TOWARDS A MACROINVERTEBRATE SAMPLING PROTOCOL FOR MONITORING WATER QUALITY OF WETLANDS IN SOUTH AFRICA

by

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ABSTRACT

The degradation of wetlands and loss of their associated ecosystem services is widely recognised in South Africa, however, at present there is no standard method of biologically assessing wetland health in this country. Internationally, particularly in the U.S.A and Australia, wetland bioassessment techniques using macroinvertebrates are well established. A number of these wetland bioassessment protocols have been derived from local river biomonitoring techniques, as there is a belief that river and wetland ecology and macroinvertebrate assemblages at family level are similar. However, some authors consider wetland macroinvertebrate assemblages and ecological processes to differ greatly from those found in rivers, and believe that such techniques are not transferable.

South Africa has a well established macroinvertebrate biomonitoring protocol for rivers called SASS5 (South African Scoring System Version 5). This study is a preliminary investigation into the extent to which the SASS5 scoring system is applicable to the assessment of nutrient enriched wetland water quality.

Macroinvertebrates are particularly suitable as biomonitoring tools: they respond to a variety of stressors, have life cycles that allow for integrated responses to episodic pollution, and are relatively easy to identify to family level. When selecting wetlands for the development of a biomonitoring protocol, wetlands should all be of the same; classification (i.e. palustrine), geomorphological and climate setting, hydrological regime and dominant vegetation class. Sampling was restricted to sedge-dominated palustrine wetlands in the midlands of KwaZulu-Natal, with similar hydro-geomorphological settings.

Due to wetlands and rivers having different biotopes (e.g. no riffles present in wetlands), the SASS5 sampling protocol could not be used, thus a pilot investigation was undertaken to derive a suitable sampling technique for

collecting a representative and diagnostic sample of aquatic macroinvertebrates from a wetland. This technique was developed based on published methods. Both sweep net and activity trap sampling were conducted, and each evaluated for their effectiveness at macroinvertebrate collection. Sweep net sampling was tested over a range of sweep intensities (2-6 sweeps), and activity traps were placed at four different depths: at the water surface, just below the surface, 0.1-0.15m below surface and on the substrate.

A total of 32 taxa identified to family level were identified in the samples. Taxon diversity and composition did not differ in the activity traps placed at the four depth locations. Taxon diversity did not differ significantly between different sweep intensities; however there was a significant difference in taxon composition between the different sweep intensities and between activity trap and sweep net samples ($p<0.05$). Sixty-eight percent of taxa appeared more frequently in sweep net sampling compared to activity trap sampling. Six taxa were found exclusively in sweep net samples, and two taxa were recorded exclusively in activity traps. There was no trend in either method collecting more or missing any unique trophic group. In conclusion, activity traps are not required to supplement sweep net data, and a technique using a sweep net with a sweep intensity of five would be suitable to collect a representative sample of wetland macroinvertebrates.

Using the derived technique, four reference and three wetlands impacted by dairy effluent were sampled. Six macroinvertebrate samples were collected from each of the seven wetland, together with data for selected physico-chemical variables, macrohabitat condition, biotope suitability and organism detectability.

For each sample, the macroinvertebrates were identified and assigned a predetermined SASS5 tolerance score between 1 and 15, with higher scores indicating increased sensitivity to poor water quality.

A total of 39 taxa, identified to family level, were collected during sampling. SASS5 scores ranged from 15-82. Five of the wetlands had mean SASS5 scores of between 46 and 59. Five of the wetlands had an intra-wetland SASS5 score range greater than 30. ASPT values ranged from 3.3 to 5.5, and few high scoring (≥8) taxa were collected. There was no significant difference in SASS5 scores between samples collected above, at and downstream of an effluent discharge point within the same impacted wetland. SASS5 scores for reference wetlands were also not significantly higher than those recorded for impacted wetlands. Comparison of ranked SASS5 scores and environmental data did suggest a relationship between the variables, but was not significant.

Based on the SASS5 score water quality guidelines, all sampled wetlands were considered to have impacted water quality; however, this was not supported by the macrohabitat and physico-chemical results. Possible reasons for the low SASS5 scores include: the lack of 'stones in/out current' biotopes in wetlands, lower levels of dissolved oxygen present compared to rivers, and the limited detectability of organisms due to large amounts of substrate in the samples. A wetland adaptation of SASS5 would require the reassignment of modified scores to certain taxa based on their distribution in wetlands of varying water quality. The SASS5 score level of 100 and the ASPT value of 6 (as specified in the SASS5 score water quality guidelines) were found to be inappropriate for wetlands. It is suggested that, either the range of taxa tolerance scores be increased (1 to >15), or the score level of 100 be lowered. The ASPT value should also be reduced. Although SASS5 appears unsuitable for assessing wetlands, variations in taxon composition between sampled wetlands, identified through CA analysis, suggests that macroinvertebrates are responsive to changes in wetland condition, and thus have potential as indicators of wetland water quality. Nine taxa responsive to the presence of nitrogen have been identified as being potentially good indicators.

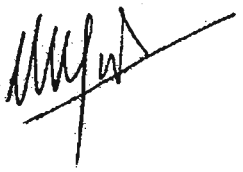
Further research should focus on the testing of SASS5 throughout the year, in a range of wetland types, and in wetlands moderately to severely impacted by pollutants other than dairy effluent. It is recommended that a habitat or biotope index be developed and used in conjunction with any future wetland macroinvertebrate bioassessment protocols.

PREFACE

The research described in this thesis was carried out at the Centre for Environment and Development, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Dr Nevil Quinn (Faculty of the Built Environment, University of the West of England, Bristol, U.K.) and Dr Donovan Kotze (Centre for Environment and Development, University of KwaZulu-Natal, Pietermaritzburg, South Africa).

The thesis represents the original work of the author and has not otherwise been submitted in any form for any degree or diploma at any university. Where use has been made of the work of others it is duly acknowledged in the text.

Rebecca Bowd



Dr. Nevil Quinn (Supervisor)

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1. INTRODUCTION

Wetlands are ecosystems that are of great importance, both for their uses to humans and for their high biodiversity value (Kotze *et al.* 1995). Wetlands support many rare and endangered species, as well as being systems which can cleanse polluted waters, minimize flooding, store water, prevent erosion and regulate stream flow (Begg 1989, Kotze 1999). Although no official national survey of wetland loss has been conducted in South Africa, studies which have been undertaken in several catchments have indicated that between 35% and 50% of the wetlands (and the benefits they provide) have been lost or severely degraded (Dini 2004). This is largely due to agricultural development, such as drainage and poor land use practices (Kotze *et al.* 1995). Currently more than half of all wetlands in KwaZulu-Natal have been modified so that their functioning is impaired (Begg 1990). Wetlands with impaired functioning cannot provide humans with the level of natural services that they normally provide, nor can they maintain high levels of ecological integrity. South Africa is a signatory of the Ramsar Convention of 1971, thus it is committed to the conservation and wise use of wetlands. South Africa's National Water Act (Act No. 36 of 1998) states that water resources (including wetlands) must be managed in an integrated, holistic manner, and that methods of monitoring the condition of water resources need to be designed and implemented.

In order for South Africa to comply with national and international legislation, and cater for human and environmental needs, methods for monitoring water quality need to be developed for all water resources, including wetlands. The purpose of this project is to contribute to the development of a wetland water quality assessment protocol. There are a variety of methods that can be employed to monitor water quality, however this study focuses on the use of aquatic macroinvertebrates for the biomonitoring of wetlands in South Africa. Biomonitoring as well as other key concepts used in this document are defined in Table 1.1.

Table 1.1 Key words used in this document

Key word	Definition
Macroinvertebrate	Animals without a vertebral column that can be caught with a 500-800 micron mesh net. Macroinvertebrates do not include zooplankton or ostracods, which are generally smaller than 200 microns in size (Helgen 2002)
Wetlands	'Wetlands are lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow water, and which land in normal circumstances supports or would support vegetation typically adapted to life in saturated soil. Wetlands must have one or more of the following attributes: 1) at least periodically, the land supports predominantly hydrophytes, 2) the substrate is predominantly undrained hydric soil, and 3) the substrate is nonsoil and is saturated with water or covered by shallow water at some time during the growing season of each year' (Cowardin *et al.* 1979 p. 3)*
Palustrine wetlands**	Wetlands which are nontidal and dominated by trees, shrubs, persistent emergents, emergent mosses or lichens, and all such wetlands that occur in tidal areas where salinity due to ocean-derived salts is below 5% (Cowardin *et al.*1979). It also includes wetlands lacking such vegetation, but which have all of the following characteristics: 1) area less than eight hectares; 2) active wave-formed or bedrock shoreline features lacking; 3) water depth in the deepest part less than two metres at low water; 4) salinity due to ocean-derived salts less than 0.5‰ (Mitsch and Gosselink 1993). Features of a palustrine wetland system are illustrated in Appendix 1
Biomonitoring	The sampling of communities and life forms which inhabit water bodies in order to provide an assessment of health, or degree of impact from human development (Hicks 2000)
Bioassessment	Using biomonitoring data of samples of living organisms to evaluate the condition or health of a wetland or stream (Helgen 2002)
Bioindicator	An organism, species, assemblage, or community characteristic of a particular biotope, or indicative of a particular set of environmental conditions (Gernes and Helgen 2000)
Reference wetland	Wetlands in which the ecological characteristics most closely represent the pristine or minimally impaired condition (Parker 2002, Butcher 2003)
Impacted wetland	Wetlands which have some degree of human influence affecting them, resulting in adverse changes to their ecological characteristics (Helgen 2002)
Sample point	The location within a wetland where a sample is taken
Picking (of a sample)	The extraction of organisms from a sample. A sample can either be picked in its entirety or subsampled. For subsampling methods see Table 2.19
Metric	An attribute with empirical change in value along a gradient of human influence (Karr and Chu 1997, Bartoldus 1999)
Index of Biological Integrity (IBI)	An integrative expression of the biological condition that is composed of multiple metrics (Teels and Adamus 2002)
Biotope	Refers to a given set of conditions which is occupied by a community (Macan 1963)

*This definition was adopted by wetland scientists in the U.S. Fish and Wildlife Service in 1979 after several years of review (Mitsch and Gosselink 1993). It also forms the basis for the wetland definition contained in South Africa's National Water Act.
**Palustrine wetlands are mainly associated with river floodplains, topographic depressions, margins of lakes and ponds, and other areas where the water table is at or near the surface for a significant period during the growing seasons (Wilen and Tiner 1993)

There are a number of different biological indicators that can be used to determine the health of a wetland. These include, amphibians, fish, diatoms, water birds and aquatic macroinvertebrates. Different taxa within each of these groups have different tolerances to pollution, thus the presence or absence of

certain taxa can be an indication of specific water quality conditions. Taxa may be ascribed scores based on their relative tolerance of changes in water quality.

Aquatic macroinvertebrates have been used extensively internationally as water quality biomonitoring tools in streams and rivers (Wissinger 1999, Hicks 2000, Butcher 2003), and are the next most popular choice of bioindicator after plants for wetland bioassessment (DWAF 2004). Little work has been undertaken on the use of aquatic macroinvertebrates in wetlands (Butcher 2003). Butcher concluded that worldwide, literature was relatively hard to find on wetland assessments and monitoring programmes, and that most countries are still at the inventory stage. Therefore well-developed wetland assessment and monitoring programmes are not in operation.

To date, there has been limited work undertaken on the assessment of wetland health in South Africa, and there is little information available on the topic. However, studies undertaken in the south-western Cape wetlands of the country, suggested that macroinvertebrates do not provide consistent and useful bioassessment information (Dr Day pers. comm. 2004).

In many cases where wetland biomonitoring programmes using aquatic macroinvertebrates have been developed, they have largely been derived from local river monitoring methods (Teels and Adamus 2002). Two common changes that have been made to adapt the river monitoring methods for wetland assessment, are an increase in score of certain taxa, and the development of a specific sampling protocol.

South Africa has a macroinvertebrate biomonitoring water quality tool developed for streams and rivers called SASS5 (South African Scoring System Version 5). SASS5 is specifically aimed at the detection of changes within the aquatic macroinvertebrate community of streams and rivers (Dallas *et al.* 1999, Dickens and Graham 2002). SASS was originally derived from the British Monitoring

Working Party system (BMWP) by Chutter (1994). Since its original derivation, the method has been modified four times. The latest modification of this method, SASS5, has been carried out by Dickens and Graham (2002). It includes a stricter definition of the technique, an improved sampling and analytical process, and the introduction of quality control procedures. Additions to the list of invertebrates have also been made. In SASS5, tolerance scores, which range from 1 to 15, are assigned to chosen aquatic invertebrates at family level. These scores are processed through simple calculations to determine river health.

SASS5 plays an important part in the South African National Rivers Health Programme (Uys *et al.* 1996), and has been employed to assist with the determination of the Ecological Reserve as required by the South African National Water Act (1998) (Dickens and Graham 2002). Water boards, irrigation boards and a number of universities and consultants have also implemented SASS5 as a method of assessing river health (Graham *et al.* 2004).

This study tests whether the current SASS5 tolerance scores are appropriate for the assessment of nutrient enriched palustrine wetlands in South Africa. Wetlands that have dairy effluent as their main source of nutrient enrichment have been used in this study. The pollution type (dairy effluent) and the wetland type (palustrine) were restricted to minimise variability of results, as pollution and wetland type can both affect taxon composition (Waterwatch, Water and Rivers Commission 1996, Batzer *et al.* 1999). The SASS5 sampling protocol cannot be used to sample wetlands, as the biotopes sampled in SASS5 are not all present in wetlands (Table 2.7). For this reason, a wetland sampling technique was developed specifically for this study.

1.1 The Use of Aquatic Macroinvertebrates as a Water Quality Biomonitoring Tool

Since the 1970s, the United States Environmental Protection Agency (U.S. EPA) has regulated wetland assessment (DWAF 2004). The U.S. EPA has conducted extensive work on the biomonitoring of different wetland types (e.g. depressional, forested and lacustrine fringe), and have commonly used amphibian and diatom assemblages. However, the use of aquatic macroinvertebrate assemblages for rapid bioassessment has been most popular since 1989 (Sutherland and Stribling 1995). Much of the U.S. EPA's work in this field has been well documented (DWAF 2004), including numerous guideline documents that describe how to develop bioassessment tools (e.g. Dentenbeck 2002, Helgen 2002, Parker 2002, Teels and Adamus 2002). This large resource of transferable information could assist South Africa to avoid unnecessary effort during the research and development stage of establishing a wetland bioassessment protocol (DWAF 2004).

In Australia and New Zealand, the use of aquatic macroinvertebrates for biomonitoring is also favoured. When the choice of bioindicator to apply to streams and wetlands is not immediately obvious, aquatic macroinvertebrate communities represent the most broadly applicable group (ANZECC and ARMCANZ 2000). Although the ecosystems of Australia and South Africa are somewhat different, both countries are semi arid and have similar levels of hydrological variability, thus the work conducted in Australia is of interest, as it may have application in South Africa (DWAF 2004).

There are a number of advantages of using biological indicators to assess the health of aquatic environments (Section 2.1). Day (2000 p. 5) concludes that 'if we learn to interpret the stories told by the plants and animals that live in our rivers, lakes and wetlands, we can save time and money and also contribute to the conservation of our aquatic ecosystems and their inhabitants'.

1.2 Problem Statement

South Africa requires a method of assessing and monitoring wetland health in order to meet both national and international legislative requirements, as well as human and environmental needs. However, there has been little work done on the assessment of wetland health in South Africa to date. At present, a scoring system, SASS5, is applicable to rivers through out the country, but it is not known to what extent SASS5 tolerance scores are suitable for assessing wetland health, or if this technique could be applicable through modifications to the scoring system.

1.3 Aim and Objectives

The aim of this research is to investigate the potential use of aquatic macroinvertebrates and the SASS5 scoring procedure for the monitoring and assessment of palustrine wetlands in South Africa, which will contribute to the development of a South African wetland water quality biomonitoring programme.

To achieve this aim, this study has the following objectives:

1. Evaluate international and regional approaches to the use of aquatic macroinvertebrates in wetland biomonitoring
2. Develop an aquatic macroinvertebrate sampling technique for use in sedge-dominated palustrine wetlands in the KwaZulu-Natal midlands
3. Collect macroinvertebrate data from selected wetlands with the derived technique, and evaluate whether SASS5 tolerance scores and scoring system are useful in assessing the health of nutrient enriched sedge-dominated palustrine wetlands in the KwaZulu-Natal midlands
4. Make recommendations for the development of an aquatic macroinvertebrate biomonitoring protocol for use in sedge-dominated palustrine wetlands in the KwaZulu-Natal midlands

1.4 Methods

This study examined the literature concerning wetland biomonitoring the relevant elements of river biomonitoring. Based on the review findings, an aquatic macroinvertebrate sampling technique was developed for use in KwaZulu-Natal palustrine wetlands. This was done within a pilot study that tested two sampling techniques, sweep net sampling and activity traps. Quantitative data were used to determine the optimum number of sweeps required to sample a wetland effectively. Activity traps were tested to determine i) if macroinvertebrate composition varied between different water column depths, and ii) if they collected additional taxa to those collected by sweep net sampling. The data obtained from this testing were quantitative. Results were statistically analysed, and a suitable sampling method was derived, based on the results.

