauw & Vanhooren 1983). Such reference sites are particularly important in countries spread over a wide range of geographic regions, such as South Africa. Riverine biotic communities are affected by a number of physical, chemical and biological factors. Because many of these factors vary geographically (e.g. water chemistry: Day *et al.* 1994, Dallas *et al.* 1995), the resultant biotic communities may also vary geographically. Preliminary assessments using SASS illustrate these differences in that certain benthic macroinvertebrate families are rare in the Eastern Transvaal and Natal but common in the south-western Cape. When south-western Cape data were used in isolation to categorize water quality, ranges for Total Scores and ASPT values were generally higher than those calculated from national SASS data. These intrinsic biogeographic differences will result in differences in biotic scores, which need to be taken into account when using a rapid bioassessment technique such as SASS to assess the impairment of water quality on a national basis.

There is a need to establish SASS scores for selected reference sites against which SASS scores from adjacent rivers can realistically be compared. Such reference sites need to be in the same geographic region, river zone (e.g. upland versus lowland rivers) and water type (e.g. acidic, brown-water, mountain streams of the south-western Cape or lowveld middle-reach rivers). The differences in SASS scores between these reference sites ("expected scores") and the SASS scores at "test" sites ("observed scores") can then be used to quantify the impairment of water quality. Reference sites should be selected within in a geographic framework so that regional differences in water quantity, water quality and biotas are taken into account. The USEPA (1989) divided the United Sates into a number of ecoregions, by examination and interpretation of spatial patterns in factors such as soil-type, vegetation, altitude and geology. Naturally occurring biotic assemblages, as components of the ecosystem, would be expected to differ among ecoregions but be relatively similar within a given ecoregion (USEPA 1989). Since biological asssessment methods such as SASS utilize this biotic component, reference sites within an ecoregion framework, could be used to

provide reasonable predictions of the biota that can be attained within an ecoregion.

One of the potential applications of SASS is as a "goalpost", i.e. a value that can be strived towards during rehabilitative measures undertaken by a particular authority. This "goalpost" needs to be realistic, and it can only be such if it is within the same ecoregion and river zone as the site undergoing rehabilitation. Reference sites that range from unimpacted or "least impacted" to "best attainable" need to be established. The latter is necessary because of the relative shortage of unimpacted sites/rivers within South Africa. Systematic monitoring to establish reference sites on a national basis will provide a far-sighted and significant contribution to the management of water quality in riverine ecosystems in South Africa.

6.5. OBJECTIVE SCORE ALLOCATION

A weakness of biotic indices is the subjective assessment which is often used to classify the tolerance/sensitivity of organisms (Herrick & Cairns 1982). An alternative method, based on extensive correlations between species presence and water quality, was used by Winget & Mangum (1979, cited by Herrick & Cairns 1982). The potential for developing an objective method of score allocation was investigated in this study by establishing the minimum-maximum ranges in which each macroinvertebrate taxon was found. Conductivity, pH, and concentrations (mg l^{-1}) of total suspended solids, organic fraction of the TSS, total dissolved solids, total alkalinity (meq l^{-1}), anions (chloride and sulphate), cations (sodium, magnesium, potassium and calcium) and nutrients (ammonium-nitrogen, nitrate-nitrogen, nitrite-nitrogen and Soluble Reactive Phosphorus) were measured simultaneously to SASS assessments. The measurements and concentrations of these variables are given in Appendix B. *In situ* measurements of temperature and dissolved oxygen were also taken, but were not used in subsequent analyses. Temperature was excluded because of the difficulties associated with spot-measurements. Dissolved oxygen was excluded because of the diurnal variability and because the relative ranges of each variable were based on maximum values of the variables whilst for dissolved oxygen, the minimum value is the critical one. Only macroinvertebrate taxa that were recorded at least five times were included. The minimum-maximum ranges were calculated for 50 benthic macroinvertebrate taxa for each water quality variable (n=16). To enable a composite measure of the tolerance of each taxon to

be established, the maximum value for each variable at which a taxon was recorded was expressed as a percentage of the maximum value for the variable. These percentages were summed and a composite maximum value (CMV) for each taxon was established (Figure 6.13). The use of maximum values as opposed to ranges was necessary because of cases where certain taxa had the same ranges, but one started at a higher concentration than the other. For example, Blephariceridae (minimum=0.01, maximum=4.599, relative range=12.7%) and Hirudinea (minimum=2.80, maximum=6.97, relative range 14.7) had very similar ranges, expressed as a percentage of the maximum range, for total suspended solids. On the assumption that it is more often the maximum value that is responsible for the critical limit of an organism, Hirudinea could be considered more tolerant and the maximum values (i.e. 4.599 and 6.97 for Blephariceridae and Hirudinea respectively) were therefore used to establish relative tolerances. The resultant composite maximum values, expressed as a percentage of the maximum value for the variable, are 12.7% and 24.6% respectively. These composite maximum values are therefore a measure of the relative tolerance of each taxon to sixteen water quality variables. The composite maximum values (as a percentage) and the current SASS scores are given (Figure 6.13) for each taxon in descending order of tolerance as calculated by this method.

Chironomidae and Corixidae were the most tolerant (>85%). Baetidae, Oligochaeta, Simuliidae, Coenoagriidae and Gyrinidae all had composite maximum values between 60 and 80%. The most sensitive taxa included Blephariceridae, Dixidae, Limnichidae, Ecnomidae, Athericidae, Ephemerellidae and Notonemouridae, which all had composite maximum values of less than 20%. If a comparison between these composite maximum values and the current SASS scores is made (Figure 6.13), it becomes apparent that in general there is a good degree of agreement between the two. Many low-scoring taxa, e.g. Chironomidae, Corixidae, Baetidae, Oligochaeta, have high composite maximum values and many high-scoring taxa, e.g. Blephariceridae, Helodidae, Athericidae, have low composite maximum values. Certain taxa are however clearly scored too high or too low. Dixidae, Limnichidae, Ecnomidae, Empididae, Hydroptilidae, Tipulidae, Hydrophilidae, Planaria, Libellulidae and Ceratopogonidae appear to be less tolerant than suggested by their SASS scores; whilst Ephemerellidae, Nymphulidae, Leptophlebiidae, Heptageniidae, Tricorythidae, Elmidae/Dryopidae, Aeschnidae, Ancylidae, Hydracarina, Caenidae and Gyrinidae appear

to be more tolerant than suggested by their SASS scores. The number of underscored taxa is approximately equal to the number of overscored taxa and this is probably why SASS, based on the current scoring system, is successful in differentiating between sites of different water quality. Three taxa which are given a strikingly lower SASS score than one would expect from the composite maximum values are Muscidae, Physidae and Culicidae. Whether this is the result of limited recordings or actual differences needs to be investigated further. Other non-water-quality factors such as flow velocity may have a greater effect on certain taxa than water quality factors. Greater refinement of the scores, on both a regional and national scale is important. As the use of SASS in the assessment of water quality increases, provision should be made for the continual refinement of the tolerance/sensitivity scores so that the scores are ecologically meaningful. By regularly calculating composite maximum values for each taxon, refinement of the scoring system will be facilitated.