Using the sampling technique derived from the pilot study, three impacted and four reference wetlands were sampled. In each wetland six samples were taken. From the impacted wetlands, two samples were taken from above the effluent discharge point, two adjacent to the effluent discharge point, and two downstream of the effluent discharge point. The results, which were both qualitative and quantitative, were then processed using the SASS5 scoring system. Based on the findings, it was evaluated whether the current SASS5 tolerance scores and calculation procedure are suitable for determining the water quality of wetlands impacted upon by dairy effluent.

1.5 Document Structure

The first chapter provides a purpose and context for the research, and outlines the aims and objectives of the study. Chapter two is a literature review which reviews all aspects associated with the development of a wetland bioassessment protocol. This chapter meets objective one. Chapter three provides the methodology that was followed to complete this study. The results of this study are presented as two journal articles written in the format of *African Journal of Aquatic Science*. The first article directly addresses objective two, and details the

pilot investigation undertaken to derive a suitable macroinvertebrate sampling technique. The second article meets objective three, and documents the procedure undertaken to test the applicability of the SASS5 scoring procedure in nutrient enriched palustrine wetlands. Both papers address objective four, and make recommendations towards the development of a South African biomonitoring protocol.

2. LITERATURE REVIEW

This literature review is divided into nine sections. Section one lists the advantages and disadvantages of using aquatic macroinvertebrates as indicators of water quality. Section two details selected case studies that form the basis for the remainder of the literature review. Section three addresses the issue of how appropriate stream biomonitoring procedures are to wetland health assessment. Section four identifies considerations that need to be addressed before sampling can be performed (e.g. wetland selection and best time of year to sample). Section five reviews five different techniques used for aquatic macroinvertebrate collection. Section six examines additional environmental data requiring collection from a wetland, and Section seven gives details on the management of samples post extraction from a wetland. Section eight discusses what level of taxonomic identification is sufficient for bioassessment. Finally, Section nine reviews invertebrate metrics and indices.

2.1 Advantages and Disadvantages of Using Aquatic Macroinvertebrates as a Biomonitoring Tool

There are many reasons why aquatic macroinvertebrates are particularly suitable as biomonitoring tools, however there are also a number of limitations associated with their use (Table 2.1).

2.2 Case Studies

Throughout this literature review, several case studies have been compared and contrasted, concerning a variety of aspects of biomonitoring. Case studies were specifically chosen that were either applicable to palustrine wetlands or addressed the issue of using aquatic macroinvertebrates for wetland biomonitoring, and which had sufficient literature available. This section provides an overview of all case studies examined (Table 2.2).

Table 2.1 Advantages and disadvantages of using aquatic macroinvertebrates as biomonitoring tools

Advantages	Disadvantages
• Invertebrates can be expected to respond to a wide array of stressors in wetlands (Helgen 2002), including fluctuations in dissolved oxygen (DO), sediment, metals, other toxins and organic enrichment (Butcher 2003) • Invertebrates have life cycles that range from weeks to months, which allows for integrated responses to historic and/or episodic pollution (Helgen 2002, Graham *et al.* 2004) • There is extensive literature on the analysis of aquatic invertebrates within biological monitoring approaches for streams (Hicks 2000, Helgen 2002) • Water quality assessment can take place in the field as invertebrates can be identified to family level without the need for a laboratory (Helgen 2002) • Invertebrates can be identified down to family level with relative ease using simple taxonomic keys (Hicks 2000) • Many invertebrates are tightly linked to wetland conditions, complete their life cycles within the same wetland, and are exposed to site specific conditions (Butcher 2003) • Invertebrates are ubiquitous in aquatic biotopes (Sharitz and Batzer 1999, Helgen 2002)	• Depending on the level of taxonomic identification, facilities for processing and identifying invertebrates may be expensive (both equipment and expertise) and time consuming (Helgen 2002, Butcher 2003) • As multi-stressors are likely to be present, it may be difficult to precisely pinpoint the cause of impairment (Chutter 1998, Helgen 2002) • Some invertebrates migrate from other water bodies, thus these taxa are not representative of the conditions of the wetland (Helgen 2002) • Invertebrate composition varies between seasons and the rate of maturity varies between different invertebrates. Thus it is difficult to determine the best time to sample (Helgen 2002) • Invertebrates have a high spatial variability due to habitat dependence (Butcher 2003) • The patchiness of biotope conditions within a single wetland will result in complex patterns of invertebrate distribution and diversity, making it very difficult to obtain quantitative data that can be analysed with traditional statistics because the variability among the samples is great (Adamus and Brandt 1990)

Table 2.2 Overview of case studies examined

Title of project	The Vermont Wetland Bioassessment Programme	The Mid-Atlantic Wetland Project	The Minnesota Pollution Control Agency Wetland Bioassessment Programme	Development of Wetland Bioassessment methods in Wisconsin	Development of Biocriteria for Wetlands in Montana
Source	Burnham (2000)	Sparling *et al.* (2000)	Gernes and Helgen (2000)	Lillie (2000)	Apfelbeck (1999)
Purpose/ objectives/ aim	**Objectives** **1)** To gather chemical, physical and biological data from seasonal pools, in order to classify wetlands and identify reference wetlands in Vermont, U.S. **2)** To use both previously and newly collected data to help identify specific biological attributes that can serve as indicators of ecological integrity	**Purpose** To develop biological metrics to assess the condition and progress of restored wetlands in Maryland, Delaware and Virginia, U.S.	**Purpose** To develop a macroinvertebrate Index of Biological Integrity (IBI) (Section 2.9.1) and a separate vegetation IBI for Minnesota depressional wetlands, U.S. with the aim of establishing water quality standards	**Purpose** To develop a biotic index for palustrine wetlands, and a biological rating system for classifying wetlands in Wisconsin, U.S.	**Objectives** **1)** To assess the status and determine the trends in wetland water quality **2)** To understand how climate, hydrologic controls and geomorphic settings influence biological communities to help classify wetlands **3)** To define the extent and degree of anthropogenic impacts to wetland water quality in Montana, U.S.
No. and state of wetlands sampled	**Total:** 18	**Total:** 30 **Restored:** 22 **Natural:** 8	**Project 1. Total:** 35 **Reference:** 32 **Disturbed:** 3 **Project 2. Total:** 27 Range in degree of human disturbance	**Total:** 104 Range from undisturbed to restored to degraded	**Total:** 80
Biota sampled	Macroinvertebrates Algae Amphibians	Macroinvertebrates Vascular Plants Amphibians	Macroinvertebrates Vascular Plants Amphibians	Macroinvertebrates Vascular Plants	Macroinvertebrates Algae Vascular Plants
Type of analysis/ other comments	-	The study was carried out over three years	The stressors affecting the sampled wetlands were: Agriculture, Storm water runoff	Using SYSTAT (SPSS 1997), metrics were developed by visual comparison of community attribute responses to suspected measures of disturbance	The stressors affecting the sampled wetlands were: Agriculture, Mining, Others (NS)

Table 2.2 Overview of case studies examined (contd.)

Title of project	The North Dakota Wetland Bioassessment Programme	The Maine Department of Environmental Protection	NEFWIBP (New England Freshwater Wetland Invertebrate Biomonitoring Protocol)	Study on the Ichauway Ecological Reserve	SWAMPS (Swan Wetland Aquatic Macroinvertebrate Sensitivity)
Source	Ell (2000)	DiFranco and Stevenson (2000)	Hicks (2000)	Battle *et al.* (2001)	Chessman *et al.* (2002)
Purpose/ objectives/ aim	**Aim** To develop a wetland water quality sampling protocol for North Dakota, U.S. **Objective** To develop biological community metrics and an IBI for temporary and seasonal depressional wetlands	**Aim** To develop a sampling protocol using aquatic macroinvertebrates and algae **Objective** To develop biological criteria for Maine wetlands (U.S.) and diagnose the stressors of degraded wetlands	**Purpose of manual** This manual gives extensive detail on how to develop a wetland sampling protocol, and explains how to use a multimetric approach to analysing the raw data obtained from the invertebrate sampling. The manual is designed for freshwater wetlands	**Purpose** To study the quality of water in the Ichauway ecological reserve in southwest Georgia, Baker County, U.S.	**Aim** To develop a biotic index for wetlands located on the Swan Coastal Plain near Perth, Western Australia **Purpose** To assess and monitor the ecological condition of wetlands, in order to guide protective and restorative work
No. and state of wetlands sampled	NS	**Total:** 20 Range from minimally disturbed to degraded	NS	NS	**Project 1. Total:** 40 (1989-1990) **Project 2. Total:** 23 (1997)
Biota sampled	Macroinvertebrates Algae Vascular Plants	Macroinvertebrates Algae	Macroinvertebrates	Macroinvertebrates	Macroinvertebrates
Types of analysis/ other comments	The stressors affecting the sampled wetlands were Agricultural	-	-	To compare: 1) Water quality among wetland types- PCA 2) Macroinvertebrate assemblages among wetland types- NMS 3) To test sensitivity of the metric- b-and-w plots	The SWAMP index has been developed for both family and species level data. This was done to test differences in index sensitivity in relation to taxonomic resolution

Key: NS: Not specified PCA: Principle Component Analysis NMS: Non-Metric Multidimensional scaling ordination b-and-w: Box and Whisker plots

2.3 Stream Biomonitoring Methods: How Appropriate are they to Wetlands?

Several studies that have developed bioassessment methods for wetlands have adapted bioassessment frameworks that were originally designed for streams (Teels and Adamus 2002). There are two views concerning the use of stream monitoring procedures in wetland assessment. Many authors support the idea that rapid wetland assessment methods should be similar to those methods being used in national river health programmes (e.g. in Australia and the U.S.) (Butcher 2003). This has largely occurred because of the belief that rivers and wetlands are ecologically similar and therefore can be monitored using the same methods (Butcher 2003). The opposing view is that aquatic macroinvertebrate assemblages vary too greatly between wetlands and rivers for the same methods to be used. This variation has been attributed to the ecological processes and biotope structure of wetlands being distinct from that of rivers (Wissinger 1999). However, Butcher (2003) has noted that there are significant overlaps in taxa between river and wetland macroinvertebrate assemblages, particularly at family level. Although some aspects of stream bioassessment may apply to wetland bioassessment, specific information from wetland assemblages is needed to develop indices and metrics for wetlands (Teels and Adamus 2002) (Section 2.9.3).

Hicks (2000) made a number of observations concerning similarities and differences between wetland and stream invertebrate assemblages. Hicks (2000) found that most of the insect families commonly found in streams also occur in wetlands; however their relative abundance is often quite different. Hicks gives two examples where abundance of particular invertebrate taxa may be greater in wetlands:

- The large amounts of vegetation in wetlands may support a high abundance of molluscs
- Wetlands have a rich organic detritus layer on the substrate, which supports large numbers of detritivores, such as isopods, amphipods and worms

There is debate over which taxa can be used for wetland bioassessment. For the development of the Montana wetland biocriteria, a number of taxa were eliminated from the original stream metric, as they were either non-benthic or semi-aquatic surface dwellers, and were considered inappropriate for reflecting water quality (Apfelbeck 1999). These taxa included Gerridae, Collembola, Dytiscidae, Hydrophilidae, Ostracoda, Anostraca, Copepoda, Cladocera, Notonectidae and Corixidae. However, Wissinger (1999) identifies a number of specialist taxa in wetlands, including some of those considered inappropriate by Apfelbeck (1999), such as Corixidae, Notonectidae and Dytiscidae. For these and other taxa that are predominantly wetland specialists, there is little or no existing information on their tolerances to human-caused impairments (Helgen 2002).

Stream invertebrate tolerance scores may not be applicable to wetland invertebrates as they are based on invertebrates that inhabit well-oxygenated riffle communities, where pollution promotes a decrease of taxa that are intolerant of lower oxygen conditions. In wetlands, many of the aquatic invertebrates are well adapted to the natural, diurnal fluctuations of oxygen (Helgen 2002) and therefore would not respond in a similar manner to lowered oxygen levels caused by pollution (Gernes and Helgen 2000).

Stream invertebrate sampling methods often have limited applicability to wetlands. The 'kick and collect' method, popular for stream sampling, cannot be used in wetlands, as it requires running water to wash organisms into the net (Hicks 2000). Dickens and Graham (2002) warn that the SASS5 collection technique is designed for low to moderate flow hydrology, and is not applicable to wetlands, impoundments, estuaries and other lentic habitats. Furthermore, as wetlands have muddy substrates and typically denser vegetation in comparison to streams, the use of stream sweep net sampling in wetlands may be challenging (Apfelbeck 1999, Hicks 2000).

It can be concluded that there are strong arguments for both the use and dismissal of applying river biomonitoring methods to wetlands. However, it is apparent that river biomonitoring methods are not directly transferable to wetlands, and that both invertebrate methods of collection and tolerance values would require some modification to be used in a wetland biomonitoring protocol.

2.4 Considerations Before Sampling Can Be Carried Out

This section considers the importance of wetland classification, gives recommendations for selecting which wetlands to sample (for both reference and impacted wetlands) and reviews the most appropriate time of year to carry out sampling.

2.4.1 The Importance of Wetland Classification

The aim of wetland classification is to group areas which, without human disturbance, have similar hydrological, geomorphological and ecological characteristics (DWAF 2004), and where responses to human disturbance are similar (Karr and Chu 1999). Wetland classification must be undertaken prior to sampling (Hicks 2000), as different wetland types may have different invertebrate assemblages. When different human influences on different wetland types are grouped in a single analysis, it becomes almost impossible to understand the causes or consequences of human versus natural events (Karr and Chu 1999).

2.4.2 Wetland Selection

Wetland selection is arguably the most important task when developing a bioassessment method (Kentula *et al.* 1992). Both reference wetlands and wetlands which have similar environments and ranges in impairment need to be selected to successfully develop a wetland bioassessment tool (Karr and Chu 1999, Hicks 2000). There are a number of factors that need to be considered when selecting which wetlands to sample:

- The time lag between stressor occurrence and aquatic macroinvertebrate responses (this can depend on type of stressor, climate, system hydrology) (Parker 2002, Butcher 2003)
- Availability of historic data (Apfelbeck 1999)
- Cooperation by land owners (Apfelbeck 1999)
- Accessibility (Apfelbeck 1999)

2.4.2.1 Selecting reference wetlands

When choosing reference wetlands for the development of a biomonitoring protocol, it is important that they are representative of the natural conditions within the same landscape setting, geomorphology (mineral or organic soils), climatic setting, hydrological regime and dominant vegetation class (Hicks 2000, Parker 2002). If a reference wetland differs from an impaired wetland in more than one of the above factors, then differences in the biological communities cannot be attributed to a single factor, and the degree of impairment cannot be determined with any certainty (Hicks 2000).

To detect significant stressor-response relationships between invertebrate attributes and water quality, sufficient numbers of both reference and impaired wetlands are required (Helgen 2002). Hicks (2000) and Parker (2002) recommend that a minimum of three reference sites should be identified. However, a challenge to the characterisation of reference sites is that there are few places that have not been affected by human actions (Teels and Adamus 2002). If three reference wetlands cannot be found within the same catchment as the respective impaired wetlands, then wetlands from neighbouring catchments that have similar characteristics to the impacted wetlands should be selected (Hicks 2000). The sampling of all wetlands (both reference and impaired wetlands) should be carried out in the same manner and within the same week (Hicks 2000).

2.4.2.2 Selecting impacted wetlands

When selecting impacted wetlands for the development of a biomonitoring protocol, it is important that they are impacted by similar stressors, as different stressors may affect aquatic macroinvertebrate communities in different ways. A number of stressors enter a wetland from a point source (e.g. nutrient enrichment via an inefficient wastewater treatment system). To study the effects of point source pollution in a wetland, the sampling of at least three sites is required, somewhere before the discharge point, at the discharge point and at a point downstream of the discharge point (Hicks 2000). Sampling should not take place around the edge of the wetland, as the sample will be representative of the immediate conditions of the surrounding upland, and not the entire wetland (Hicks 2000).