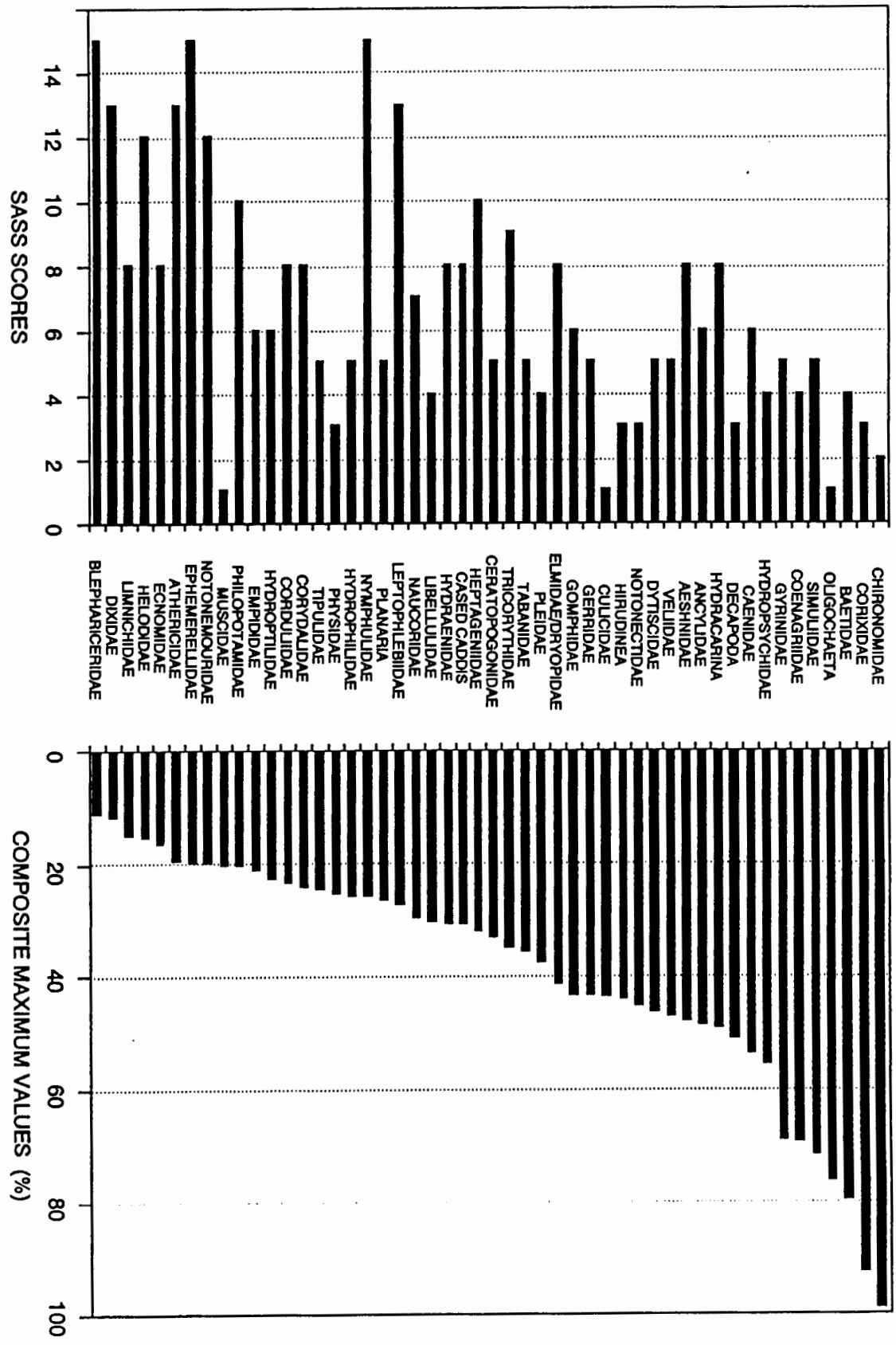


Figure 6.13. Current SASS tolerance/sensitivity scores and composite maximum values calculated for fifty benthic macroinvertebrate taxa. Taxa are arranged in descending order of tolerance.

6.6. CONCLUSIONS

Ranges of Total Score and ASPT values within each water quality category were higher in the south-western Cape than those ranges calculated on a national scale. This is probably related to the high number of endemic groups that commonly occur only in acidic, brown water streams of the south-western Cape.

Sites grouped on the basis of the degree of impact and water quality, and subsequently assessed using SASS, showed a clustering of sites within each water quality group. There was a strong positive correlation ($r=0.77$, $p<0.05$) between ASPT and Total Score. Severely impacted sites were concentrated at the lower end of the scale, moderately impacted site in the middle, and unimpacted sites at the upper end of the scale. The unimpacted sites were the most dispersed, suggesting greater variability in both ASPT and Total Score at such sites.

The influence of the variety of biotopes available for habitation by macroinvertebrates on SASS scores was investigated. Taxa present in the stones-in-current (SIC) biotope constituted the highest percentage of Total Score, followed by those taxa present in marginal vegetation, stones-out-of-current, aquatic/instream vegetation and sand in decreasing percentages respectively. Whilst Total Score and the number of taxa varied between biotopes, ASPT values remained relatively constant regardless of which biotopes were sampled.

Temporal differences in SASS scores were examined on a monthly, a seasonal (from historical data) and an annual basis. Minor variations in Total Score and ASPT values were mostly at unimpacted sites, although these variations did not mask the effects of impaired water quality. Further assessments are needed to establish if the seasonal and annual difference in SASS scores are the result of intrinsic changes within the aquatic ecosystem, or the result of impaired or improved water quality.

Total Score varied longitudinally down the river, but was generally highest in the upper mountain zones and stony-foothill zones. ASPT progressively decreased from the upper sites to the lower ones.

The importance of establishing reference sites, preferably within an ecoregion framework, such that regional and zonal differences can be accounted for, is emphasised. The rapid bioassessment method, SASS, is ideally suited to provide data for the selection of reference sites against which other sites can be compared. The deviation of the observed SASS score from the expected score at a realistically comparable reference site will provide a means of assessing the degree of impact. Rehabilitative measures can then be undertaken and audited using SASS within this reference system.

A comparison of current SASS tolerance/sensitivity score and composite maximum values calculated for 50 benthic macroinvertebrate taxa indicated that certain taxa are overscored and others are underscored. The importance of incorporating a feedback loop to facilitate continual refinement of SASS scores is recommended.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

The relatively scarce surface water resources in South Africa, are under increasing pressure through greater consumption of water resulting from population, industrial and economic growth. The quality of the water resource is affected by this growth through increased water pollution, and the need to establish an effective water quality management plan has been recognised by the Department of Water Affairs & Forestry. A resource-based approach, termed "Receiving Water Quality Objectives" (RWQO), which aims to allocate waste loads for non-hazardous substances on a catchment-by-catchment basis, has been implemented. To enable the overall effectiveness of this approach to be evaluated, case studies that establish baseline characteristics of the physical attributes, chemical constituents and biota are needed. Changes in these characteristics subsequent to implementation of the RWQO plan, can then be assessed by monitoring. Comprehensive monitoring programmes have long been established for chemical and physical characteristics, while biological monitoring is at its infancy stage.

Many water quality variables have a direct or indirect effect on the biotic component of riverine ecosystems by influencing the structure of the biotic communities and thus the functioning of the system. The biotic component therefore has great potential to be used in assessing water quality of riverine ecosystems because the biota integrate effects such as multiple effluent discharges and accumulation of toxins, and reveal deleterious effects of pulses of pollution, which are often missed by chemical and physical monitoring, unless such monitoring is on a very short time-scale.

Numerous methods of biological assessment that use different components of riverine biotas have been developed. Benthic macroinvertebrates have commonly been used in South Africa, traditionally via quantitative box- or Surber-sampling methods. It is impractical, however,

to incorporate such labour- and time-consuming methods into a monitoring programme. A rapid bioassessment method, the South African Scoring System (SASS), developed recently to assess water quality (Chutter 1994b) in riverine ecosystems, has the potential to become an integral part of a monitoring programme. This biotic index produces two scores: a Total Score and an Average Score per Taxon and interpretation of these scores enables inferences regarding water quality to be made. Given the imminent expansion of the use of SASS within South Africa, the need to evaluate SASS as a tool for rapid bioassessment of water quality was recognised and has been focused on in this study. Aspects of SASS investigated include sample variability and replication of both biological assessment methods, namely quantitative box-sampling and SASS; the ability of each method to differentiate between sites that differ in water quality; and potential problems associated with SASS.

This discussion is structured around the seven attributes of an "ideal" biotic index as proposed by Cook (1976) and Sheehan (1984). A biotic index should:

1. be sensitive to the effects of pollution,
2. have general application to different types of streams,
3. provide a continuous assessment from unpolluted to polluted sites,
4. be independent of sample size,
5. have relatively simple data gathering and index calculations,
6. have the ability to distinguish the cyclical and natural variability of the system, and
7. be ecologically meaningful.

The degree to which SASS fulfils each of these attributes is discussed. Points one and three and closely linked and are therefore discussed jointly.

Is SASS sensitive to the effects of pollution and does it provide a continuous assessment from unpolluted to polluted sites?

Based on a comparison of the Family-level benthic macroinvertebrate faunal groups generated by cluster and ordination analysis for quantitative box-sampling and SASS sampling, it is clear that both methods differentiate between sites of different water quality. The three sites

focused on for detailed analysis differed in water quality as follows: Site 1 was an unimpacted mountain stream, acidic, mineral-poor and poorly buffered; Site 2 had elevated levels of dissolved organics and nutrients; and Site 3 had elevated conductivity and concentrations of total dissolved solids. At least two distinguishing taxa were found to be common between the two methods. Distinguishing taxa at Site 1 were Notonemouridae and Leptophlebiidae, at Site 2 they were Leptoceridae, Libellulidae and Caenidae, and at Site 3 they were Tricorythidae and Chironomidae. A factor that may be of significance in undertakings such as environmental impact assessments, in which rarer taxa may be of importance, is the inability of SASS sampling to detect rare taxa that are often cryptic or closely adherent. Whilst such differences resulted in differences in Total Scores calculated from each method, ASPT values were very similar, suggesting that the scores of these additional rarer taxa were not necessarily high (sensitive) or low (tolerant) but that they ranged between the two extremes. The importance of using both Total Score and ASPT in interpretation is clearly demonstrated.

The differentiation of forty sites into three categories of water quality on the basis of proximity and exposure to pollution sources or impacts, facilitated the calculation of ranges for Total Scores and ASPT values for each category. Sites within each category differed significantly from sites in other categories. Total Score was significantly positively correlated with ASPT and severely impacted sites, with poor water quality grouped at the bottom end of the scale, whilst unimpacted sites with good water quality grouped at the upper end of the scale. Moderately impacted sites with intermediate water quality ranged from the upper point of the severely impacted sites to the lower end of the unimpacted sites.

The sensitivity of the SASS technique was further demonstrated at sites exposed to effluent from a fish farm. Five sites 0, 50, 250, 500 meters and 1 km below an effluent outlet, exhibited a steady increase in both Total Score and ASPT, indicating that even on a spatially small scale, SASS is sufficiently sensitive to pick up these changes.

These ranges in Total Scores and ASPT values suggest that SASS is sensitive to the effects of pollution and can provide a continuous assessment from unpolluted to polluted sites.

Does SASS have general application to different types of streams?

This attribute can be interpreted in two ways. The first relates to the applicability of the technique and associated scoring system, and the second to the interpretation and conclusions drawn from SASS assessments undertaken in different types of streams.

The first interpretation would in general hold for SASS, in that the technique is widely applicable in flowing riverine ecosystems. The ephemeral nature of many rivers in South Africa, and the seasonal flooding of others, would limit the use of SASS during such periods. The scoring system is generally applicable nationally, with the exception of one macroinvertebrate family that has been allocated two scores on the basis of its dependence on pH. Certain families commonly occur in a particular geographic region and may never be recorded elsewhere. This however does not affect the scoring system, merely the resultant scores and interpretation.

Based on data examined in this study it is unlikely that SASS would fulfil the second interpretation of this attribute: "biotic scores should have general applicability to different types of streams". Given the number of geological, climatic and geographic regions within South Africa, it seems likely that intrinsic characteristics of water quality and the biota would differ regionally. Such regional differences are clearly illustrated by the number of endemic species present in the acid, brown-water streams of the south-western Cape. In addition to these regional difference, rivers differ longitudinally and distinct differences occur between upper rivers (e.g. mountain streams and stony-foothills, and lowland rivers). Natural changes in environmental factors (e.g. flow velocity, water temperature, dissolved oxygen and food sources) down the longitudinal profile of river systems exert a direct control on the population dynamics of aquatic organisms, resulting in characteristic biological communities. The driving physical factor is flow rate, which is high in the upper reaches and declines steadily as stream order increases. This results in three distinct zones: a fast-flowing erosive headwater zone; a slower-flowing, partly-erosive middle zone; and a slow-flowing, low-lying, lower reach or mature river where materials eroded in the upper reaches are deposited.

Clearly such inherent regional and longitudinal differences should be taken into account when

biological assessments using SASS are undertaken. The establishment of reference sites within clearly defined ecoregions would ensure that realistic comparisons and interpretations are made of water quality. By ensuring a selection of sufficient representative reference sites on a national scale, monitoring programmes using SASS would provide a means of auditing the national water quality situation in a pre-determined time frame, e.g. annually. The initial effort needed to select and assess appropriate reference sites would be great, but the long term advantages in implementing the use of reference sites in a national monitoring network are significant.

Is SASS independent of sample size?

Independence of sample size could imply independence in the number of samples, i.e. sample replication, or in the actual size of the sample, i.e. area. Neither quantitative box-sampling nor SASS sampling were independent of sample size. A minimum of twelve and four quantitative samples is needed to ensure collection of 95% and 75% of the macroinvertebrate taxa respectively. Sampling within a single biotope component, such as a "riffle" or "run" reduced the number of replicates needed to a minimum of nine and three for riffles, and six and two for runs (95% and 75% of the taxa respectively). The number of SASS samples needed to ensure collection of 95% and 75% of the taxa within a single biotope, stones-in-current, are four and two respectively. The SASS technique is however designed such that a single sample which incorporates all available biotopes is taken per site. The maximum Total Scores that one sample would produce as a percentage of twenty samples, is 28%, 59% and 45% at Sites 1, 2 and 3 respectively. Clearly Total Score increases as sampling effort increases. The ASPT values however changed very little with sampling effort and the importance of using the ASPT in the interpretation of a SASS assessment is again clearly demonstrated. The size of the sample, i.e. area, was not investigated in this study. SASS is a qualitative method of benthic macroinvertebrate sampling and therefore is dependent on the number of biotopes available for sampling as opposed to area.

The SASS technique requires that a net of 950 μm-mesh diameter is used, primarily because field identification of organisms <950 μm to family level is extremely difficult. Laboratory separation of quantitative samples into different size class, indicated that the >950 μm size

fraction generally includes between 86% and 94% of the taxa present. This has important implications in terms of how representative a SASS assessment is of the actual benthic macroinvertebrate assemblage.

In relation to sample size, an important feature that was continually noted throughout this study, was the high variability of samples from unimpacted sites. Both quantitative box-sampling and SASS sampling at the unimpacted site required a high number of replicates before the sampled community could be considered representative of the actual community and before Total Scores did not increase with increasing number of samples. In quantitative box-sampling, selection of sampling points to take into account habitat heterogeneity would reduce error resulting from sample variability. To reduce error resulting from sampling variability in the single-sample, multiple-biotope SASS sampling method, the sampling period should be increased. Careful note should be taken of all the possible biotopes present at a site and sampling should be conducted for a longer time period, particularly in the stones-in-current and stones-out-of current biotopes. This is especially important if SASS data are collected for reference sites against which sites with impaired water quality are compared.