2.4.3 Determining the Most Appropriate Time of Year to Sample Wetlands

Determining the most appropriate time of year to sample wetlands is not an easy task, as different taxa have different life cycles (Lillie 2000). Ideally, sampling should be carried out when the invertebrate community is the most mature, when the maximum number of invertebrates are identifiable, and when the taxa are the most representative of wetland condition (Helgen 2002).

Other recommendations include:

- Wetlands should be sampled in more than one season (Helgen 2002)
- Wetlands should be sampled near the same date, time and weather conditions in order to assure a valid comparison between sites (Hicks 2000, Reber *et al.* 2000)
- If sampling is performed too late in the year, there may be adults present in a wetland that have arrived from other water bodies

A number of sources have recommended certain times of year in which to carry out sampling (Table 2.3).

Table 2.3 Recommended time of year to sample wetlands

Source	Region	Season	Month
Adamus and Brandt (1990)	General U.S.	Late wet season Beginning of dry season	April to June
Teels and Adamus (2002)	General U.S.	Late summer	September to mid October
Hicks (2000)	New England U.S.	Spring or late summer	April to May September to October
Karr and Chu (1999)	General U.S.	Late summer before Autumn rains	September to October
Sparling *et al.* (2000)	Mid-Atlantic U.S.	Mid to late summer	Late May to October
Lillie (2000)	Wisconsin U.S.	Early spring	April
Apfelbeck (1999)	Montana U.S.	Beginning of spring to end of summer	April to September
Gernes and Helgen (2000)	Minnesota U.S.	Mid summer	June to July
DiFranco and Stevenson (2000)	Maine U.S.	Mid to late summer	June to August
Gray *et al.* (2000)	Ohio U.S.	Early spring to mid summer	March to July
Brown (2000)	Florida U.S.	Spring to late summer	April to October
Burnham (2000)	Vermont U.S.	Spring to mid summer	April to June
Ell (2000)	North Dakota U.S.	Mid summer	June
Battle *et al.* (2001)	George U.S.	Spring Late summer	February to April August to September

Note: All case studies in Table 2.2 took place in the Northern hemisphere, thus boreal seasons are used

Spring sampling in New England is the most desirable, as it is prior to invertebrate emergence and when larvae are at their maximum size (to facilitate identification) (Hicks 2000). In Wisconsin, spring sampling is also favoured to minimise influences of immigration-emigration (Lillie 2000). Another good time to sample is late summer, as it is generally the time of year when wetlands are most stressed (Hicks 2000), and when the greatest diversity (Gernes and Helgen 2000, Sparling *et al.* 2000), abundance (Sparling *et al.* 2000) and maximum taxa maturity (Gernes and Helgen 2000) occurs.

2.5 Aquatic Macroinvertebrate Collection Methods

A description of five different types of wetland sampling techniques, along with their advantages and disadvantages is given in this section. Evaluation of these techniques is important, as an understanding of the limitations of any sampling method is essential to the interpretation of the data collected (Bidlingmayer 1967).

2.5.1 Sweep Net Sampling

Sweep net sampling is a widely used technique for collecting macroinvertebrates (Table 2.5). Details of the sweep net sampling method along with its advantages and disadvantages are given in Table 2.4.

Table 2.4 Details of sweep net sampling and its associated advantages and disadvantages

Method description:

A long wooden or metal pole with a deep net attached to one end is passed through water and vegetation to collect invertebrates

Advantages	Disadvantages
• Experienced persons can collect samples quickly from a wide range of different biotopes (Helgen 2002) • Collects a higher diversity of taxa in comparison to other methods, including; the Gerking box sampler (Cheal *et al.* 1993); the stovepipe sampler and artificial substrate (Mackey *et al.* 1984) • Time and cost efficient	• May miss some motile invertebrates (e.g. large predatory coleopterans and hemipterans). (However, this can be overcome by combining the use of sweep nets and activity traps (Helgen 2002)) • The amount of vegetation which gets trapped in the sample adds to the time needed to pick the invertebrates (Helgen 2002)

Other Comments:

Helgen (2002) lists ways to assure repeatability in sweep net sampling protocols:

- Defining the number of sweeps
- Defining the distance of sweeps
- Defining the amount of time for sweeps
- Doing consistently repeated efforts at each site

Sweep net sampling is the most common method used for the sampling of aquatic macroinvertebrates in shallow water bodies (Helgen 2002)

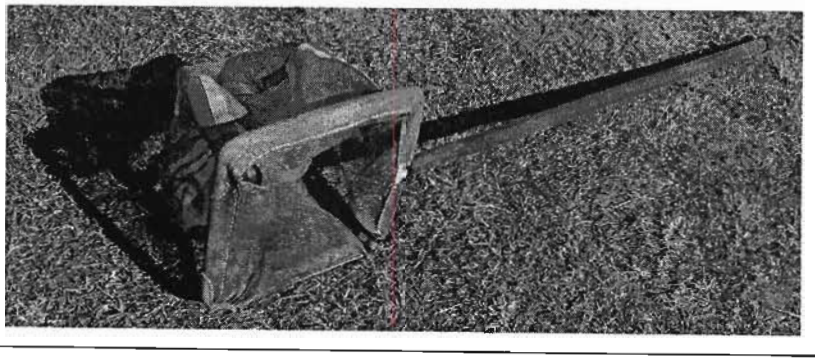


Table 2.5 Details of the sweep net protocols used in selected case studies and SASS5

Source	Type of wetland/ water body sampled	No. of samples taken from a wetland	No. of sweeps in each sample/ Length of time/ No. of organisms required	Technique	The biotope/location samples are collected from	Multi or single biotope sampling	Other comments
Apfelbeck (1999)	All wetland types which have surface water	3 or 4	1 minute, or until 300 organisms are collected	NS	Near-shore emergent zone less than one metre in depth	Multi-bio. sampling	Sample locations chosen based on ease of accessibility and best representation of wetland
Gray *et al.* (2000)	Depressional, forested, emergent shrub-shrub	NS	30 minutes	The net is placed in water for a total of 30 minutes and every microhabitat is visited	NS	Multi-bio. sampling	Invertebrates are hand picked from substrate that could not be sampled by sweep net
Gernes and Helgen (2000)	Depressional, emergent vegetation	4	3 to 5 sweeps are carried out twice for each sample	The sweep net is dragged strongly through water column and downwards towards the bottom	Emergent vegetation zone less than one metre in depth	NS	Two samples make up one site sample
Helgen (2002)	Depressional, emergent, herbaceous, forest	3 (minimum)	20 sweeps	Each sweep is 0.5 metres in length	Near shore emergent zone less than one metre in depth	Multi-bio. sampling	Sweeps are distributed in proportion to the representation of biotope type, with emphasis on the 'productive biotopes'
Battle *et al.*(2001)	NS	3	5 sweeps	Each sweep is one metre in length	NS	NS	
Ell (2000)	Depressional (prairie potholes)	2	NS	A one metre area of wetland vegetation and benthos is 'jabbed' and then a sweep net is swept through the area twice	Emergent vegetation	Single bio. sampling	Each wetland visited twice
DiFranco and Stevenson (2000)	Depressional, riverine, lacustrine fringe	3	10 sweeps	Each sweep is one metre in length	Near shore emergent zone less than one metre in depth	Multi-bio. sampling	
Lillie (2000)	Palustrine	2	3 sweeps	Each sweep is one metre in length	Sample perimeter of wetland in water < 60cm	NS	All major plant communities are sampled

Table 2.5 Details of the sweep net protocols used in selected case studies and SASS5 (contd.)

Source	Type of wetland/ water body sampled	No. of samples taken from a wetland/river	No. of sweeps in each sample/ Length of time/ No. of organisms required	Technique	The biotope/location samples are collected from	Multi or single biotope sampling?	Other comments
Burton (2001)	Vernal pools, ponds	3 samples are taken at each biotope	NS	The sweep net is swept through water column at three depths, (surface, mid and bottom)	Benthos, aquatic veg. and water column	Muliti-bio. sampling	
Chessman *et al.* (2002) Project 1.	Coastal floodplain wetlands	6	10 sweeps	The net is held vertically and moved from the water surface to the bed ten times over a distance of ten metres	NS	NS	Each wetland visited three times
Chessman *et al.* (2002) Project 2.	NS	2 major biotopes are sampled at a minimum of 4 sites within a wetland	Each biotope is sampled for 2 minutes	In open water and submerged macrophytes- the net is moved in a zigzag trajectory between the water surface and bed. Among emergent macrophytes- the net is forced vigorously from the base of plants to the waters surface	Submerged macrophytes (which includes bare substrate), emergent or fringing macrophytes (sedges and rushes)	Multi-bio. sampling	Individual sample sites should be positioned in the north, south, east and west sectors of the wetland. Long linear wetlands may require subdivision and separate assessment. Separate sampling of submerged and emergent plant biotope is preferred
Hicks (2000)	Freshwater wetlands with standing water. Not for forested wetlands and wet meadows	Variable	1 sweep The timing of each sweep is kept constant	At a depth of 0.3 metres, the net is fully extended to the right-hand side of the body. Starting at the surface, the net is slowly swept down in a 180 degree arc that descends through the veg. and the water column and then upwards to complete the sweep on the left-hand side. The net is then brought up to the surface	NS	NS	Each sample site has three sampling stations (one for each replicate sample). The disadvantages of this technique are (Hicks 2000): • Samples are full of organic detritus, making it time-consuming to sort • Difficult to standardise the sampling
Dickens and Graham (2002)	Streams and rivers in South Africa	3 (Table 2.7)	Total 5 minutes + sampling 3metres of veg. (Table 2.7)	See Table 2.7	Stones, veg., gravel, sand and mud (Table 2.7)	Multi-bio. sampling	

Key: NS: Not specified bio.: biotope Veg.: vegetation

2.5.1.1 Deriving the optimum number of sweeps, activity traps or samples required to extract a representative sample from a wetland

A common procedure to investigate the optimum number of sweeps, activity traps (Section 2.5.2) or samples required to extract a representative invertebrate sample, is to produce a graph which has the number of sweeps/activity traps/samples along the x-axis and the cumulative number of taxa along the y-axis. This technique allows easy statistical identification of the effort required (Teels and Adamus 2002). However, there is doubt over the usefulness of this technique, as a plateau may not be reached even with many samples (Mackey *et al.* 1984).

Two studies that have used the above procedure concluded that:

- Three to five sweeps collects most of the taxa present in a single wetland biotope (Reber *et al.* 2000)
- Two to five sweeps collects eighty percent of taxa present in a particular aquatic plant bed (Friday 1987)

2.5.1.2 Multi-biotope versus single biotope sampling

The selection of wetland biotopes for sampling is important, and needs to be investigated before field data collection commences (Hicks 2000). There is still debate over whether single or multiple biotope sampling is best when using invertebrates (Karr and Chu 1999). Some authors consider a single biotope to be adequate, others insist that sampling multiple biotopes is essential (Table 2.5). SASS5 works best when there are a wide variety of biotopes, which includes riffles and rapids (Dickens and Graham 2002). In Table 2.6, the methods of multi-biotope and single biotope sampling have been explained, and advantages and disadvantages for each listed.

Table 2.6 Multi-biotope versus single biotope sampling

Type of Sampling	Advantages	Disadvantages
Multi-biotope Sampling: Samples are taken from a number of different biotopes within a wetland. Sampling effort is distributed proportionally among the biotope types present (Helgen 2002)	• The sample represents the complexity of the wetland (Helgen 2002) • Collects the majority of the invertebrate taxa present (Helgen 2002)	• Wetlands differ in biotope type, thus comparison between different wetlands may be inaccurate/difficult (Helgen 2002) • A time-constrained sweep net protocol may be difficult when biotopes are far apart (Helgen 2002) • Does not work well for collecting representative chironomid taxa (DiFranco and Stevenson 2000)
Single Biotope Sampling: Samples are taken from a single type of biotope within a wetland. The biotope type sampled is generally the type that is representative of the wetland (Teels and Adamus 2002)	• More time efficient as time is not spent calculating the amounts of each type of biotope needed to be sampled • Less statistical analysis is required as all samples are from the same biotope • Reduces variability in results based on sampling efficiency of different biotopes	• Less accurate representation of invertebrate community in a wetland (Brown 2000)

This debate exists because there are typically a variety of biotopes within a wetland (Butcher 2003), and each one supports a slightly different group of taxa. Statements made by a number of sources enforce the disproportionate distribution of invertebrates, for example:

- Vegetation is the primarily influence on aquatic macroinvertebrate assemblages (Battle *et al.* 2001)
- Emergent vegetation has greater diversity in comparison to open water areas that lack submerged vegetation (Olson *et al.* 1995)
- The water surface has a higher diversity of Hemiptera and Coleoptera in comparison to other aquatic biotopes (Hicks 2000)
- Vegetated biotopes have more chironomids (Driver 1977) and Coleoptera (Aitkin 1991)

Helgen (2002) does not agree with sampling every biotope, and states that the aim is not to measure every attribute of a wetland, but rather to find effective indicators of health that are expressed satisfactorily with a minimal amount of

sampling. Helgen (2002) recommend that, to assess a wetland's condition, either a sufficient number of biotopes are selected to make the sample representative of the wetland, or the area most sensitive to impairment should be sampled. Three recommendations for selecting which biotopes to sample are (Helgen 2002):

- Sample in the zone which has the greatest variety and production of aquatic macroinvertebrates
- Sample in the zone which is the most vulnerable or most effected by human disturbance
- Choose a biotope type that is representative of the wetland

Helgen (2002) considers it beneficial to keep samples from different biotopes separate, as this might assist with determining which biotopes show the most response to human disturbance. The SASS5 sampling protocol requires the sampling of three separate biotopes (Table 2.7). Table 2.7 gives details of how these three biotopes (referred to as habitats by SASS5) are sampled. The samples collected from the three biotopes are kept separate.

Table 2.7 The SASS5 sampling habitats

Habitat		
Stones	***Vegetation****	***Gravel, sand and mud***
Stones in and out of current are kicked, rubbed with hands or boots, or turned over against each other to dislodge invertebrates. For stones in current, this procedure is carried out for two minutes, for stones out of current for one minute	Marginal vegetation (both in current and out of current) is swept with a net for a total of two metres over one or more locations. All present vegetation types are sampled by forcefully pushing the sweep net back and forwards through the same area (Dickens and Graham 2002). Aquatic vegetation (both in current and out of current) is swept for one metre over the location. This is done by repeatedly pushing the sweep net through and against the vegetation under the water (Dickens and Graham 2002)	Gravel, sand and mud are disturbed by shuffling the feet whilst sweeping the sweep net over the area to collect the dislodged invertebrates (Dickens and Graham 2002). This is done for one minute

* The hand-picking of invertebrates from vegetation in and along the waterline of the river is also carried out for one minute. Any additional taxa found along the waterline are then added to the SASS5 score sheet (Appendix 2) under the biotope with which they are most closely associated (Dickens and Graham 2002)

2.5.2 Activity Trap Sampling

Table 2.8 gives details of the activity trap sampling method along with its advantages and disadvantages. Table 2.9 gives details on the use of activity traps in selected case studies.