Does SASS have simple data gathering and index calculations?

SASS is a field-based sampling method that uses minimal equipment, is rapid and is usable by technicians trained to identify macroinvertebrate taxa to family level. The fact that it is field-based ensures that the bulk of the fauna within a sample is returned to the river from which it was collected, i.e. faunal sampling is non-destructive. Voucher samples may be taken if identifications need to be confirmed or at sites sampled for the first time. The standard SASS scoring sheet ensures uniformity in data gathering and entering and Total Score and ASPT calculations are simple. The establishment of a SASS database that is linked to a national monitoring programme would ensure that SASS records are accessible and usable.

The final two attributes are ones added by Sheehan (1984).

Is SASS able to distinguish between the cyclical and natural variability of a system?

Running waters in regions of seasonal climates exhibit daily and seasonal periodicity. Many organisms are adapted so that seasonal changes, for example in temperature, act as cues for the timing of migration, spawning and emergence. Physical attributes such as turbidity show natural seasonal variability, the extent of which is governed by the basic hydrology and geomorphology of the particular region. pH, which is affected by the rates of photosynthesis, respiration and decomposition, fluctuates widely over a 24-hour period. Cyclical and natural variations such as these are often reflected in changes in biotic community assemblages. SASS assessments should therefore take account of potential differences that result, in particular, from seasonal variability. Preliminary examination of SASS data collected monthly suggests that whilst Total Score and ASPT values varied monthly, the variation was not of a magnitude to mask the differences resulting from the effects of water quality impairment. From this study no conclusions can be drawn regarding the effect of seasonal and annual variation. Given the intrinsic seasonal and inter-annual changes in many water quality variables however, it seems likely that SASS scores would vary. Resh & Jackson (1993) assessed the effect of season on the accuracy of rapid bioassessment measures and found that there were seasonal differences in almost all of the measurements. Initial inclusion of SASS in a national monitoring system should take cyclical and natural variation into account, particularly if comparisons between sites with naturally different water quality are undertaken. Given sufficient SASS assessments, the differentiation of cyclical and natural variability of a system from variation or changes resulting from the impairment of water quality should be possible.

Is SASS ecologically meaningful?

Biotic indices are calculated from scores allocated to organisms on the basis of their sensitivity or tolerance to pollution. Generally each organism is assigned a score subjectively

as decided by expert opinion. The degree to which these allocated scores correspond with scores determined in a less subjective manner, would be one way of indicating how ecologically meaningful such an index is. Winget & Mangum (1979, cited by Herricks & Cairns 1982) proposed a biotic condition index based on extensive correlations between species presence and water quality. Difficulties arise however in relating an integrated biological score to an instantaneous physical or chemical measurement. In this study the current SASS scores of each macroinvertebrate taxon were compared with the composite maximum values of each macroinvertebrate taxon. Certain taxa were clearly scored too high or too low. The establishment of a feedback loop that facilitates modification and refinement of SASS scores is important. This will ensure that the scores on which SASS is based are scientifically sound and through this, that the index is ecologically meaningful.

Potential applications of SASS as a tool for rapid bioassessment of water quality

SASS has enormous potential as a tool for rapid bioassessment of water quality and as a tool for assessing the ecological integrity of riverine ecosystems. It is suited to determining the ecological state of rivers in South Africa. Such an environmental audit would enable the ecological status of each river to be established, and rivers or reaches requiring urgent attention and those of particular ecological importance which warrant greater protection could be identified. SASS can be used to establish reference sites to facilitate the quantification of impairment of water quality. The percentage degradation of a "test" site relative to the reference site would result in a numerical value easily interpretable by water quality managers or river authorities. Such reference sites could also act as "goalposts" against which rehabilitative measures undertaken by a river authority or polluter can be evaluated and audited. Regular monitoring using SASS would facilitate assessment of the effectiveness of the Receiving Water Quality Objectives management plan and validation of water quality guidelines developed from laboratory tolerance/toxicity tests. Incorporation of SASS in baseline studies which form part of the Instream Flow Assessment (IFA) methodology provides a good indication of current ecological status of rivers and sites on rivers under investigation. The use of SASS in the control of pollution and checking on compliance of polluters has legal implications in that conclusions drawn may form part of a legal action.

The importance of founding SASS on a sound scientific base is clearly therefore of primary importance.

As a result of this study certain research needs have become apparent. Whilst temporal and longitudinal variability were addressed in this study, the data used were of a preliminary nature. A more detailed investigation into the influence of seasonal, annual and longitudinal variation on SASS scores is therefore advocated. Given the variability of Total Score at unimpacted mountain stream sites, and the probable use of such sites as reference sites against which water quality impairment is measured, it is of particular importance that this variability be determined. Such an investigation would need to be conducted within-regions because of inherent geographical differences in water quality. The discussion and promotion of the use of reference sites in an ecoregion framework as part of a water quality monitoring programme has been central to much of this thesis. Investigations into the establishment and implementation of such a system should be conducted. A preliminary examination of an objective method of SASS tolerance/sensitivity assignment in this thesis indicated that certain scores need to be modified. The establishment of a formal feedback loop that would enable continual refinement of these scores is proposed.

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APPENDIX A. Site code, river, site description, biotopes sampled (1=stones-in-current, 2=stones-out-of-current, 3=marginal vegetation, 4=aquatic/instream vegetation, 5=sand) and co-ordinates (LONG=longitude and LAT=latitude, degrees and minutes), of 49 sites assessed using SASS. The physical attributes and chemical characteristics were measured simultaneously at most sites, except for those indicated with an asterix.

SITE CODE	RIVER	SITE DESCRIPTION	BIOTOPE	LONG	LAT
B1	BERG	MOUNTAIN STREAM IN FRANSCHHOEK FORESTRY RESERVE	1,2	33 59	19 04
B2	BERG	BELOW FORESTRY OPERATIONS	1,2	33 59	19 04
B3	BERG	ABOVE THEEWATERSKLOOF WATER TRANSFER TUNNEL	1,2,3,5	33 57	19 04
B4	BERG	BELOW THEEWATERSKLOOF WATER TRANSFER TUNNEL	1,2	33 54	19 03
B5	BERG	BELOW DEWDALE TROUT FARM	1,2,3	33 54	19 03
B6	BERG	UPSTREAM OF THE JIM FOUCHE BRIDGE, BELOW CONFLUENCE OF FRANSCHHOEK TRIBUTARY	1,2,3,5	33 52	19 02
B7	BERG	CECILIAS DRIFT ABOVE PAARL	1,2,3,5	33 46	18 58
B8	BERG	DALJASOPHAT IN PAARL, BELOW SEWAGE TREATMENT WORKS	1,3,5	33 42	18 53
B9	BERG	UPSTREAM OF THE LADY LOCH BRIDGE BELOW WELLINGTON	2,3,5	33 38	18 58
B10	BERG	HERMON ROAD BRIDGE	1,2,3,5	33 26	18 57
B11	BERG	ROAD BRIDGE AT GOEDVERWAG BELOW GOUDA	3,5	33 15	18 57
B12	BERG	BELOW DRIE HEUWELS WEIR	1,3,5	33 08	18 52
B13	BERG	BRIDGETOWN FARM	1	33 05	18 51
B14	BERG	BELOW ROADBRIDGE ON N7 NEAR PIKETBERG	1,3,5	32 58	18 45
B15	BERG	SANDRIFT FARM	3,5	32 54	18 35
B16	BERG	KERSEFONTEIN FARM	3,5	32 54	18 20
T1	ASSEGAAIBOSCH	FRANSCHHOEK FORESTRY RESERVE	1,2,3,5	33 58	19 05
T2	FRANSCHHOEK	AT BRIDGE TO WINEFARM "LA PROVENCE"	1,5	33 54	19 06
T3	WEMMERS	AT ROADBRIDGE, BELOW SPILLAGE DAM	1,2,3	33 51	19 02
T4	DWARS	AT ROADBRIDGE NEAR RHODES FRUIT FARMS	1,2,3,5	33 52	18 59
T5	MAATJIES	AT MAATJIES RIVER GAUGING WEIR	1,2,3,5	33 03	18 50
T6	SOUT	AT BRIDGE TO FARM "HAZEKRAAL" NEAR HOPEFIELD	2,3,5	33 01	18 22
T7	TWENTY-FOUR	BELOW ROADBRIDGE AT HALFMANSHOF	1,2,3	33 10	18 56
T8	KROM	TRIBUTARY OF MAATJIES RIVER, ON ROUTE TO FARM "MATJIESRIVIER"	1,3,5	33 01	18 50

APPENDIX A. (CONT.)

SITE CODE	RIVER	SITE DESCRIPTION	BIOTOPE	LONG	LAT
K1	KRAALSTROOM	MOUNTAIN STREAM ABOVE TROUT FARM	1,2	19 08	33 46
K2	KRAALSTROOM	IMMEDIATELY BELOW TROUT FARM EFFLUENT OUTLET	1,2	19 08	33 46
K3	KRAALSTROOM	50 METRES BELOW TROUT FARM EFFLUENT OUTLET	1,2	19 08	33 46
K4	KRAALSTROOM	250 METRES BELOW TROUT FARM EFFLUENT OUTLET	1,2	19 08	33 46
K5	KRAALSTROOM	500 METRES BELOW TROUT FARM EFFLUENT OUTLET	1,2	19 08	33 46
K6	KRAALSTROOM	1000 METRES BELOW TROUT FARM EFFLUENT OUTLET	1,2,3	19 08	33 46
EL*	ELANDSPAD	100 METRES ABOVE CONFLUENCE WITH KRAALSTROOM RIVER	1,2,3,4	19 07	33 46
PD*	PERDEKLOOF	MONT ROCHELLE NATURE RESERVE, FRANSCHHOEK	1,2	19 09	33 53
RV*	RIVIERSONDEREND	IN GRABOUW STATE FOREST, 10 KM UPSTREAM OF THEEWATERSKLOOF DAM	1,2,3,4	19 04	34 03
M1	MOLENAARS	DU TOITS PASS. ABOVE BRIDGE CONSTRUCTION	1,2,3,5	19 08	33 43
M2	MOLENAARS	DU TOITS PASS. BELOW BRIDGE CONSTRUCTION	1,2,3,5	19 11	33 43
E1	JONKERSHOEK	MOUNTAIN STREAM IN JONKERSHOEK STATE FOREST	1,2,3,4	18 56	33 58
E2	EERSTE	ABOVE STELLENBOSCH SEWAGE WORKS	1,2,3,5	18 50	33 56
E3	EERSTE	BELOW STELLENBOSCH SEWAGE WORKS	1,2,3,5	18 48	33 58
L	LANG	MOUNTAIN STREAM IN JONKERSHOEK STATE FOREST	1,2	18 58	33 59
PL	PLANKENBERG	BELOW STELLENBOSCH INDUSTRIAL AREA	1,2,3	18 49	33 57
O*	OLIFANTS	AT ALGERIA CAUSEWAY	1,2,3,5	18 57	32 22
R*	RONDEGAT	ABOVE ALGERIA CAMPSITE, CEDERBERG	1,2,4,5	19 03	32 23
D*	DRIEHOEK	SANDDRIFT, CEDERBERG	1,2,3,5	19 15	32 29
B*	BRANDKRAALS	NEAR FARM "VOELFONTEIN, CEDERBERG	1,2,3,4,5	19 22	32 34
G*	GROOT	NEAR FARM "GROOTRIVIER", CEDERBERG	1,2,3	19 24	32 39
P1	PALMIET	MOUNTAIN STREAM IN NUWEBERG STATE FOREST	1,2,3,4	19 03	34 02
P2	PALMIET	AT ROADBRIDGE BELOW NUWEBERG DAM	1,2,3,5	19 03	34 05
P3	PALMIET	BELOW KOGELBERG DAM	1,2	18 59	34 13
P4	PALMIET	KOGELBERG NATURE RESERVE	1,2,3,4	18 58	34 19

APPENDIX B. Physical attributes and concentrations of chemical constituents measured at 41 sites in the south-western Cape. * indicate missing values.

SITE CODE	DATE	TEMPERATURE	PH	CONDUCTIVITY	DISSOLVED OXYGEN
		oC		mS/m	mg/l
B1	02.93	17.8	4.96	2.98	9.3
B1	09.93	13.0	4.50	2.05	8.6
B2	09.93	15.0	4.01	2.09	
B3	09.93	14.5	4.38	2.46	7.7
B4	09.93	17.0	6.46	4.06	7.8
B5	09.93	17.0	5.85	3.74	7.7
B6	02.93	21.3	6.26	6.78	8.4
B6	09.93	16.5	6.73	5.89	10.1
B6	12.93	27.0	6.40	7.19	8.5
B6	02.94	18.5	6.22	10.55	9.2
B6	03.94	20.0	6.25	9.66	8.2
B7	09.93	16.5	8.09	11.06	10.2
B8	09.93	16.5	6.63	14.53	10.0
B8	12.93	30.0	6.77	12.20	8.0
B8	02.94	23.0	6.33	14.06	9.3
B8	03.94	23.0	6.70	12.87	6.7
B9	09.93	17.0	6.60	16.76	6.0
B10	09.93	17.0	6.87	28.30	9.6
B11	09.93	18.0	7.20	32.90	8.8
B12	02.93	24.2	6.87	22.00	6.4
B12	09.93	18.0	7.18	37.80	7.6
B13	09.93	18.0	7.33	41.00	5.9
B14	09.93	18.5	8.06	97.60	8.5
B14	12.93	27.5	7.50	65.30	7.9
B14	02.93	26.0	7.52	71.90	7.0
B14	03.93	25.5	7.28	51.60	6.1
B15	09.93	19.0	7.69	57.30	9.4

APPENDIX B CONT.

SITE CODE	DATE	TEMPERATURE	PH	CONDUCTIVITY	DISSOLVED OXYGEN
		oC		mS/m	mg/l
B16	09.93	20.0	7.70	130.90	8.9
T1	09.93	13.0	5.62	3.00	9.5
T2	09.93	18.0	7.18	3.87	8.6
T3	09.93	15.0	5.14	5.76	9.3
T4	09.93	15.5	6.49	8.10	19.5
T5	09.93	21.5	7.91	164.00	7.8
T6	09.93	23.0	8.27	1257.00	6.0
T7	09.93	23.0	5.95	4.53	7.3
T7	12.93	31.0	8.17	12.47	6.2
T7	02.93	29.0	8.94	16.93	6.5
T8	09.93	21.5	7.88	623.00	10.3
K1	12.93	19.8	6.40	2.79	7.3
K1	02.94	20.0	6.65	3.42	9.0
K1	03.94	17.0	6.02	4.41	9.4
K2	12.93	20.0	5.38	4.62	7.5
K2	02.94	18.5	5.95	6.42	8.4
K2	03.94	19.0	5.85	6.49	7.4
K3	12.93	20.0	5.65	4.33	7.6
K3	02.94	18.0	5.58	5.60	8.2
K3	03.94	19.5	5.95	6.46	7.7
K4	12.93	20.0	5.88	4.01	7.6
K4	02.94	18.0	5.99	5.60	8.9
K4	03.94	19.5	6.09	6.26	8.9
K5	12.93	19.3	6.09	4.18	8.0
K5	02.94	17.5	6.09	5.63	7.6
K5	03.94	19.5	6.39	6.05	8.9
K6	12.93	19.0	6.10	3.33	7.8
K6	02.94	16.5	5.95	5.90	8.0

APPENDIX B CONT.