Table 2.8 Details of activity trap sampling and its associated advantages and disadvantages

Method description:

Aquatic macroinvertebrates are trapped when they pass the neck of the funnel (see diagram below). It is recommended that these traps are left for between 24 and 48 hours so that both diurnal and nocturnal invertebrates can be collected (Gernes and Helgen 2000)

Advantages	Disadvantages
• Inexpensive and lightweight (Hanson *et al.* 2000) • Little training is needed to set out and collect apparatus (Helgen 2002) • Can be used in both still-water and slow-flowing wetlands (Adamus and Brandt 1990) • Traps motile wetland invertebrates better than sweep nets (Hilsenhoff 1987, DWAF 2004) • Gathers a representative sample (Brinkman and Duffy 1996) • Reduces the possibility of missing key invertebrates due to inappropriate time of visit (Adamus and Brandt 1990) (as traps are left in place for at least 12 hours) • Can obtain a sample from areas (e.g. with dense vegetation) which are difficult to sample by other means (Adamus and Brandt 1990) • The sample has only little or no vegetation so requires less processing time (Helgen 2002)	• A need to revisit the site after 24/ 48 hours • Only a limited range of invertebrates are collected as activity traps are deployed as submerged samplers, thus missing surface-associated taxa (Hanson *et al.* 2000) • Predators in the trap might eat other trapped invertebrates, thus altering the invertebrate composition of a sample (Helgen 2002) • Large numbers of tadpoles collected in the trap might be so dense that they exclude aquatic macroinvertebrates (Helgen 2002) • Decomposition of invertebrates may take place within 24 hours in the water (Sparling *et al.* 1995) • Dead organisms in a trap might attract predators into the trap, thus influencing taxon composition, however this has not been proven (Helgen 2002)

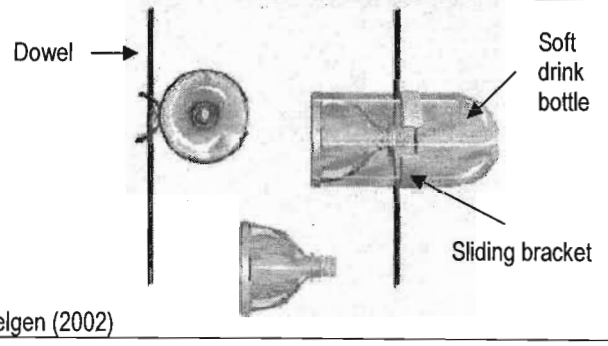
Other Comments:

There are a number of aspects concerning the use of activity traps which require research (Helgen 2002). These include:

- The size of the funnel and how it affects the size of the organisms, including vertebrate predators, that can enter the trap
- The volume of the trap and whether the trap is enclosed (glass, plastic) or open (screen)
- The consequence of declining oxygen levels in the enclosed traps
- The relationship between water temperature and efficiency of funnel traps for active aquatic macroinvertebrates

Activity trap construction:

A funnel is cut from the top end of a two litre soft drink bottle, and four grooves of 3mm x 55mm are cut into the rim of the funnel to attach it to the bottle. The trap is attached to a dowel by a sliding PVC bracket. The dowel is then pressed into the substrate



Source: After Helgen (2002)

Table 2.9 Details of activity trap protocols used in selected case studies

Source	No. of traps used	No. of hours left in place	Depth of activity trap placement	Location of trap placement	Other comments
Burnham (2000)	NS	24	NS	Placed ten metres apart	Wetland visited twice
Gernes and Helgen (2000)	10	12	In shallow water placed on bottom just under surface. In deeper water placed 15-20cm beneath surface	Placed from nearest shallow shore edge to inner side of the deepest emergent vegetation zone	Traps placed in water no deeper than one metre. Traps are back filled to leave no air bubbles inside, thus reducing activity of trapped predators
Gray *et al.* (2000)	10	NS	NS	15-30cm from edge of wetland	
Burton (2001)	NS	NS	NS	NS	Traps used as sweep nets underestimated certain invertebrates, e.g. Dytiscids and Corixids
Helgen (2002)	10	NS	In water up to one metre	Near shore area. Placed immediately on top of substrate or on vegetation mats growing on substrate	Predaceous coleopterans and hemipterans targeted

Key: NS: Not specified

2.5.3 Gerking Box Sampling

Table 2.10 gives details of the Gerking box sampling method along with its advantages and disadvantages. Table 2.11 gives details of the Gerking box used in the Mid-Atlantic Wetland Project.

Table 2.10 Details of the Gerking sampler used in the Mid-Atlantic Wetland Project and its associated advantages and disadvantages

Method description:

The sampler is lowered into the water until the bottom is pushed into the sediment leaving the open top projecting above the water's surface. Any vegetation beneath the Gerking sampler is usually cut at the mud-water interface and preserved for sampling. The screen door of the Gerking box is then slowly closed. The box is then shaken to allow the soil material to be sieved through the screen

Advantages	Disadvantages
• Permits the calculation of the number of invertebrates per unit area of wetland bottom (a quantitative estimate) (Karr and Chu 1999) • Invertebrates are captured from the benthos, vegetation and water column • Collects larger numbers of invertebrate taxa than activity traps, stovepipe samplers or artificial substrates (Brinkman and Duffy 1996) • As effective as sweep nets when sampling the water column (Kaminski and Murkin 1981)	• Cannot be used in areas with large amounts of woody vegetation • Is heavy and requires two people to carry it and three to four people to operate it (Helgen 2002) • A large amount of labour is require to process the sample

Description of the Gerking box sampler:

A 60cm tall aluminium box with a base approximately 40cm by 80cm. There is a sliding screen door at the bottom (Sparling *et al.* 2000)

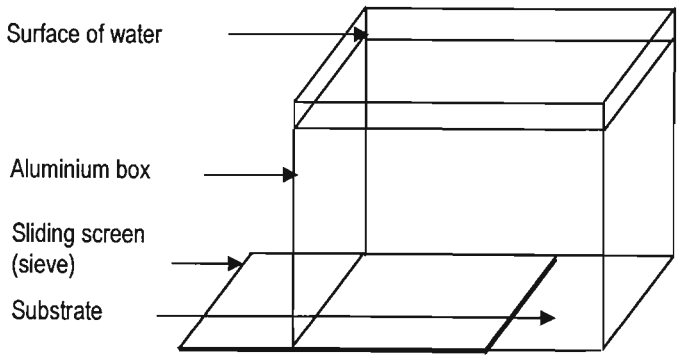


Table 2.11 Details of Gerking box sampler used in the Mid-Atlantic Wetland Project (Sparling *et al.* 2000)

Source	No. of times wetland sampled	Depth of Gerking box placement	Location of Gerking sampler placement	Other Comments
Mid-Atlantic Wetland Project	Four times a year (six week intervals)	Three depths • 15cm • 15 to 45cm • > 45cm	Sampling is done along transects following compass coordinates starting from the deepest part of each wetland. The transect coordinates are randomly selected	These samples are collected to determine invertebrate relative abundance, diversity, and relative biomass of each wetland

2.5.4 Stovepipe Sampler

Table 2.12 gives details of the stovepipe sampler method along with its advantages and disadvantages. Table 2.13 gives details of the stovepipe sampler used by the Maine Department of Environmental Protection (DiFranco and Stevenson 2000).

Table 2.12 Details of the stovepipe sampler used by the Maine Department of Environmental Protection and its associated advantages and disadvantages (DiFranco and Stevenson 2000)

Method description:

The sampler is pressed into the wetland substrate, and the contents of the sampler are then agitated (DiFranco and Stevenson 2000). Substrate, organic matter and invertebrates are then removed with a net and placed into a benthic sieve. The sieve is then rinsed so that only invertebrates remain

Advantages	Disadvantages
• Takes less time to collect samples compared to the Gerking box sampler • Good for capturing oligochaetes, benthic molluscs and chironomids (Helgen 2002) • Effective for wetlands which have shallow water or saturated conditions (Helgen 2002) • Quantitative results can be calculated based on the sampler's bottom area (Helgen 2002)	• Apparatus is heavy and expensive (Fredrickson and Reid 1988) • Does not capture motile taxa • Organisms have to be extracted from mud which is time consuming • Stovepipe samples contain less invertebrate taxa than Gerking box or sweep net samples (Hyvonen and Nummi 2000)

Other Comments:

The stovepipe sampler is similar to the Gerking box sampler, however has a much smaller bottom area. The stovepipe sampler can be made from a 20 litre bucket with the bottom removed

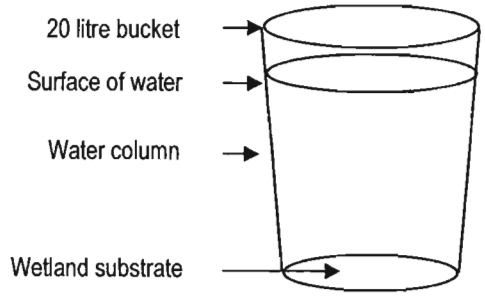


Table 2.13 Details of stovepipe sampling used by the Maine Department of Environmental Protection (DiFranco and Stevenson 2000)

Source	No. of replications at each wetland	Depth of placement	Placement of stovepipe sampler
Maine Department of Environmental Protection	3	Less than 0.5 metres	Near-shore emergent zone

2.5.5 Artificial Substrate Sampling

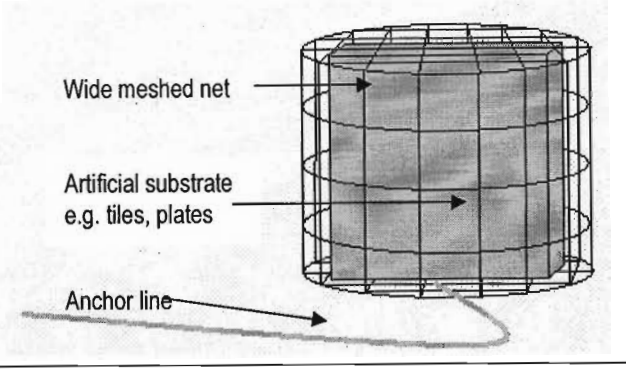
Table 2.14 gives details of the artificial substrate sampling method along with its advantages and disadvantages.

Table 2.14 Details and advantages and disadvantages of artificial substrate sampling

Method description:

Items which are made from hard substrate (such as plates, tiles or other objects which mimic the natural substrate), are placed in a wide mesh net bag and left in a wetland for a few weeks to allow colonisation by aquatic invertebrates. When the artificial substrate is removed, it is collected in a sweep net so organisms are not lost when it is taken from the water

Advantages	Disadvantages
• Can be used in areas difficult to sample by other means (Adamus and Brandt 1990) • Easy to deploy in the field • Clean sample with little debris • Collects chironomids, oligochaetes, molluscs and other epiphytic taxa	• Sampler must be left in-situ for several weeks • Possible loss or disturbance of sampler over time • As samples only epiphytic species, representativeness can be questioned (Adamus and Brandt 1990)



2.6 Environmental Data Required From a Wetland

As well as sampling aquatic macroinvertebrates, all case studies reviewed collect additional environmental data to determine wetland health. To accurately assess the health of a wetland, the degree of impairment should be determined by assessing the combination of disturbances present within both the landscape and the wetland, not from a single source of disturbance (Teels and Adamus 2002). Habitat and catchment/landscape assessment (Kentula *et al.* 1992, Hicks 2000), hydrological and chemical water quality measures (Adamus and Brandt 1990), as well as biological indices/metrics (Section 2.9.3) should be incorporated. Both landscape setting (hydrogeomorphic type) and geographical factors (climate, geologic setting) are expected to affect both water quality and biotic communities

(Detenbeck 2002). Several states in the U.S. have developed data forms to assist with biotope assessment (Table 2.15). Environmental data collected from a wetland often forms part of an IBI (Section 2.9.1).

Table 2.15 Additional methods used in selected case studies to assist with the determination of wetland health

State	Florida	Ohio	Maine	Minnesota	Montana	New England
Source	Brown (2000)	Gray *et al.* (2000)	DiFranco and Stevenson (2000)	Gernes and Helgen (2000)	Apfelbeck (1999)	Hick (2000)
Additional method	Land use Develop-ment Index	Ohio Rapid Assessment Method (ORAM)	Landscape disturbance score, with hydrologic alterations and physico-chemical data	Landscape, hydrologic alternation and chemistry data combined into a single score of human influence	Assessed landscape disturbance, hydrological alteration and chemistry data combined into a single score of human influence	A scoring system which has a variety of biotope indicators which are scored between zero and 20. Results (expressed as a %) can fall into one of four categories*

* Not impaired, somewhat impaired, moderately impaired, severely impaired

It is apparent that certain measurements are commonly required to help assess wetland impairment. These measurements have been divided into four sections: physico-chemical measurements, emergent vegetation measurements, riparian zone information and soil data.

2.6.1 Physico-chemical Measurements Taken From a Wetland

Certain water chemistry measurements and other water quality data are collected to identify what pollutants are present in a wetland, and thus assist with identifying the level of human impairment (Table 2.16).

Table 2.16 Summary of water chemistry and other parameters measured in selected case studies

Parameters	Source												
	Waterwatch, Water and Rivers Commission (1996)	Dickens and Graham (2002)	Butcher (2003)	Gernes and Helgen (2002)	Gernes and Helgen (2000)	Ramsar (2002)	Teels and Adamus (2002)	Hicks (2000)	Battle *et al.* (2001)	Burnham (2000)	Lillie (2000)	Burton (2001)	Adamus and Brandt (1990)
Water chemistry testing													
Temperature	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	
pH	✓	✓	✓		✓	✓		✓	✓	✓	✓		✓
Dissolved oxygen*	✓	✓	✓		✓	✓	✓	✓	✓			✓	✓
Dissolved carbon									✓				
Total organic carbon							✓		✓				✓
Nitrogen (general) Nitrates Ammonia Nitrites			✓	✓	✓	✓	✓		✓ ✓ ✓			✓	✓
Sulphate												✓	
Phosphorus				✓	✓	✓	✓		✓				✓
Chloride				✓	✓		✓					✓	
Chlorophyll				✓		✓							
Electrical conductivity	✓	✓	✓		✓	✓	✓			✓	✓		✓
Total suspended solids				✓	✓		✓						
Total dissolved solids			✓										
Calcium			✓		✓								
Metals			✓	✓			✓			✓			✓
Other parameters													
Depth								✓			✓	✓	✓
Colour			✓						✓		✓		
Turbidity		✓	✓	✓		✓					✓		
Current velocity		✓											✓

*Oxygen levels in wetlands fluctuate over the course of a day (being very high during the day and very low during the night), thus dissolved oxygen is not a reliable indicator of human impact (Hicks 2000)

2.6.2 Sample Vegetation Assessment

As wetland biotopes (particularly the composition and diversity of plant communities) influence the composition and diversity of macroinvertebrate communities (Davis *et al.* 1993, Mitsch and Gosselink 2000, Chessman *et al.* 2002), there is a need to employ a method of biotope assessment to be used in conjunction with a macroinvertebrate bioassessment protocol (Chessman *et al.* 2002). Chessman *et al* (2002) recommends that a rapid assessment of wetland biotopes, which focuses on vegetation, and assesses the composition and abundance of submerged, emergent and fringing vegetation should be incorporated into macroinvertebrate bioassessment protocols. Adamus and Brandt (1990), Danielson and Hoskins (2002) and Teels and Adamus (2002) all recommend that the general distribution of wetland vegetation, and ratio of open water to vegetated area, are important features to note at a study site. Biotope assessment can provide information on the quantity, quality and suitability of the sampling environment, and thus enable inferences to be made as to whether a low macroinvertebrate bioassessment score reflects a poor biotope condition or poor water quality, or both (Chessman *et al.* 2002, DWAF 2004).

The U.S. Fish and Wildlife Service developed a Habitat Evaluation Procedure (HEP) manual for wetlands in the early 1980s. This manual documents the quality and quantity of available habitat for various wetland biota (USFWS 1980), and is presently still in use (Bartoldus 1999).

Although the scope of this study does not allow for a detailed review of vegetation bioassessment methods, vegetation bioassessment has briefly been examined to identify potential methods for assessing sample site vegetation. Many methods of wetland bioassessment using plants have been developed and well documented by the U.S. EPA (e.g. Mack 2001, Simon *et al.* 2001). Just as macroinvertebrate bioassessments frequently use the multimetric approach to derive a wetland's condition (see Section 2.9), so to does plant bioassessment.

DeKeyser *et al.* (2003), Gernes and Helgen (1999) and Fennessy *et al.* (2002) have all used a three value scoring criteria to score their vegetation metrics:

5 If the metric being assessed is of a high quality or quantity or both

3 If the metric being assessed is of a fair quality or quantity or both

1 If the metric being assessed is of a poor quality or quantity or both

It depends on the individual metric as to whether quality, quantity or both of these aspects are assessed when scoring a metric.

2.6.3 Riparian Zone Information

The type of land cover that surrounds a wetland is generally one of the most dominant influences on wetland condition (Danielson and Hoskins 2002, van der Valk 2002). Lillie (2000) suggests that riparian vegetation cover type for an area 100 ft (~ 30m) adjacent to the wetland should be considered in an assessment. Teels and Adamus (2002) suggest that the land use in a radius of 300m around the wetland should be considered. They also recommend that greater weight should be given to land use in areas upstream or upslope of the wetland. Helgen (2002) mentions that when considering the influence of particular land uses, it is important to consider their distance from a wetland, the intervening slope, and the time period (current or recent or distant past) at which they occurred.

2.6.4 Soil Data

Teels and Adamus (2002) recommend the following soil data should be recorded in each vegetated community type:

- Thickness of organic layer
- Soil texture
- Colour as determined by a Munsell (1975) soil colour chart
- Presence of mottles and their size and colour as well as the presence of oxidised root channels

2.7 The Management of Samples Post Extraction from Wetland

Different studies use a variety of methods to deal with invertebrate samples following their collection from a water body. From reviewing SASS5 and selected case study sampling protocols, six different aspects have been identified:

1. The location where vegetation is removed from a sample (in the field or laboratory)
2. The method used to separate invertebrates from debris
3. The method used to preserve invertebrates in the field
4. Container used to transport sample from field to the laboratory
5. The method used to pick invertebrates from a sample
6. The site where invertebrates are picked from a sample (in the field or under laboratory conditions)

Methods used in different case studies and SASS5 are summarised in Table 2.17. For additional information on these aspects, see Table 2.18. Details for picking invertebrates using subsampling methods are given in Table 2.19. For recommendations made by Helgen (2002) concerning the management of samples post extraction from a wetland see Section 2.7.1.