SITE	DATE	TEMPERATURE	PH	CONDUCTIVITY	DISSOLVED OXYGEN
		oC		mS/m	mg/l
K6	03.94	20.0	6.11	5.42	8.5
M1	12.93	24.0	5.35	3.01	7.1
M2	02.94	26.5	6.05	4.83	9.0
M2	03.94	19.0	5.55	4.81	9.6
E1	12.93	20.5	5.58	4.02	8.5
E1	02.94	19.5	5.53	6.17	9.4
E1	03.94	19.5	5.60	5.60	8.8
E2	12.93	26.0	8.00	31.60	9.9
E2	02.94	22.5	6.68	17.99	6.8
E2	03.94	21.0	6.61	14.60	7.4
E3	12.93	28.5	7.91	66.30	7.6
E3	02.94	25.0	7.04	50.90	6.5
E3	03.94	21.0	7.05	46.20	6.7
L	03.94	16.5	5.52	4.90	9.4
PL	12.93	25.0	6.74	83.10	1.2
PL	02.94	22.0	6.38	67.90	0.9
PL	03.94	18.0	6.83	47.00	1.8
P1	12.93	21.5	4.22	3.87	8.9
P1	02.94	22.5	4.64	6.51	8.3
P1	03.94	17.5	4.26	6.70	8.8
P2	12.93	19.3	4.51	4.02	8.9
P2	02.94	18.5	4.46	6.30	8.8
P2	03.94	19.8	4.76	5.50	8.7
P3	12.93	29.5	7.07	14.56	6.4
P3	02.94	21.5	6.34	16.38	7.3
P3	03.94	24.5	6.44	15.87	8.1
P4	12.93	26.0	6.64	18.56	8.4
P4	02.94	22.0	5.82	15.12	7.8
P4	03.94	24.5	6.45	14.36	7.9

APPENDIX B CONT.

SITE CODE	DATE	TDS	TSS	ORGANIC FRACTION OF TSS
		mg/l	mg/l	mg/l
B1	02.93	10.00	0.001	*
B1	09.93	12.50	0.083	*
B2	09.93	5.88	0.600	0.400
B3	09.93	8.88	0.750	*
B4	09.93	19.50	1.100	0.700
B5	09.93	19.50	7.286	2.143
B6	02.93	37.05	4.300	2.550
B6	09.93	34.35	5.800	2.600
B6	12.93	39.88	2.600	1.500
B6	02.94	35.50	1.750	1.550
B6	03.94	56.62	2.414	1.410
B7	09.93	67.25	7.600	2.000
B8	09.93	79.13	8.800	2.800
B8	12.93	52.63	6.933	2.667
B8	02.94	87.63	5.500	3.400
B8	03.94	61.25	6.062	2.590
B9	09.93	99.25	7.000	2.400
B10	09.93	155.25	9.571	2.000
B11	09.93	181.75	18.800	2.800
B12	02.93	101.11	18.400	3.700
B12	09.93	213.25	9.571	2.000
B13	09.93	220.88	13.000	3.200
B14	09.93	481.88	7.800	3.800
B14	12.93	288.63	8.800	3.867
B14	02.93	286.63	13.760	3.500
B14	03.93	235.90	15.773	4.209
B15	09.93	312.00	8.600	3.000
B16	09.93	723.75	26.800	5.200
T1	09.93	12.47	1.400	0.967

APPENDIX B CONT.

SITE CODE	DATE	TDS	TSS	ORGANIC FRACTION OF TSS
		mg/l	mg/l	mg/l
T2	09.93	69.63	9.400	2.600
T3	09.93	17.50	4.000	1.900
T4	09.93	43.38	6.400	2.600
T5	09.93	1027.50	6.000	2.200
T6	09.93	8553.50	19.000	5.429
T7	09.93	28.88	1.000	*
T7	12.93	60.50	*	*
T7	02.93	179.13	1.633	1.383
T8	09.93	4574.25	7.300	2.600
K1	12.93	8.38	0.600	*
K1	02.94	54.50	0.546	0.546
K1	03.94	20.88	0.400	0.400
K2	12.93	30.13	6.470	4.533
K2	02.94	45.60	4.957	3.482
K2	03.94	58.10	5.900	4.775
K3	12.93	29.00	6.450	4.550
K3	02.94	46.25	4.882	3.761
K3	03.94	23.88	5.900	4.100
K4	12.93	31.63	4.850	3.617
K4	02.94	40.00	3.333	2.730
K4	03.94	16.93	4.100	3.634
K5	12.93	30.13	3.000	2.250
K5	02.94	20.38	2.067	1.933
K5	03.94	53.20	5.609	3.355
K6	12.93	26.75	3.600	2.500
K6	02.94	97.38	1.900	1.833
K6	03.94	44.75	2.495	2.091
M1	12.93	23.25	0.600	*

APPENDIX B CONT.

SITE CODE	DATE	TDS	TSS	ORGANIC FRACTION OF TSS
		mg/l	mg/l	mg/l
M1	02.94	35.25	0.667	0.546
M1	03.94	9.75	0.467	0.467
M2	12.93	25.63	1.300	1.300
M2	02.94	14.75	0.909	0.788
M2	03.94	18.60	0.733	0.550
E1	12.93	27.88	0.300	*
E1	02.94	24.63	0.706	0.529
E1	03.94	23.88	0.914	0.800
E2	12.93	128.38	3.000	1.700
E2	02.94	68.38	2.800	1.900
E2	03.94	53.38	4.020	1.707
E3	12.93	297.88	6.500	3.000
E3	02.94	188.12	5.900	3.100
E3	03.94	201.88	6.972	1.787
L	03.94	22.88	0.627	0.572
PL	12.93	407.50	12.800	9.200
PL	02.94	251.12	7.000	6.800
PL	03.94	177.25	5.015	4.328
P1	12.93	24.75	*	*
P1	02.94	31.62	0.629	0.514
P1	03.94	36.25	0.400	0.400
P2	12.93	25.25	1.067	1.067
P2	02.94	28.75	1.417	1.417
P2	03.94	64.40	3.883	2.650
P3	12.93	71.88	1.500	1.300
P3	02.94	87.40	2.600	1.900
P3	03.94	74.63	1.050	0.850
P4	12.93	83.75	0.383	*
P4	02.94	57.75	1.771	1.429
P4	03.94	20.30	1.733	1.400

APPENDIX B CONT.