Table 2.17 Management of samples post abstraction from a water body for a number of case studies

Source	Sampling method	Location of veg. removal	Method used to separate invertebrates from debris	Method of invertebrate preservation	Container for sample transport-ation	Picking method*	Site of sample picking	Other comments
Ell (2000)	Sweep net	Field	Sample placed in shallow pan and excess debris removed	Formalin 10%	Jar	S, F E if < 300 organisms	Lab	In the laboratory samples are washed through a 0.6mm sieve to remove further debris
Sparling *et al.* (2000)	Gerking box	Lab.	NS	NS	Plastic bag	NS	Lab	
DiFranco and Stevenson (2000)	Stove pipe	Field (large pieces)	Sediment and vegetation sieved into a bucket	NS	Plastic bag	E	Lab	Small pieces of vegetation left in sample to be taken to the laboratory
Burton (2001)	Sweep net	Field	Sample placed in white tray and invertebrates picked	Ethanol 95%	NS	T, F	Field	
Chessman *et al.* (2002)	Sweep net	Field	Sample placed in white tray and invertebrates picked	Alcohol 70%	NS	T	Field	Samples are stored at 5 °C until they are sorted
Hicks (2000)	Sweep net	Field	The contents of the sweep net are washed into a bucket and then washed through a 0.6mm brass sieve. The contents of the sieve are then placed in a white tray	Alcohol > 70%	Zip lock plastic bag	S, F E if < 100 organisms	Lab	To transport samples from field to laboratory, samples are placed in cooler containers with ice to prevent heating. On return from field, samples are stored in an air-conditioned laboratory for no longer than two weeks, before the invertebrates are sorted from the debris
Gernes and Helgen (2000)	Sweep net	Field	The Minnesota debris removal method, and the glass tray and light box method (Table 2.18)	Alcohol	NS	E	Lab	
Lillie (2000)	Sweep net	NS	NS	Ethanol	NS	S, F	Lab	
Dickens and Graham (2002)	Sweep net	Field	Sample placed in white tray and clean water added until sample is immersed	Cold preservation or 10% formalin	NS	T	Field	Three trays are used, one for each biotope. Samples are left standing to allow invertebrates to emerge from veg.

Key: NS: Not specified * A sample can be picked: E: Entirely T: Under timed conditions F: To a fixed count S: Using the 'square method' (Table 2.19)

Table 2.18 Additional information on the management of invertebrates following collection from a wetland

The site of vegetation removed from a sample (in the field or laboratory)

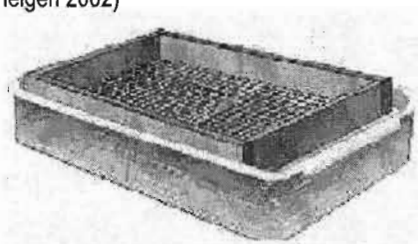
	Advantage	*Disadvantage*
Removal of vegetation in the field	Less material needs to be preserved (Helgen 2002)	Slows down field work considerably (Helgen 2002)

Methods used to separate invertebrates from debris

There are three identified methods to assist in the separation of invertebrates from debris:

The Minnesota debris removal method

The contents of the sweep net are placed on a wood frame, which has a piece of hardware cloth 30cm by 40cm stretched over it. This frame is set over a tray filled with water, and for ten minutes the contents of the sample is evenly spread over the hardware cloth. The invertebrates crawl through the hardware cloth and drop down into the water. The water and organisms are then poured through a 200 micron sieve to separate out the chironomids. This process is carried out twice for each sample (Helgen 2002)



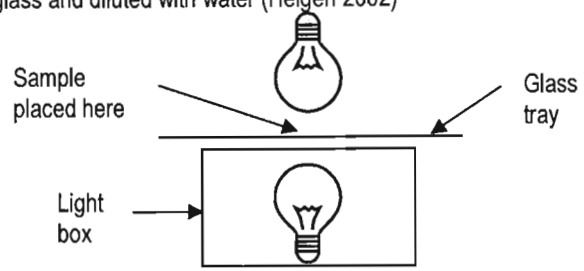
Source: Helgen (2002)

The white tray method

The white tray method is the most commonly used method to separate invertebrates from debris. The tray is made of plastic, which makes it durable and light weight to carry in the field. The colour white is preferred, as invertebrates are easier to identify when placed on a light background. This method is the most inexpensive

The glass tray and light box method

The glass tray and light box apparatus enables light to come from both above and below the sample, thus improving organism detectability. However, if there are large amounts of debris in the sample, small proportions of the sample should be placed over the glass and diluted with water (Helgen 2002)



Methods used to preserve invertebrates in the field

There are two methods to preserve samples, cold preservation and chemical preservation
Cold preservation: Sample is drained and placed into a plastic container. The sample is refrigerated at just above freezing (for up to 72 hours). For identification to take place, the sample is placed into a white tray with clean water and allowed to heat up to room temperate for 30 minutes (Dickens and Graham 2002)

Type of preservation	*Advantages*	*Disadvantages*
Cold preservation	• Organisms retain their colour • When brought back to ambient temperature, retain their behavioural characteristics	• Sample must be processed in a short time (Helgen 2002)
Chemical preservation	• Samples can be stored for a long time and can be re-examined at a later date	• Must be stored in a flame proof environment • Loss of colour and behavioural characteristics

Table 2.18 Additional information on the management of invertebrates following collection from a wetland (contd.)

Container used to transport sample from field to the laboratory		
There are two types of containers which can be used to transport samples from the field to the laboratory; plastic jars/bags and glass jars (Helgen 2002)		
Container	***Advantages***	***Disadvantages***
Plastic jar/bag	• Light weight and unbreakable	• Inappropriate for long term storage
Glass jar	• Good for long term storage • Allows one to observe the sample whilst contained	• Glass is breakable and heavy
Methods used to pick invertebrates from a sample		
Subsampling is the picking of a fraction of a sample under predetermined constraints (Table 2.19)		
Picking method	***Advantages***	***Disadvantages***
Picking the entire sample	• Gives maximum information on taxa present in a sample • Allows better estimation of relative abundances	• Much effort is required
Subsampling	• Picking effort is reduced when a lot of debris is present (Hicks 2000) • Appropriate when financial resources and time are limited (Hicks 2000) • Standardises the effort between samples	• Lower taxon diversity and more variability in data for metrics (Burton 2001) • In some cases subsampling procedure may be more time consuming than counting and removing all organisms (Hicks 2000) • Courtemanch (1996) does not consider subsampling to be a requirement, and subsampling is viewed as inappropriate by some scientists
The site where invertebrates are picked from a sample		
Picking location	***Advantages***	***Disadvantages***
Picking invertebrates in field	• Reduces time needed to pick samples in the laboratory	• Slows down field work • Weather and light conditions may result in an inconsistency in quality of picking (Helgen 2002)
Picking invertebrates in laboratory	• Picking samples under controlled conditions reduces the possibility of bias in results (Barbour *et al.* 1999)	• Time consuming

Table 2.19 Details for picking invertebrates using subsampling methods

Subsampling method	Under timed conditions	Picking to a fixed count	The 'Square method'
Explanation	A sample is picked for a predetermined length of time	A sample is picked until a predetermined number of organisms have been collected	The sample is placed into a white tray with approximately 24 squares (Lillie 2000) or 5cm by 5cm squares (Hicks 2000) marked out on the bottom. Randomly selected squares are then chosen (using a random numbers table) and invertebrates are picked from those squares. If there is a large amount of debris, the sample can be separated into multiple batches (Hicks 2000)
Disadvantages of methods	Picking may be biased towards larger, more visible invertebrates		Rare taxa are more likely to be missed
Source			
Chessman *et al.* (2002)	Pick invertebrates for 30 minutes	-	-
Dickens and Graham (2002)	Each of the three biotopes are viewed for 15 minutes and recorded separately on a SASS5 score sheet (Appendix 2)	-	-
Burton (2001)	Pick invertebrates for 30 minutes, after which the number of invertebrates is counted. Picking then continues until the next highest interval of 50 (with 150 being the maximum number of invertebrates collected)		-
Hicks (2000)	-	If the total number of organisms for each sample exceeds 100, the 'square' method is used. All squares are sorted fully until the total number of invertebrates are within ten percent of 100	
Lillie (2000)	-	First the fixed count method is used to pick 100 organisms from a sample. Then the 'square method' is employed, and the rest of the sample is processed except for the dominant taxa which appeared in the 100 organism count. The 'square method' is used so not to miss organisms in the sample	
Ell (2000)	-	If there are less than 300 organisms the whole sample is picked. If there are more, then the 'square method' is used. Randomly chosen squares are selected until 300 organisms have been identified	

2.7.1 Helgen (2002) Recommendations Concerning the Management of Samples Post Extraction from Wetland

Helgen (2002) recommends the following procedure:

- Preservation in the field is favoured so picking can take place under controlled laboratory conditions
- Picking the sample should be done in a glass tray over a light box with a magnifying lamp
- The sample should be picked into partly sorted categories to assist identification
- The entire sample is picked if feasible. 'When wetlands are sampled with repeatable, consistent sampling effort, picking the entire sample improves proportion metrics of total sample count, taxon diversity and variability in metrics, and allows better comparability among sites' (Doberstein *et al.* 2000 cited in Helgen 2002 p. 15).

2.8 What Level of Taxonomic Identification is Sufficient?

Many authors support the idea that macroinvertebrate identification to family level is sufficient for the biomonitoring of wetlands. In Australia, Britain and Europe, identification to family level has been used extensively in stream assessment (Adamus and Brandt 1990). Lillie (2000) is in favour of identifying organisms to family level, and states that it is adequate for developing wetland macroinvertebrate metrics. Hilsenhoff (1988) is in support of family level identification for stream biomonitoring. Adamus and Brandt (1990) believe that identification to family level for wetland biomonitoring is adequate in most cases, and consider a small loss of precision to be acceptable if there is a relative saving in time and cost. Fredrickson and Reid (1988) state that family level is usually adequate for management studies, however, they recommend that identification to genus level may be appropriate for research efforts. Identification to family level is also in keeping with the SASS5 protocol.

However, several authors believe that identification must be down to genus or species level (Table 2.20). Helgen (2002) found that when only identifying taxa to family level, there are often differences in sensitivities to factors causing impairment.

Table 2.20 The taxonomic level used for biomonitoring in selected studies

Source	Brown (2000)	Gray *et al.* (2000)	DiFranco and Stevenson (2000)	Gernes and Helgen (2000)	Ell (2000)	Sparling *et al.* (2000)	Lillie (2000)	Dickens and Graham (2002)
Level of Taxonomic identification	Genus, Species	Genus, Species	Genus	Family (Sphaeriidae) Genus, Species,	Genus, Species	Genus, Species	Family	Family

A study conducted by Chessman *et al.* (2002) investigated whether there is a significant difference between identifying taxa to family level and identifying taxa to species level when assessing the impairment of a wetland. Chessman (2002) assigned a score to each invertebrate at both species and family level. For both taxonomic levels, scores were given between 1 (most tolerant) and 100 (most sensitive) which reflected the sensitivities of those taxa to anthropogenic disturbances.

Chessman *et al.* (2002) found that species scores within a family sometimes varied widely, particularly for the families:

- Chironomidae (Diptera) with its three subfamilies (Chironominae, Orthocladiinae and Tanypodinae)
- Corixidae (Hemiptera)
- Chydoridae (Cladocera)
- Daphniidae (Cladocera)
- Cyprididae (Ostracoda)
- Centropagidae (Copepoda)

Chessman *et al.* (2000) produced correlations to display both the family and species scores against environmental variables. It was found that, in most cases,

where the correlation was significant, it was higher for species than for family. Family scores were somewhat less repeatable than species scores. Hilsenhoff (1988) also found this with studies done on rivers. Species scores were more variable than family scores among wetland trophic types; they were also more variable within each trophic type, most probably in response to factors other than trophic state (Adamus and Brandt 1990). Chessman *et al.* (2002) concluded that the lower accuracy of family level scoring is the result of there being a difference in sensitivities among co-family species when data are combined at the family level.

In conclusion, taxonomic identification can be to any level, but must be done consistently among samples. Genus and species level provide more accurate information on environmental relationships and sensitivity to impairment. However, identification to family level provides a higher degree of precision and repeatability among samples, requires less expertise and speeds up assessment results (Barbour *et al.* 1999).

2.9 Evaluation of Methods Used to Assess Wetland Health

The two most common methods used to calculate wetland health are indices of biological integrity (IBI) and multivariate analysis. This section explains what IBIs are, and reviews in detail various invertebrate metrics and indices used in selected case studies.

2.9.1 Indices of Biological Integrity (IBI)

An IBI combines multiple indicators of biological condition, called metrics, into an easy-to-understand index value (see Section 2.9.3 for wetland invertebrate metrics). The aim is to identify metrics, which are attributes that reveal an empirical and predictable change in value along a gradient of human disturbance (U.S. EPA 1998). The index value can be compared to reference values, and the state of a wetland's health can be derived (U.S. EPA 2002). One of the strengths of IBIs is that they summarise and present complex biological information in a

format that is easily communicated to managers and the public. Most people can relate more easily with faunal IBIs than with complex statistical calculations, or chemical and physical wetland functions (U.S. EPA 2002).

To construct an IBI, Hughes and Noss (1992), Karr and Chu (1999), Hicks (2000) and Helgen (2002) recommend that at least five (ideally 8 to 12), metrics should be defined and employed in a single study. Hughes and Noss (1992) recommend that each metric should reflect the quality of a different aspect of biota that responds in a different way to disturbances in wetlands. Karr and Chu (1999) and Helgen (2002) state that metrics which represent patterns or responses to changes in the physical, chemical and biological integrity of the wetland should be chosen. Karr *et al.* (1997) recommend that the performance of each attribute should be evaluated by assessing how well it does the following:

- Increase or decrease along a gradient of human disturbance
- Separate the least from the most impaired sites
- Provide similar values for similarly impaired sites

The process for selecting metrics for use in an IBI requires the testing of a large set of biological attributes, and then selecting the ones that are most sensitive to various aspects of human disturbance (Teels and Adamus 2002). In Teels and Adamus (2002), two graphical techniques and statistical tests that can evaluate the performance of attributes are documented. These methods are summarised below:

- Create bar graphs or box plots that show means or medians and variances of a particular attribute at sites believed to be least and most impaired (Mundahl and Simon 1999). The degree of separation between the least and most impaired sites can then form the basis for retaining or discarding the attribute for subsequent analysis. The statistical significance of the separation can be confirmed using standard statistical tests such as *t* tests
- Compare attribute data from both extreme sites as well as all sites across the spectrum of human disturbance. For the comparison, the disturbance gradient

can be based on either a single or multivariable human disturbance. The relationship can be expressed graphically or by a comparison of correlation coefficients (i.e. Pearson's correlation coefficient). From the results of the correlation, attributes can either be retained or eliminated

2.9.2 Multivariate Analysis

Multivariate analysis is statistical analysis that examines many variables simultaneously. Data from communities of organisms are multivariate because there are several taxa that respond differently to a number of environmental factors (Helgen 2002). Reynoldson (1997) and others found that accuracy and precision estimates were higher when multivariate techniques were used for data analysis, compared with multimetric (IBI) methods.

2.9.3 IBI Invertebrate Metrics and Indices Used By SASS5 and Selected Case Studies

This section details a number of invertebrate metrics and indices used in seven different case studies. These case studies were selected based on the availability of relevant documentation. There is a wide range of metrics/indices employed, with relatively few case studies using the same metrics/indices (Table 2.21). A possible explanation for this is that wetland bioassessment, using aquatic macroinvertebrates, is a new field of research, thus little testing has been carried out on which metrics/indices are the most appropriate.

Table 2.21 Invertebrate metrics/indices used in selected case studies

Metric/index	Source						
	Helgen (2002)	Gernes and Helgen (2000)	Battle *et al.* (2001)	Lillie (2000)	Dickens and Graham (2002)	Apfelbeck (1999)	Hicks (2000)
Taxon diversity	✓D	✓D	✓D	✓D	✓D	✓D	✓D
No. of intolerant taxa % of intolerant taxa (EOT)	✓D	✓*D					 ✓D
% contribution of dominant taxa						✓I	✓V
Functional feeding groups	✓?						✓$
OET count (Odonata, Ephemeroptera, Trichoptera)						✓D	✓D
No. of Chironomidae taxa % of Chironomidae taxa		✓D				✓D ✓I	 ✓I
No. of Hirudinea		✓D				✓D	
Total abundance				✓?		✓D	
% of tolerant taxa	✓I	✓I					
Condition or health of individual invertebrates	✓D						
% Corixidae of all coleopterans and hemipterans from activity trap samples (Corixidae ratio)		✓I					
% Hirudinea, Erpobdella in activity trap and sweep net samples of the total sweep net abundance		✓I					
% of three most dominant taxa of total invertebrate abundance in sweep net sample		✓I					
No. of genera of Ephemeroptera and Trichoptera plus presence of Sphaeriidae and Odonata		✓D					
No. of genera of Odonata and Zygoptera		✓D					
No. of mollusc taxa		✓D					
No. of coleopteran taxa			✓?				
% of Isopoda of the composition			✓D				
% of scavengers of the trophic			✓D				
Abundance of selected organisms				✓^?			
Non-insect diversity				✓?			
% Trichoptera				✓D			
Invertebrates given tolerance scores					✓D		
Average score per taxa (ASPT) (Section 2.9.3.2)					✓D		
No. of Crustacea/Mollusca taxa % of Crustacea/Mollusca taxa						✓D ✓D	
Community taxa similarity index							✓D
Community trophic similarity index							✓D
Family biotic index							✓I
Invertebrate community index							✓D

Key: I: Increase with impairment V: Variable with impairment D: Decrease with impairment ?: Not given

^ Molluscs, Annelids, Anostraca, Zygoptera, Culicidae, Stratiomyidae

* *Laucorrhinia*, *Libella*, *Tanytarus*, *Procladius*, *Triaenode*, *Oecetis*

$ % Predators D; % Corixidae I; % Lestidae I

2.9.3.1 Taxon diversity

Taxon diversity is the number of different types of invertebrates collected from a sampling effort. The diversity of taxa commonly declines as human disturbance increases (Barbour *et al.* 1996, Hicks 2000, Chessman *et al.* 2002); however, there can be exceptions. Taxon diversity may increase if forested canopy areas are opened up over wetlands that were previously covered and less productive before forest clearing (King *et al.* 2000), or if low nutrient wetlands receive some nutrient input (Rader and Richardson 1994). In a study by Growns *et al.* (1992), it was concluded that moderately nutrient-enriched wetlands had significantly higher taxon diversity and higher numbers of rare taxa. This was explained by increased productivity at moderate levels of enrichment, which leads to increased areas of macrophytes and thus greater variety in the system, allowing more taxa to coexist. However, taxon diversity decreases with excessive nutrient enrichment, as increased amounts of algae results in a lack of oxygen, which affects most invertebrates (Growns *et al.* 1992). Livestock, fertilizer application, ineffective wastewater treatment systems and urban runoff (Adamus and Brandt 1990) can all cause eutrophication in a wetland.

Taxon diversity can be plotted against the level of impairment to produce a response curve. If the response is a peak of taxon diversity at the intermediate level of impairment, this metric may not be purposeful (Helgen 2002). All seven studies examined used this metric.

2.9.3.2 Tolerance metrics / biotic indices

Aquatic macroinvertebrate tolerance of pollution is based on the fact that every organism requires a certain range of chemical and physical conditions in which to survive. Some organisms can survive in a wide range of conditions and are considered tolerant organisms. Others are sensitive to changes, can only survive specific conditions, and are referred to as intolerant organisms (Waterwatch, Water and Rivers Commission 1996). Over time, taxa assemblages have evolved that are capable of withstanding or rapidly recovering from most natural

perturbations. However, changes in the chemical, physical and biological environment caused by humans can cause a loss of intolerant organisms (Teels and Adamus 2002).

The NEFWIBP (Hicks 2000), SASS5 (Dickens and Graham 2002) and Chessman *et al.* (2002) all use biotic indices to grade a water body's health (Appendix 3). Biotic indices give scores (values) to certain invertebrates that reflect their tolerance of impairment (Hicks 2000). See Figure 2.1 for examples of high scoring and low scoring SASS5 taxa.

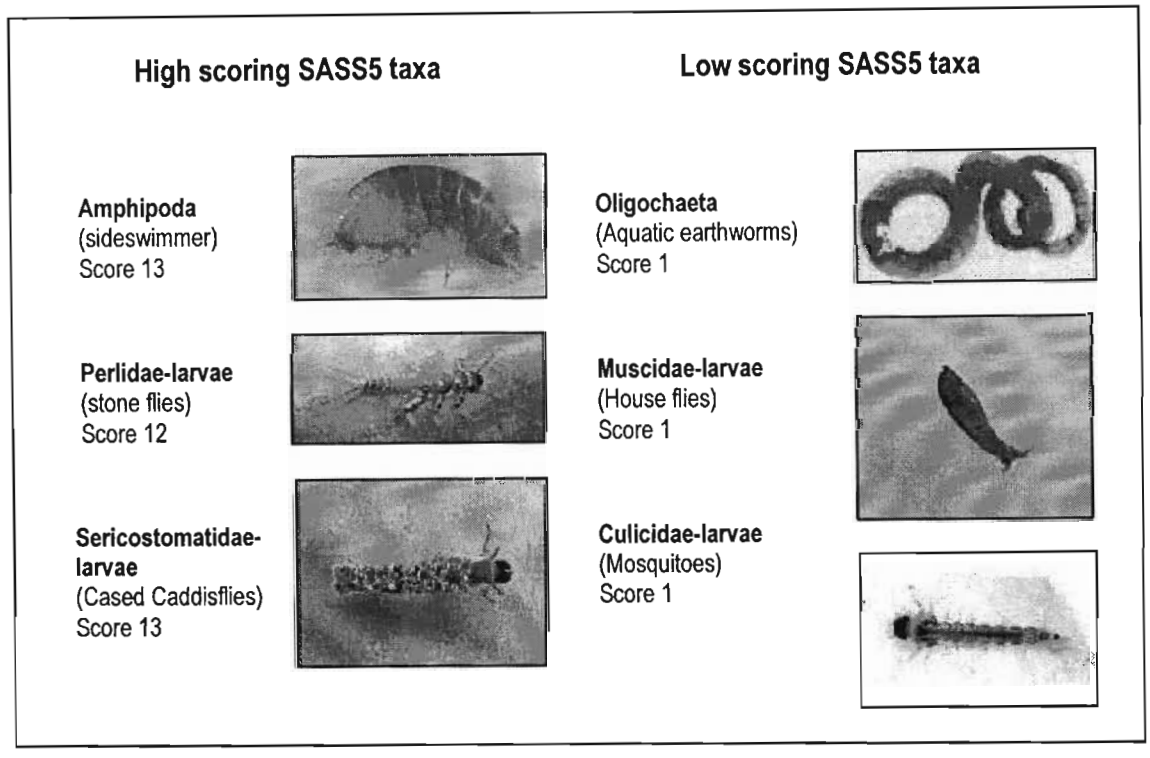


Figure 2.1 Selected examples of SASS5 high and low scoring invertebrates

Intolerant taxa are more likely to disappear with impaired conditions (Karr and Chu 1999). This may be due to the direct effects of water chemistry (Palmer *et al.* 1994, Suren 1994) or more tolerant organisms out-competing less tolerant organisms (Waterwatch, Water and Rivers Commission 1996, Hicks 2000). To determine which taxa are intolerant of impaired conditions, an examination of the

data sets to identify which taxa become disproportionately represented under greater impairment should be carried out (Helgen 2002).

With increased impairment, the number of tolerant taxa may not change, but the relative abundance of tolerant organisms tends to increase (Helgen 2002). This can be measured by the proportion of known taxa or by the proportion represented by the dominant two or three taxa of the total sample count (Helgen 2002).

Gernes and Helgen (1999) believe that the mere presence of intolerant taxa is a strong indicator of good biological conditions. Karr *et al.* (1986) warns that endangered and threatened taxa must not be considered intolerant, as their low numbers might be due to factors other than human disturbance. Teels and Adamus (2002) believe that presence alone of tolerant taxa reveals little about the biological conditions of a wetland, as tolerant taxa inhabit a range of conditions.

To calculate the water quality of rivers in South Africa, Chutter (1998) uses three metrics (Table 2.21). The calculation procedure for SASS is explained below:

1. Each taxon is assigned a predetermined quality value (for SASS5 this value lies between 1 and 15* (Dickens and Graham 2002)) (Appendix 2)
2. The total SASS score for a site is calculated by summing the taxon scores from the three different biotopes
3. The average score per taxon (ASPT) is then calculated by dividing the total SASS score by the number of taxa found
4. Using both the SASS score and ASPT, reference is then made to Table 2.22, to determine the water quality and habitat diversity of a river

*A score of one is assigned to macroinvertebrates which have a high tolerance of pollution. A score of 15 is assigned to those which cannot survive in highly polluted water, thus their presence is indicative of good water quality

Table 2.22 SASS5 score water quality guidelines

SASS5 Score	ASPT	Water quality (WQ) and habitat diversity (HD)
>100	>6	WQ Natural HD High
<100	>6	WQ Natural HD Reduced
>100	<6	Borderline case between WQ natural and some deterioration in WQ
50-100	<6	Some deterioration in WQ
<50	variable	Major deterioration in WQ

Source: Chutter (1998)

2.9.3.3 Dominant taxa

Hicks (2000) believes that a wetland, which has five or more family groups that appear in relatively high numbers, can indicate a balanced, healthy community, thus indicating good water quality. Gernes and Helgen (2000) used the percentage of the top three dominant taxa as one of their metrics. The highest score (indicating good water quality) was assigned when the total percentage of the top three dominant taxa was less than 35 percent. The higher the percentage of the top three dominant taxa, the greater the degree of water quality impairment.

2.9.3.4 Functional feeding groups/ trophic index

Functional feeding groups relate to the dietary specialisation of invertebrates. They include herbivores, predators, omnivores and detritivores. It is expected that the density of predators will decrease as impairment increases (Kerans and Karr 1994, Hicks 2000). However, surface water level can also influence the number of predators present, and in most natural wetlands water level fluctuates widely (Wissinger 1999).

Predatory taxa are more dominant in systems with longer hydroperiods, and this has an influence on the lower trophic level structure (Detenbeck 2002). Kerans and Karr (1994), in a study on streams, found that the proportion of grazers and predators decreased, and the proportion of filter feeders increased with increased human disturbance. One explanation for this is that collectors and filter feeders have a broader range of acceptable food material in comparison to predators and

grazers (Barbour *et al.* 1999). Within streams, specialised feeders, such as scrapers, piercers and shredders, are the most sensitive organisms, and are thought to be well represented in healthy systems (Barbour *et al.* 1999). Battle *et al.* (2001) found that a higher percentage of scavengers were found in reference wetlands. Hicks (2000) developed an index called Community Trophic Similarity Index. This index measures trophic (feeding group) similarities between impaired and reference wetlands.

Although a few taxonomic groups can be entirely classed in one functional feeding group (e.g. Odonata as predators (Helgen 2002)), most groups belong to a variety of feeding groups. Thus, Helgen (2002) recommends that each organism be identified to the lowest taxonomic level to achieve a more accurate result (Section 2.8). More work is required to test the attributes of functional feeding groups against degrees of human disturbance in wetlands (Helgen 2002).

2.9.3.5 The percent and number of selected taxa

The use of a small number of selected taxa for rapid assessment techniques has been suggested, but the correlative work showing the effectiveness of this method has not been performed (Butcher 2003). However, many case studies employ metrics that are specific to certain groups of taxa (Table 2.21). For example, both Apfelbeck (1999) and Hicks (2000) have used the orders of Odonata, Ephemeroptera and Trichoptera, which are commonly used in stream biomonitoring. These orders are known to contain many intolerant families. Wetlands that have a high number of families within these orders, are more likely to be in a less impaired state than those wetlands that have few or none (Hicks 2000). Metrics using Chironomidae are also widely used. This family has many members highly tolerant of impairment (Hicks 2000). Battle *et al.* (2001), who also uses specific taxa, found that oligochaetes, molluscs and Hirudinea were more common in degraded sites compared to reference sites, although their numbers were highly variable. However, Gernes and Helgen (2000) predict that

molluscs and Hirudinea numbers would decrease with increased human impairment.

Odonata and molluscs have long life spans in comparison to other aquatic invertebrates (Helgen 2002). These organisms are very suitable for metrics, as they experience longer exposure to wetland conditions. Invertebrates with shorter life cycles (e.g. Culicidae), will respond to human disturbances faster than long-lived organisms, however, they may recover more rapidly, either from recolonisation by adult insects, or by resting eggs (Helgen 2002).

Hicks (2000) presents the predicted responses of certain organisms to increased impairment (Table 2.23). Although not all taxa present in Table 2.23 have their own metrics, it is interesting to compare which families of taxa increase or decrease with human impairment.

Table 2.23 Responses of invertebrate families to increased impairment

Expected responses of invertebrates to increased impairment					
Oligochaeta	Rise	Decapoda	Variable	Hemiptera-Homoptera	Rise
Hirudinea	Rise	Hydrachnida	Decline	Coleoptera	Rise
Gastropoda	Rise	Ephemeroptera	Decline	Trichoptera	Decline
Pelecypoda	Rise	Lestidae (Odonata)	Rise	Lepidoptera	Decline
Isopoda	Rise	Other Odonata	Decline	Chironomidae	Rise
Amphipoda	Variable	Megaloptera	Decline	Other Diptera	Variable

Source: Hicks (2000)

Impairment as a result of increased nutrients can affect the relative ratio of certain groups of invertebrates (e.g. taxa feeding groups) (Table 2.24). Adamus and Brandt (1990) have documented a list of invertebrates that indicate eutrophic conditions in wetlands. The organisms are identified to species level. It is noted that the listed species may also occur in wetlands that are not eutrophic, although usually in smaller proportion relative to other species.

Table 2.24 Relative ratio response of taxa groups which indicate increased nutrients

Source	Ratio
Waterwatch, Water and Rivers Commission (1996)	Increase in grazers to other feeding groups
Hicks (2000)	Increase in Corixidae to other orders (as these organisms are particularity tolerant of low dissolved oxygen in waters)
Hicks (2000)	Increase in Lestidae to other orders
Teels and Adamus (2002)	Increase in herbivorous taxa to non-herbivorous taxa
Ferrington and Crisp (1989)	Increase in Tubificids to aquatic insects
Ferrington and Crisp (1989)	Increase in chironomid subfamilies, Tanypodinae and/or Chironominae to the subfamily Orthocladiinae
Ferrington and Crisp (1989)	Increase in cladocerans to rotifers

2.9.3.6 Abundance

Apfelbeck (1999) and Lillie (2000) only use the metric of abundance (total number of organisms). Apfelbeck (1999) and Hicks (2000) state that density will decrease with increased impairment. However, Karr and Chu (1999) and Hicks (2000) both consider this metric to be a poor candidate for a multimetric index. This is because total abundance varies too much, even when human disturbance is minimal, and it is difficult to measure and score (Karr and Chu 1999).

2.9.3.7 Condition or health of individual invertebrates

Helgen (2002) uses the condition or health of individual invertebrates as a metric. This metric looks at the deformities and lesions of invertebrates. This attribute has been used successfully in conjunction with the fish IBI, however, little work has been done in this area on invertebrates. It has been found that this attribute can only be used effectively in highly contaminated areas (Helgen 2002). A study conducted by Cushman and Goyert (1984) has looked at malformations in chironomid mouthparts. This metric was used to indicate impairments to water bodies from sedimentation, contamination and eutrophication (Warwick 1980).

2.9.3.8 Community taxa similarity index

This index determines the degree of similarity between two sites based on a comparison of dominant families or groups. Only those families or groups that exceed four percent of the total composition at either the reference or the impacted site are used. The absolute differences of the two sites is summed, the

total divided by two, and then subtracted from 100 to derive a percentage. Results range from zero (no similarity) to 100 (complete similarity). A table of assessment criteria is then consulted to make a qualitative interpretation of the taxonomic similarity between the reference and impaired wetland (Hicks 2000).

2.9.3.9 Community trophic similarity index

This index measures the trophic similarity of an impaired wetland to a reference wetland (Hicks 2000). The calculation procedure for this index is the same as described above for the community taxa similarity index.

2.9.3.10 Family biotic index

This index summarises and averages the various eutrophic tolerance values of the families that make up the aquatic community. Each family is assigned a value from zero to ten, with zero being intolerant, and ten being the most tolerant (Hicks 2000). For each of the wetlands sampled, the number of organisms within each family is multiplied by its given value. These values are summed, and the total is then divided by the total number of invertebrates found for each wetland. The family biotic index assessment criteria table is then consulted to interpret the results. The general trend is that the index increases in positive correlation with eutrophication (Hicks 2000).

2.9.3.11 Invertebrate community indices

The Invertebrate Community Index (ICI) integrates the results of between eight and twelve of the metrics or indices used for a study. For each metric/index, the reference wetland score is divided by the impaired wetland score and then multiplied by 100. The derived percentage is then compared to a biological condition scoring criterion and assigned a score. The scores of all the metrics/indices are then summed, and a table of assessment criteria is then consulted to interpret the results (Hicks 2000).