SITE CODE	DATE	NITRATE	NITRITE	AMMONIUM	SOLUBLE REACTIVE PHOSPHORUS
		mg/l N	mg/l N	mg/l N	mg/l
B1	02.93	0.0034	0.0008	0.0109	0.046
B1	09.93	0.0108	0.0007	0.0148	0.013
B2	09.93	0.0089	0.0014	0.1546	0.000
B3	09.93	0.0130	0.0013	0.0374	0.000
B4	09.93	0.0708	0.0015	0.1436	0.001
B5	09.93	0.1880	0.0044	0.0362	0.000
B6	02.93	0.1168	0.0023	0.0403	0.000
B6	09.93	0.5640	0.0036	0.0409	0.000
B6	12.93	0.0070	0.0020	0.0268	0.002
B6	02.94	0.0189	0.0020	0.0272	0.000
B6	03.94	0.0032	0.0028	<0.1	0.012
B7	09.93	0.9594	0.6456	0.1499	0.018
B8	09.93	1.4008	0.0087	0.0151	0.031
B8	12.93	0.0116	0.0056	0.0256	0.000
B8	02.94	0.1740	0.0036	<0.1	0.000
B8	03.94	0.0073	0.0032	<0.1	0.000
B9	09.93	1.8102	0.0016	0.0472	0.030
B10	09.93	0.3748	0.0085	0.0327	0.113
B11	09.93	0.8438	0.0045	0.0409	0.043
B12	02.93	0.0214	0.0019	0.0263	0.056
B12	09.93	1.2083	0.0045	0.1306	0.022
B13	09.93	0.3765	0.0038	0.0358	0.023
B14	09.93	0.9118	0.0067	0.1480	0.023
B14	12.93	0.1037	0.0008	0.0287	0.000
B14	02.93	0.0007	0.0008	0.0279	0.000
B14	03.93	0.0014	0.0476	<0.1	0.000
B15	09.93	0.9547	0.0053	0.0413	0.022
B16	09.93	0.1953	0.5221	0.0287	0.007
T1	09.93	0.0399	0.0009	0.0122	0.000

APPENDIX B CONT.

SITE CODE	DATE	NITRATE	NITRITE	AMMONIUM	SOLUBLE REACTIVE PHOSPHORUS
		mg/l N	mg/l N	mg/l N	mg/l
T2	09.93	1.6870	0.0084	0.0393	0.006
T3	09.93	0.0698	0.0012	0.0413	0.037
T4	09.93	0.8102	0.0049	0.0409	0.010
T5	09.93	0.3832	0.0068	0.0358	0.043
T6	09.93	0.0145	0.0018	0.0370	0.160
T7	09.93	0.0162	0.0013	0.0409	0.016
T7	12.93	0.1715	0.0015	0.0287	0.000
T7	02.93	0.0006	0.0024	<0.1	0.000
T8	09.93	0.0129	0.0018	0.1460	0.000
K1	12.93	0.0025	0.0013	0.0224	0.000
K1	02.94	0.1029	0.0008	0.0287	0.000
K1	03.94	0.4116	0.0049	<0.1	0.000
K2	12.93	0.0851	0.0111	0.0429	0.063
K2	02.94	0.0437	0.0115	0.3888	0.194
K2	03.94	0.1392	0.0190	0.6691	0.284
K3	12.93	0.0014	0.0108	0.0496	0.052
K3	02.94	0.0542	0.0115	0.0445	0.000
K3	03.94	0.3402	0.0371	0.4797	0.274
K4	12.93	0.1498	0.0167	0.0512	0.034
K4	02.94	0.1250	0.0182	0.5732	0.201
K4	03.94	0.0014	0.0305	0.9702	0.248
K5	12.93	0.0014	0.0294	0.0421	0.061
K5	02.94	0.0290	0.0277	0.5038	0.000
K5	03.94	0.2047	0.0519	0.6796	0.282
K6	12.93	0.0469	0.0381	*	0.022
K6	02.94	0.1551	0.0381	0.1309	0.000
K6	03.94	0.0014	0.0587	0.3510	0.204
M1	12.93	1.5950	0.0015	0.0283	4.979
M1	02.94	0.0088	0.0015	0.0244	*

APPENDIX B CONT.

SITE CODE	DATE	NITRATE	NITRITE	AMMONIUM	SOLUBLE REACTIVE PHOSPHORUS
		mg/l N	mg/l N	mg/l N	mg/l
M1	03.94	0.1477	0.0008	<0.1	0.019
M2	12.93	0.0675	0.0013	0.0264	0.004
M2	02.94	0.0029	0.0015	0.0236	0.000
M2	03.94	0.1331	0.0013	<0.1	0.017
E1	12.93	0.3402	0.0015	0.0228	0.000
E1	02.94	0.0377	0.0013	0.0248	0.106
E1	03.94	0.2526	0.0013	<0.1	0.000
E2	12.93	0.4494	0.0042	0.0256	0.000
E2	02.94	0.0115	0.0020	0.0050	0.000
E2	03.94	0.0140	0.0024	0.0050	0.004
E3	12.93	0.0106	0.2699	3.3770	5.080
E3	02.94	0.0014	0.3175	0.5732	2.600
E3	03.94	0.1106	0.0722	<0.1	3.560
L	03.94	0.1515	0.0008	<0.1	*
PL	12.93	0.1126	0.0024	0.0425	0.007
PL	02.94	0.2797	0.0008	0.0323	0.124
PL	03.94	0.0193	0.0015	1.4111	0.000
P1	12.93	0.1798	0.0008	0.0236	0.014
P1	02.94	0.3006	0.0015	<0.1	0.000
P1	03.94	0.1163	0.0015	<0.1	0.000
P2	12.93	0.0041	0.0013	0.0291	0.000
P2	02.94	0.5236	0.0014	0.0283	0.000
P2	03.94	0.1632	0.0024	<0.1	0.000
P3	12.93	0.0577	0.0020	0.0228	0.000
P3	02.94	0.0147	0.0032	0.0232	0.000
P3	03.94	0.0126	0.0024	<0.1	0.021
P4	12.93	0.1155	0.0032	0.0287	0.006
P4	02.94	0.3524	0.0028	0.0386	0.000
P4	03.94	0.2190	0.0013	<0.1	0.000

APPENDIX B CONT.

SITE CODE	DATE	TOTAL ALKALINITY	CHLORIDE	SULPHATE
		meq/l	mg/l	mg/l
B1	02.93	0.012	4.206	0.630
B1	09.93	0.045	3.462	0.737
B2	09.93	0.064	3.296	0.686
B3	09.93	0.055	3.704	0.662
B4	09.93	0.146	5.703	2.007
B5	09.93	0.117	5.572	1.537
B6	02.93	0.125	11.602	2.574
B6	09.93	0.144	9.603	3.482
B6	12.93	0.173	10.862	3.310
B6	02.94	0.168	11.349	3.065
B6	03.94	0.175	12.379	3.301
B7	09.93	0.292	17.817	6.805
B8	09.93	0.370	19.423	9.196
B8	12.93	0.273	16.564	6.327
B8	02.94	0.354	17.741	5.202
B8	03.94	0.276	16.564	4.312
B9	09.93	0.454	23.664	9.566
B10	09.93	0.593	43.052	11.829
B11	09.93	0.566	53.369	12.342
B12	02.93	0.501	33.779	7.213
B12	09.93	0.592	70.153	15.599
B13	09.93	0.636	7.175	1.683
B14	09.93	0.867	163.492	28.992
B14	12.93	1.050	114.544	24.628
B14	02.93	1.001	94.952	20.843
B14	03.93	0.924	104.235	17.021
B15	09.93	0.696	131.798	28.415
B16	09.93	0.832	339.961	53.796
T1	09.93	0.117	5.082	0.858

APPENDIX B CONT.

SITE CODE	DATE	TOTAL ALKALINITY	CHLORIDE	SULPHATE
		meq/l	mg/l	mg/l
T2	09.93	0.262	15.590	6.461
T3	09.93	0.085	8.217	1.627
T4	09.93	0.226	12.517	4.235
T5	09.93	1.528	338.914	69.561
T6	09.93	2.237	4070.055	533.337
T7	09.93	0.083	12.669	1.668
T7	12.93	0.198	20.018	2.594
T7	02.93	0.402	21.666	1.203
T8	09.93	0.165	2011.578	376.656
K1	12.93	0.124	0.260	0.000
K1	02.94	0.120	4.751	0.557
K1	03.94	0.113	3.570	0.644
K2	12.93	0.207	4.663	0.972
K2	02.94	0.174	4.824	0.947
K2	03.94	0.288	*	*
K3	12.93	0.195	4.783	0.994
K3	02.94	0.162	4.409	0.891
K3	03.94	0.233	7.061	0.882
K4	12.93	0.182	4.544	0.959
K4	02.94	0.192	5.155	0.796
K4	03.94	0.240	7.090	0.878
K5	12.93	0.183	4.600	1.026
K5	02.94	0.096	5.112	0.816
K5	03.94	0.164	13.882	2.093
K6	12.93	0.155	3.564	0.902
K6	02.94	0.132	4.921	0.804
K6	03.94	0.182	5.386	0.904
M1	12.93	*	4.979	1.013
M1	02.94	0.078	5.244	0.682

APPENDIX B CONT.