3. METHODOLOGY

3.1 Selecting Wetlands to Sample

Only permanent, palustrine sedge-dominated wetlands in the midlands of KwaZulu-Natal, South Africa were sampled. This wetland type was selected for several reasons. Firstly, sedge-dominated wetlands have been selected, as they are a common type of wetland in the midlands of KwaZulu-Natal. Secondly, vegetation type may affect aquatic macroinvertebrate composition (Lillie 2000), thus using only sedge-dominated wetlands reduces the variability due to vegetation type. Thirdly, only permanent wetland areas have been examined, as a wetland's hydroperiod is likely to affect aquatic macroinvertebrate composition (Detenbeck 2002). Finally, sampling was confined to the KwaZulu-Natal midlands, as it is important that all wetlands have similar climatic and geographical characteristics to reduce bias (Karr and Chu 1999).

Both impacted and reference wetlands needed to be identified, so comparison could be made between the aquatic macroinvertebrate assemblages of polluted and non-polluted wetlands. Only wetlands that had a dairy effluent input and an obvious flow direction were considered for use as impacted sites (Section 3.4.1). Potentially suitable wetlands were identified by:

- Discussion with persons familiar with the area (Gernes and Helgen 2000)
- Examining land use activities adjacent to wetland (Teels and Adamus 2002)
- Carrying out biotope assessment (Hicks 2000)

A number of attributes needed to be assessed before site selection took place:

- Size of wetland
- Vegetation type (Lillie 2000)
- Altitude and topography
- Permanence of water (Detenbeck 2002)

These attributes were determined by inspection of 1:50 000 maps, consultation and site visits. Where possible, sites that had similar attributes were selected.

3.2 Selecting Which Biotope to Sample

Although there are arguments for carrying out multi-biotope sampling, the advantages of single biotope sampling out weigh these; for time and cost efficiency as well as complexity of analysis (Section 2.5.1.2). Although the SASS5 sampling protocol requires the sampling of three different biotopes (Table 2.7), sampling for this study only took place in emergent sedge vegetated areas. This biotope was selected for two key reasons. Firstly, in the majority of case studies, sampling was performed in emergent vegetated areas (Table 2.5). Secondly, emergent vegetation has greater taxon diversity in comparison to open water areas that lack submerged vegetation (Olson *et al.* 1995). Sedge vegetated areas were favoured, as i) they provide a dense vegetation in which aquatic macroinvertebrates can hide, ii) this type of vegetation provides a large surface area on which aquatic macroinvertebrates can live, and iii) it is easier to drag a sweep net through sedges in comparison to *Typha* and *Phragmites* (Dickens pers. comm.). The robust stems of *Typha* and *Phragmites* also provide a biotope in which invertebrates are highly accessible to predators, resulting in elevated levels of predation (Dickens pers. comm.).

3.3 Pilot Study

A pilot study was performed to derive a suitable technique for collecting a representative sample of invertebrates from a wetland. As recommended by Helgen (2002), two different sampling methods were tested to evaluate their efficacy at invertebrate collection. These methods were sweep net and activity trap sampling. Sweep netting was chosen as, i) it is the technique performed in the SASS5 protocol, ii) it is the most commonly used sampling technique in wetland biomonitoring (Table 2.4), iii) it is relatively economical, quick and simple to employ, iv) the apparatus required is readily available. The SASS5 sampling

protocol was not used in this study, due to two biological differences between rivers and wetlands:

- Physical structure
  - Palustrine wetlands do not have the biotopes that are sampled using the SASS5 protocol (Table 2.7)
  - A wetland's vegetation structure is typically much denser than that of rivers (Apfelbeck 1999, Hicks 2000). Because of this, it is not practical to use the SASS5 time and area constraints (Table 2.7)
- Flow velocity
  - Palustrine wetlands generally lack a clearly defined channel, thus sampling in and out of flow (as specified by the SASS5 sampling protocol) is not possible. The flow of water in a wetland is generally slow to nonexistent, thus trapping organisms in the sweep net without a current would also be difficult

A range of sweep intensities were tested to determine the number of sweeps required to collect an accurate representative sample of invertebrates. Activity traps were tested to determine whether they were required in addition to sweep netting to achieve a representative sample, as activity traps may collect additional taxa (e.g. Hirudinea, Coleoptera and Hemiptera) (Hilsenhoff 1987). Other methods, such as artificial substrate and the Gerking box were not tested due to time and funding limitations, and equipment availability. All sweep intensity testing and activity trap sampling was carried out in a single wetland, in order to keep other variables constant. This wetland was a reference wetland, so sample composition was not affected by pollution. The selected wetland had a large amount of suitable sampling biotope (as defined above). This was preferable, as the need for a sample site biotope assessment metric would have complicated and potentially compromised the accuracy of the results.

3.3.1 Sweep Intensity Testing

There are three different sweep techniques, however, the 'fixed number of sweeps' method is used in the majority of case studies (Table 2.5). The number of sweeps used varies between case studies:

- Three to five sweeps per sample (Gernes and Helgen 2000)
- Two to five sweeps per sample (Friday 1987)
- Three to five sweeps per sample (Reber *et al.* 2000)

For this study, an intensity range of two to six sweeps was tested. This range was chosen as it covers all sweep intensities recommended in the above case studies. Due to time and resource constraints, sampling of each intensity was carried out ten times, thus a total of 50 samples were collected. Each sample was collected with consistently repeated efforts as recommended by Helgen (2002).

From reviewing the different sweep netting techniques, the Gernes and Helgen's (2000) method was chosen for a number of reasons:

- The technique is rapid and simple, and therefore, easily repeatable
- The technique incorporates samples from the water's surface, water column and substrate (Hicks 2000, Burton 2001, Chessman *et al.* 2002)

Description of Gernes and Helgen's (2000) sweep technique

Starting at the surface, the sweep net is dragged strongly through the water column at a 45 degree angle, until the net arrives at the bottom of the wetland. The net is then brought up to the surface with the mouth facing the surface (this is a description of a single sweep).

3.3.1.1 Procedure for sweep intensity sampling

For every sweep sample, the following steps were followed:

Step one — Appropriate vegetation site was located and approached with as little disturbance as possible

Step two	One sweep sample was performed
Step three	Sweep net contents were placed into a white tray filled with water and large pieces of vegetation removed
Step four	Contents of white tray were washed though sieve (mesh size 1mm)* and organisms placed in a labelled plastic jar with clean water
Step five	Samples were stored in a cooler box until transported to the laboratory
Step six	Once in laboratory, the contents of the jars were emptied into a white tray and all large pieces of debris were removed, ensuring that all organisms were removed first. The sample was then left to stand for five minutes*
Step seven	All organisms in each sample were identified to family level using identification books and recorded on a SASS5 score sheet*
Step eight	Unidentifiable organisms were preserved in 70% ethanol and were later identified by an entomologist. A selection of identified organisms were examined by an entomologist for auditing purposes* (as suggested by Hicks 2000, Chessmen *et al.* 2002 and Stuckenberg, pers. comm.)

* In keeping with SASS5 protocol

The procedure of removing large amounts of vegetation in the field (as recommended by DiFranco and Stevenson 2000) was included, as i) there was likely to be a large amount of debris present in the sample (Helgen 2002), and ii) the majority of studies employ vegetation removal in the field (Table 2.17). Not all vegetation was removed in the field, as this would have taken too much time and, if rushed, organisms might have been missed.

3.3.2 Activity Traps

Activity traps were constructed following Helgen (2002).The activity traps were placed at four depths in sedge vegetated areas:

- At the waters surface (with bottle mouth half filled with water) in order to sample surface dwelling invertebrates (Hanson *et al.* 2000)
- Just below surface (Gernes and Helgen 2000)
- Between 15-20cm below the surface (Gernes and Helgen 2000)
- Resting on the substrate (Helgen 2002)

Sampling at each of the four depths was carried out twenty times, thus a total of 80 samples were collected. This sample size was chosen to allow for statistically valid analysis, given the time and resource constraints. Traps were left for in place for 24 hours, as this allowed both nocturnal and diurnal invertebrates to be collected (Helgen 2002).

3.3.2.1 Procedure for activity trap sampling

For each activity trap, the following steps were followed:

Step one	Appropriate vegetation site was located and approached with as little disturbance as possible
Step two	Physico-chemical measurements were taken (Section 3.3.3) and relevant information recorded (Appendix 4)
Step three	Activity traps were placed at specified depth
Step four	After 24 hours, the activity traps were collected and their contents placed in a plastic jar
Step five	Samples were stored in a cooler box until transported to the laboratory
Step six	Once in laboratory, the contents of the jars were emptied into a white tray and then left to stand for five minutes*
Step seven	All organisms in each sample were identified to family level using identification books and recorded on a SASS5 score sheet*

Step eight	Unidentifiable organisms were preserved in 70% ethanol and were later identified by an entomologist. A selection of identified organisms were examined by an entomologist for auditing purposes* (as suggested by Hicks 2000, Chessmen *et al.* 2002 and Stuckenberg, pers. comm.)

* In keeping with SASS5 protocol

Activity traps collected very little debris, thus no debris removal was necessary. Traps were initially back-filled with water, in order to remove any air bubbles inside. This was done to reduce the activity of collected predators (Gernes and Helgen 2000). However, following the first sampling session where many frogs and tadpoles were trapped and suffocated, back filling was abandoned and air was purposely left in the traps.

3.3.3 General Information Concerning Sweep Net and Activity Trap Testing

- Although preservation in the field is popular (Table 2.17), live samples were taken from the field and identified in the laboratory. This was because: it is much easier to find and identify the majority of invertebrates when alive; it is in keeping with the SASS5 protocol; and it is unnecessary to kill organisms which are easy to identify and release
- The white tray method was chosen to separate the organisms from debris for a number of reasons: it is inexpensive (in comparison to other methods, see Table 2.18); it is quick and simple to use; it is a popular choice with other studies (Table 2.17); and it is in keeping with the SASS5 protocol. There was a large amount of debris in some of the samples, therefore, the glass tray and light box was not used, as it is not effective under these conditions (Helgen 2002)
- The chemical measurements taken were pH, temperature, electrical conductivity and total dissolved solids (TDS). The tests were performed to ensure water quality was similar at all sample points in the wetland, and to confirm that the wetland was not polluted. Chemical testing took place within

every square metre containing an activity trap. For sweep net sampling, areas which were sampled had the chemical measurements taken prior to invertebrate collection

- Every sweep net sample and placement of activity trap was conducted in a different location, as disturbance may have caused bias in other samples
- Although there is debate over what level of taxonomic identity is sufficient for the biomonitoring of wetlands (Section 2.8), for this study, all invertebrates were primarily identified to family level. The key reason for this was that it is in keeping with the SASS5 protocol
- Identification of organisms took place under laboratory conditions (as recommended by Barbour *et al.*1996 and Helgen 2002) as varying light conditions in the field can cause a bias in the results. Although the SASS5 identification procedure requires the identification of organisms in the field, this study's aim required the accurate detection of all organisms within a sample, thus identification took place under controlled conditions. The contents of the jars were placed in a white tray and allowed to settle. After adding water to the tray (if required), the invertebrates were removed with a pipette or tweezers and examined
- Picking of the entire sample was carried out, as this study required the accurate detection of all organisms within a sample. Although subsampling may be appropriate when there is limited time and financial resources available, it can be more time consuming than identifying all organisms with in sample (Hicks 2000). Other authors who support the picking of the entire sample include Courtemanch (1996), Doberstein *et al.* (2000 cited in Helgen 2002) and Helgen (2002) (Table 2.17)

3.3.4 Analysis of Pilot Study Results

Results from both sweep net and activity trap sampling were analysed to determine the sampling protocol to be used in the main study.

3.3.4.1 Sweep net intensity derivation

Analysis of Variance (ANOVA) was used to investigate the effect of sweep intensity on taxon diversity. This procedure tested whether the mean number of taxa collected for different sweep intensities differ significantly

Four statistical procedures were carried out to determine the effect of sweep intensity on taxon composition:

- **Nonmetric Multi-Dimensional Scaling (NMDS) ordination** (Ter Braak and Šmilauer 2003) This method provided a visual representation that depicted the degree of similarity within and between the taxon compositions of the different sweep intensities
- **Multi-Response Permutation Procedures (MRPP)** This procedure tested for significant differences in taxon composition among sweep intensities, and identified if any sweep intensity differed from other intensities
- **Indicator Species Analysis (ISA) (McCune and Mefford 1999) and the Monte Carlo Test of Significance** These procedures identified which taxa are statistically more likely to occur in a specific sweep intensity. Indicator values were calculated using the Dufrene and Legendre (1997) method, which is based on combining the values of relative abundance and relative frequency

3.3.4.2 Activity trap invertebrate diversity and composition analysis

Two statistical procedures were carried out using activity trap collected data:

- **ANOVA** This procedure tested whether the mean number of taxa collected from the four different depth locations differed significantly
- **NMDS ordination** (Ter Braak and Šmilauer 2003) This method provided a visual representation that depicted the degree of similarity within and between the taxon compositions of the different depth locations

3.3.4.3 Activity trap and sweep net invertebrate composition comparison

Three statistical procedures were used to compare taxon composition between sweep netting and activity trapping:

- **MRPP** This procedure tested whether the taxon composition of the sweep net samples was significantly different from that of the activity trap samples
- **ISA and the Monte Carlo Test of significance** These procedures identified if there were any taxa favoured by one or the other of the two methods

3.4 Main Study

The main study tests the applicability of SASS5 using the sampling technique derived from the pilot study.

3.4.1 Sweep Net Sampling

In the impaired wetlands, the dairy effluent discharge point was located, and the direction of flow determined. Knowledge of the direction of flow was required to study the effects of point source pollution, as sampling took place in three specific areas (Hicks 2000):

- At a point before (upstream) of the effluent discharge point
- At the effluent discharge point
- At a point downstream of the effluent discharge point

In each of the three areas, the derived method was carried out twice at two separate locations (Figure 3.1), thus a total of six samples were collected from each impacted wetland. Six samples were also collected from each of the reference wetlands, however, the only influence on sample location for these wetlands was the availability of suitable sampling biotope. The collection of six samples from each wetland was sufficient to obtain a representative aquatic macroinvertebrate sample (Table 2.5).

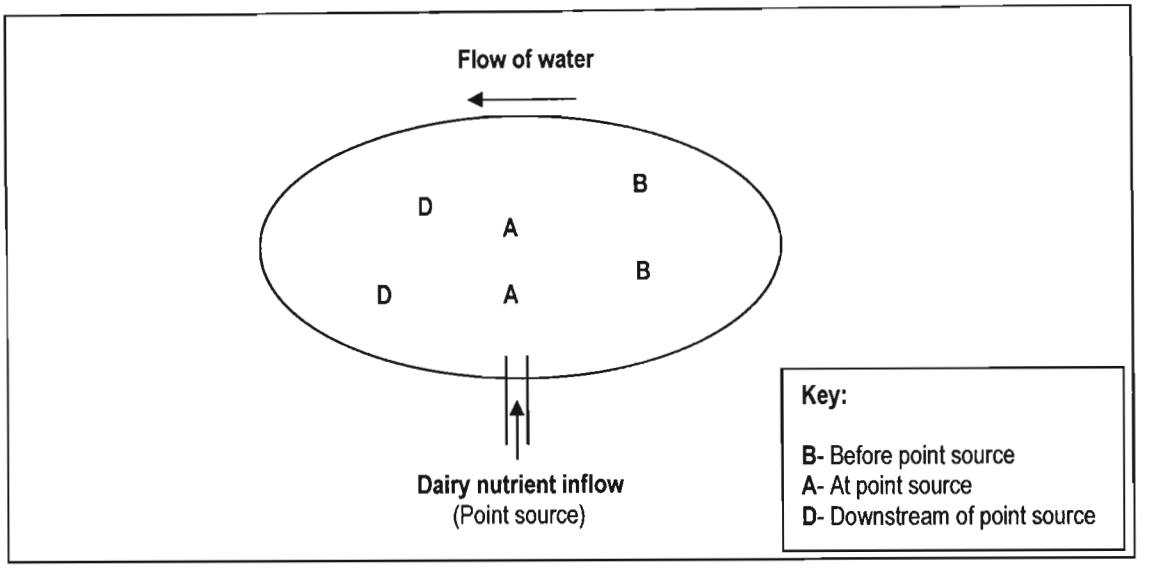


Figure 3.1 Sample points in a point source impacted wetland

Apfelbeck (1999) recommends that 75% of wetlands in a study should be reference sites, and the remaining 25% should be impaired. For this project, three impaired sites and a further four reference sites were sampled (a minimum of three reference sites is recommended by Hicks 2000 and Parker 2002). Thus, a total of seven wetlands (24 samples) were selected to allow for the intensive sampling of each site.