SITE CODE	DATE	TOTAL ALKALINITY	CHLORIDE	SULPHATE
		meq/l	mg/l	mg/l
M1	03.94	0.091	*	*
M2	12.93	0.090	4.979	1.013
M2	02.94	0.072	5.528	0.713
M2	03.94	0.096	5.358	0.748
E1	12.93	0.085	7.417	1.256
E1	02.94	0.094	8.031	1.664
E1	03.94	0.090	9.023	0.961
E2	12.93	0.763	39.649	11.937
E2	02.94	0.317	16.912	4.710
E2	03.94	0.330	15.216	4.572
E3	12.93	1.362	71.534	25.004
E3	02.94	0.882	47.318	16.663
E3	03.94	0.858	41.491	16.017
L	03.94	0.120	6.902	0.738
PL	12.93	2.796	92.431	12.031
PL	02.94	1.579	89.372	11.110
PL	03.94	1.219	66.035	9.059
P1	12.93	0.049	6.259	1.057
P1	02.94	0.050	7.890	0.587
P1	03.94	0.038	6.320	0.413
P2	12.93	0.060	7.434	1.255
P2	02.94	0.047	9.202	1.148
P2	03.94	0.098	0.700	0.068
P3	12.93	0.440	26.959	4.677
P3	02.94	0.240	21.432	4.698
P3	03.94	0.295	16.524	3.926
P4	12.93	0.180	1.194	0.140
P4	02.94	0.132	25.469	2.674
P4	03.94	0.175	3.115	1.152

APPENDIX B CONT.

SITE CODE	DATE	SODIUM	POTASSIUM	MAGNESIUM	CALCIUM
		mg/l	mg/l	mg/l	mg/l
B1	02.93	4.000	0.310	0.480	0.980
B1	09.93	2.250	0.860	0.240	0.950
B2	09.93	4.000	1.390	0.320	1.490
B3	09.93	3.500	0.850	0.260	1.430
B4	09.93	6.000	1.690	1.010	3.300
B5	09.93	1.880	0.700	0.200	1.180
B6	02.93	7.500	2.500	2.040	2.580
B6	09.93	6.620	2.640	1.590	2.260
B6	12.93	8.130	2.280	2.040	2.550
B6	02.94	7.284	0.996	1.853	2.635
B6	03.94	7.273	0.974	1.677	2.241
B7	09.93	69.500	3.740	2.300	3.830
B8	09.93	12.600	5.230	3.650	4.880
B8	12.93	10.800	3.760	2.810	4.100
B8	02.94	*	*	2.020	*
B8	03.94	9.405	1.730	2.076	3.709
B9	09.93	15.300	5.880	4.540	4.540
B10	09.93	32.600	5.830	6.310	6.200
B11	09.93	23.800	4.250	5.190	5.900
B12	02.93	16.100	3.350	9.120	3.850
B12	09.93	47.100	5.750	9.480	6.700
B13	09.93	40.600	5.480	10.900	7.300
B14	09.93	79.100	7.530	31.100	13.600
B14	12.93	37.800	5.160	10.400	6.200
B14	02.93	33.100	4.563	6.734	16.569
B14	03.93	54.056	3.543	10.869	11.688
B15	09.93	80.500	6.040	14.800	8.800
B16	09.93	122.000	6.180	33.200	14.500
T1	09.93	6.750	1.640	0.670	1.550

APPENDIX B CONT.

SITE CODE	DATE	SODIUM	POTASSIUM	MAGNESIUM	CALCIUM
		mg/l	mg/l	mg/l	mg/l
T2	09.93	11.900	5.040	3.110	5.300
T3	09.93	6.000	1.890	0.690	1.840
T4	09.93	9.500	3.610	1.940	3.000
T5	09.93	139.000	4.580	15.900	9.180
T6	09.93	692.000	26.800	97.300	38.000
T7	09.93	4.750	1.130	0.830	1.260
T7	12.93	10.600	3.110	3.390	3.190
T7	02.93	10.985	0.944	3.244	3.892
T8	09.93	354.600	23.400	132.000	40.800
K1	12.93	4.250	1.110	0.720	1.110
K1	02.94	3.972	0.346	0.693	0.698
K1	03.94	3.534	0.241	0.721	0.641
K2	12.93	4.130	1.990	0.650	1.260
K2	02.94	4.489	0.862	0.800	1.456
K2	03.94	3.990	0.887	0.700	1.163
K3	12.93	4.750	1.810	0.720	1.120
K3	02.94	4.250	0.761	0.770	1.172
K3	03.94	4.342	0.743	0.743	1.032
K4	12.93	5.750	0.580	0.620	0.810
K4	02.94	3.592	0.606	0.578	0.774
K4	03.94	3.998	0.887	0.706	1.191
K5	12.93	2.980	0.700	0.383	0.493
K5	02.94	*	*	0.673	1.084
K5	03.94	3.923	0.741	0.643	1.084
K6	12.93	5.630	0.700	0.490	1.100
K6	02.94	4.268	0.788	0.787	1.140
K6	03.94	3.249	0.683	0.588	0.964
M1	12.93	4.740	1.740	0.640	1.410
M1	02.94	2.881	0.307	0.394	0.366

APPENDIX B CONT.

SITE CODE	DATE	SODIUM	POTASSIUM	MAGNESIUM	CALCIUM
		mg/l	mg/l	mg/l	mg/l
M1	03.94	3.773	0.731	0.527	0.670
M2	12.93	5.000	1.040	0.650	1.140
M2	02.94	4.042	0.550	0.547	0.907
M2	03.94	3.788	0.641	0.468	0.668
E1	12.93	6.380	1.080	0.660	1.440
E1	02.94	6.003	0.631	0.689	0.488
E1	03.94	5.629	0.577	0.580	0.453
E2	12.93	25.300	2.650	6.350	7.600
E2	02.94	10.753	1.191	2.134	4.231
E2	03.94	10.916	1.406	2.324	4.698
E3	12.93	63.800	29.200	7.360	11.900
E3	02.94	43.038	11.387	3.676	11.507
E3	03.94	47.340	12.512	4.079	12.020
L	03.94	5.048	0.519	0.493	1.424
PL	12.93	35.100	17.600	18.500	19.100
PL	02.94	44.603	6.883	9.261	21.167
PL	03.94	36.461	5.399	7.528	17.726
P1	12.93	5.750	0.840	0.740	1.460
P1	02.94	4.653	0.052	0.619	0.142
P1	03.94	4.665	0.047	0.693	0.245
P2	12.93	6.880	1.090	0.790	1.150
P2	02.94	5.399	0.197	0.748	0.279
P2	03.94	5.495	0.184	0.660	0.259
P3	12.93	12.900	0.580	2.990	5.700
P3	02.94	11.018	1.364	2.020	5.352
P3	03.94	8.540	1.270	1.330	3.130
P4	12.93	18.600	2.580	3.680	2.280
P4	02.94	11.876	0.403	1.907	1.509
P4	03.94	12.377	0.771	2.218	2.278