3.4.2 Activity Trap Sampling

If it was found that activity traps collect different invertebrates compared to sweep nets, ten traps would be placed in suitable biotope within each reference wetland, and thirty traps would be placed in each impacted wetland (ten above, ten at and ten below the point nutrient output) (Gernes and Helgen 2000, Gray *et al.* 2000, Helgen 2002).

3.4.3 Environmental Measurements

Three pre-designed forms were developed to collect additional environmental measurements from each wetland- the macrohabitat score sheet (Appendix 5), the physico-chemical data sheet (Appendix 6) and the sample biotope and detection assessment (SBDA) index (Appendix 7). These forms allowed for

hydrological and catchment/landscape assessment (similar to the HGM approach (Hauer and Smith 1998)), physico-chemical measurements (Section 2.6.1) and biotope assessment (as recommended by Chessman *et al.* 2002). The Macrohabitat and physico-chemical forms were derived from Hicks (2000) and Helgen and Gernes (2002). For each reference wetland, one macrohabitat score sheet and SBDA index was completed. For the impacted sites, three macrohabitat score sheets and SBDA index were completed (one for each specific sampling area (Section 3.4.1)). A physico-chemical data sheet was completed for each sample point.

3.4.3.1 Macrohabitat data sheet

The macrohabitat score sheet has six key aspects (Appendix 5). These six aspects cover a range of disturbances within both the landscape and the wetland (as suggested by Teels and Adamus 2002, see Section 2.6). For each question there is a choice of four answers which range in degree of disturbance, and have relative scores (taken from Gernes and Helgen 2002). For five of the aspects, there is a list of features that must be assessed at each site. This list has been produced to provide guidance when scoring a site, and to allow for comparison of environmental factors between wetlands. To help assess the degree of impairment of an impacted wetland, the dairy farmer was asked to provide extra information regarding their dairy (Appendix 5). For each site, a diagram detailing the surrounding land use was also produced to allow for comparison of sites. The environmental scores for each aspect were combined to derive the degree of impairment of each site (as recommended by Teels and Adamus 2002, see Section 2.6). The wetlands were then ranked in order of impairment.

When scoring the six questions, the distance, type of alteration, land use and severity of impact were considered. Also considered was the time period (current, recent or distant past) at which alteration/land use change occurred (Section 2.6.3). Greater weight was given to alterations/land use changes in areas

upstream or upslope of the site (as recommended by Teels and Adamus 2002 and van der Valk 2002).

3.4.3.2 Physico-chemical data sheet

The physico-chemical measurements collected were nitrogen, ammonia, phosphorus, TDS, electrical conductivity, pH and temperature (Appendix 6). Nitrogen, ammonia and phosphorus testing was performed to obtain an indication of the level of dairy effluent reaching a wetland, as these compounds are some of the main pollutants released from a dairy (DWAF 1996). Other activities that can cause increased levels of these three compounds are fertilizer application, ineffective wastewater treatment systems, fossil fuel and urban runoff (Adamus and Brandt 1990, DWAF 1996). Thus, the presence of these sources were taken into account during the analysis of results. Electrical conductivity and TDS tests were performed to obtain a general indication of differences in water quality between sites (DWAF 1996). Temperature and turbidity measurements were also taken at each sample point, to assist with the analysis of other chemical data.

Temperature, pH and electrical conductivity measurements were taken, as the SASS5 sampling protocol requires these measurements to assist with determining water quality. These three measurements are also included in the majority of studies examined (Table 2.16). Although many case study sampling protocols require the measurement of dissolved oxygen, this parameter was not measured, as oxygen levels in wetlands naturally fluctuate over the course of a day. Thus dissolved oxygen is not a good indicator of an impact (Hicks 2000).

3.4.3.3 Sample biotope and detection assessment (SBDA)

As it is possible that poor sample site biotope, or excess sediment and/or vegetation in a sample may result in a low macroinvertebrate SASS5 score that may not be indicative of actual wetland health (and thus the applicability of SASS5 not being fairly assessed), a sample biotope and detection assessment

(SBDA) index was derived (as recommended by USFWS 1980, Chessman *et al.* 2002, DWAF 2004).

The SBDA index has five metrics (Appendix 7). As the composition and diversity of plant communities in wetlands influence the composition and diversity of macroinvertebrate communities (Davis *et al.* 1993, Mitsch and Gosselink 2000, Battle *et al.* 2001, Chessman *et al.* 2002), two metrics relate to availability of suitable biotope (metric 1 and 2). Three metrics are concerned with the detectability of organisms once a sample has been placed in the white tray for identification.

Aspects of the metrics and scoring procedure were derived from DeKeyser *et al.* (2003), Fennessy *et al.* (2002), McMillan (2002) and Gernes and Helgen (1999). Each metric was assigned one of three possible scores, which were based on biotope quality and detectability of invertebrates:

5 good
3 fair
1 poor

The results of the SBDA were reviewed in conjunction with the macroinvertebrate SASS5 scores, to assist with providing a more accurate interpretation of the results, thus allowing the researcher to assess whether the derived SASS5 scores were influenced by poor quality of sampling biotope, or detectability of macroinvertebrates in a sample. This index has been specifically developed for this study, and may not be appropriate for application elsewhere.

3.4.4 Analysis of Main Study Results

Results of the following analysis will collectively determine if the current SASS5 total score and calculation procedure is potentially applicable to palustrine wetlands in South Africa.

3.4.4.1 Initial processing of raw data

Macroinvertebrates

For each individual sample point, the macroinvertebrates were identified and the SASS5 tolerance scores assigned. These scores were then summed, and processed using the SASS5 calculation procedure (Section 2.9.3.2).

Macrohabitat score sheet

For each individual sample site, the score of each aspect on the macrohabitat score sheet were summed.

Sample biotope and detection assessment (SBDA) index

For each sample site, the score of each metric in the SBDA index was summed, and then classed into one of three possible classifications:

5-11	poor biotope / detectability
12-18	fair biotope / detectability
19-25	good biotope / detectability

3.4.4.2 Identifying variations in taxon composition between reference and impacted wetlands

Correspondence analysis (CA) was used to reveal patterns in the variation of taxon composition among and between wetlands.

3.4.4.2 Variations in SASS5 score, ASPT and taxon diversity between wetlands

Two statistical procedures were used to identify if there was a significant variation in SASS5 scores, ASPT and taxon diversity between wetlands:

- **ANOVA** was used to identify if there was a significant variation in SASS5 scores, ASPT and taxon diversity between the wetlands
- **Tukey *Post hoc* honest statistical difference (HSD) test** was used to identify how SASS5 scores, ASPT and taxon diversity differed between wetlands

3.4.4.3 SASS5 score, ASPT and taxon diversity wetland ranking

Tukey *Post hoc* HDS test was used to rank SASS5 score, ASPT and taxon diversity for all the sampled wetlands

3.4.4.4 Examining the relationship between SASS5 score, ASPT, physico-chemical results and macrohabitat score rankings

Spearman's rank correlation coefficient was used to identify if SASS5 score, ASPT, physico-chemical results and macrohabitat score rankings for each wetland were significantly related to one another

3.4.4.5 Potential indicator taxa

Canonical Correspondence analysis (CCA) using taxon composition data and physico-chemical data was first used to identify which chemical variable had the greatest effect on taxon composition. CCA was again used to identify which taxa had the greatest response to the identified chemical variable

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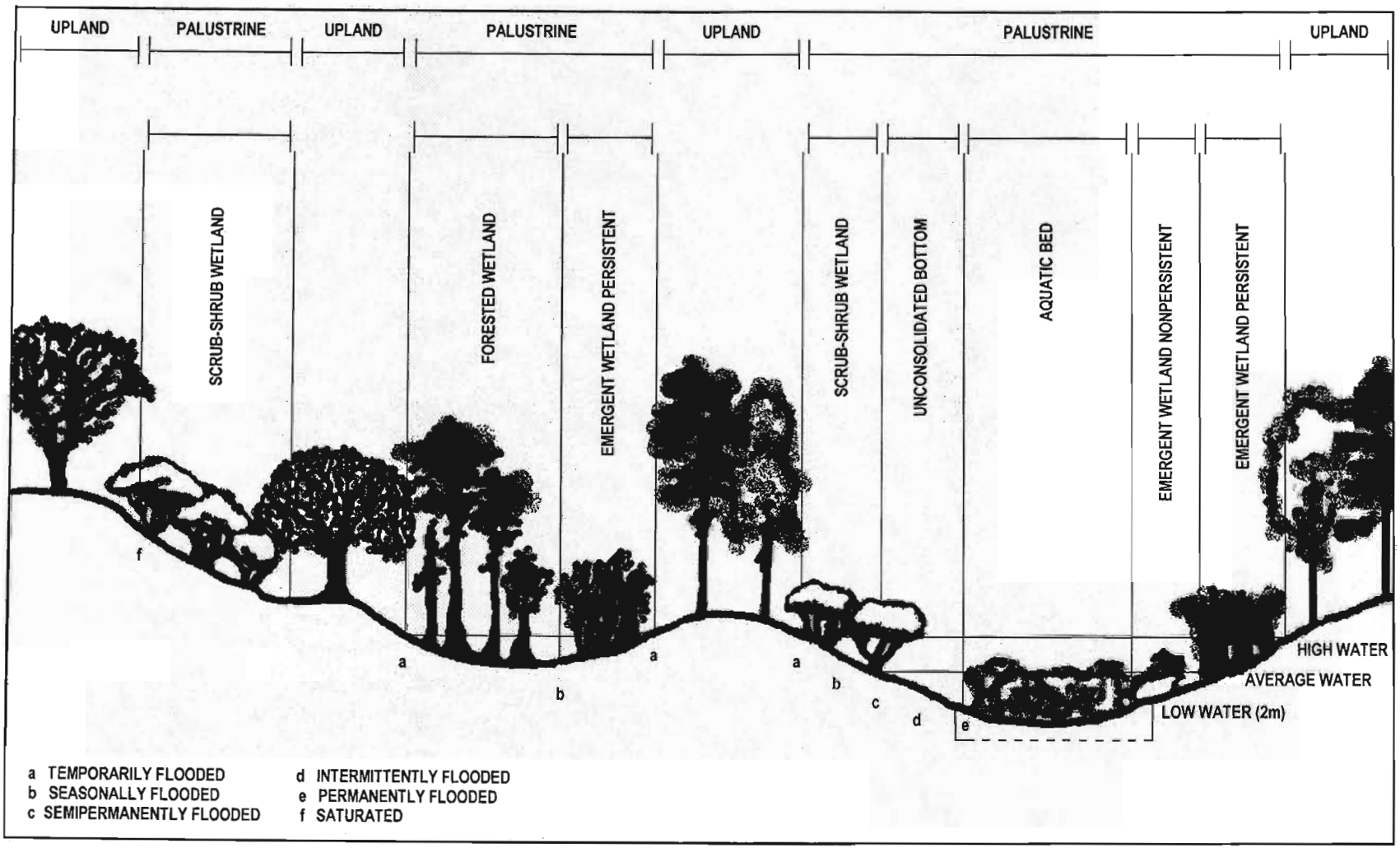
APPENDIX 1. Features and Examples of a Palustrine System



After Cowardin *et al.* (1979 p. 14)

APPENDIX 2. SASS5 Score Sheet

SASS Version 5 Score Sheet

Date: / /200__

Collector:.....

Grid Reference: WGS-84 | Cape datum

S: ° ' , E: ° ' , "

S (dd): E (dd):

Altitude:

Site code:.....

River:.....

Site description:.....

Temp:.....°C pH:.....

DO:.....mg/l Cond:.....mS/m

Biotopes sampled:

SIC .....Time.....minutes

Rate SIC Biotope - 1 2 3 4 5

SOOC.....Time.....minutes

Rate SOOC Biotope - 1 2 3 4 5

Average size of stones.....cm

Bedrock.....Rate Biotope - 1 2 3 4 5

Aquatic veg'n Rate biotope- 1 2 3 4 5

MvegIC..... Rate biotope- 1 2 3 4 5

MvegOOC.... Rate biotope- 1 2 3 4 5

Gravel.....Rate Biotope - 1 2 3 4 5

Sand.....Rate Biotope - 1 2 3 4 5

Mud.....Rate Biotope - 1 2 3 4 5

Hand picking/Visual observation.....

Flow: Low/Medium/High/Flood

Turbidity: Low/Medium/High

Riparian land use:

Disturbance in the river: eg. sandwinning, cattle drinking point, floods etc.

Observations: eg. smell and colour of water, petroleum, dead fish, etc.

Taxon		S	Veg	GSM	TOT
PORIFERA	5				
COELENTERATA	1				
TURBELLARIA	3				
ANNELIDA					
Oligochaeta	1				
Leeches	3				
CRUSTACEA					
Amphipoda	13				
Potamonautidae*	3				
Atyidae	8				
Palaemonidae	10				
HYDRACARINA	8				
PLECOPTERA					
Notonemouridae	14				
Perlidae	12				
EPHEMEROPTERA					
Baetidae 1sp	4				
Baetidae 2 sp	6				
Baetidae > 2 sp	12				
Caenidae	6				
Ephemeridae	15				
Heptageniidae	13				
Leptophlebiidae	9				
Oligoneuridae	15				
Polymitarcyidae	10				
Prosopistomatidae	15				
Teloganodidae SWC	12				
Tricorythidae	9				
ODONATA					
Calopterygidae ST,T	10				
Chlorocyphidae	10				
Chlorolestidae	8				
Coenagrionidae	4				
Lestidae	8				
Platycnemidae	10				
Protoneuridae	8				
Aeshnidae	8				
Corduliidae	8				
Gomphidae	6				
Libellulidae	4				
LEPIDOPTERA					
Pyralidae	12				

Taxon		S	Veg	GSM	TOT
HEMIPTERA					
Belostomatidae*	3				
Corixidae*	3				
Gerridae*	5				
Hydrometridae*	6				
Naucoridae*	7				
Nepidae*	3				
Notonectidae*	3				
Pleidae*	4				
Veliidae/M...veliidae*	5				
MEGALOPTERA					
Corydalidae	8				
Sialidae	6				
TRICHOPTERA					
Dipseudopsidae	10				
Ecnomidae	8				
Hydropsychidae 1 sp	4				
Hydropsychidae 2 sp	6				
Hydropsychidae > 2 sp	12				
Philopotamidae	10				
Polycentropodidae	12				
Psychomyiidae/Xiphocentronidae	8				
Cased caddis:					
Barbarochthonidae SWC	13				
Calamoceratidae ST	11				
Glossosomatidae SWC	11				
Hydroptilidae	6				
Hydrosalpingidae SWC	15				
Lepidostomatidae	10				
Leptoceridae	6				
Petrothrincidae SWC	11				
Pisuliidae	10				
Sericostomatidae SWC	13				
COLEOPTERA					
Dytiscidae*	5				
Elmidae/Dryopidae*	8				
Gyrinidae*	5				
Haliplidae*	5				
Helodidae	12				
Hydraenidae*	8				
Hydrophilidae*	5				
Limnichidae	10				
Psephenidae	10				

Taxon		S	Veg	GSM	TOT
DIPTERA					
Athericidae	10				
Blepharoceridae	15				
Ceratopogonidae	5				
Chironomidae	2				
Culicidae*	1				
Dixidae*	10				
Empididae	6				
Ephydridae	3				
Muscidae	1				
Psychodidae	1				
Simuliidae	5				
Syrphidae*	1				
Tabanidae	5				
Tipulidae	5				
GASTROPODA					
Ancylidae	6				
Bulininae*	3				
Hydrobiidae*	3				
Lymnaeidae*	3				
Physidae*	3				
Planorbinae*	3				
Thiaridae*	3				
Viviparidae* ST	5				
PELECYPODA					
Corbiculidae	5				
Sphaeriidae	3				
Unionidae	6				
SASS Score					
No. of Taxa					
ASPT					

Sample collection effort exceeds method? .....

Other biota including juveniles:

Comments:

Procedure Kick SIC & bedrock for 2 mins, max. 5 mins. Kick SOOC & bedrock for 1 min. Sweep marginal vegetation (IC & OOC) for 2m total and aquatic veg 1m². Stir & sweep gravel, sand, mud for 1 min total. * = airbreathers

Hand picking & visual observation for 1 min - record in biotope where found. Score for 15 mins/biotope but stop if no new taxa seen after 5 mins.

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

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

APPENDIX 3. Tolerance Score Comparison Table

Order	Family	Functional Trophic Group	NEFWIBP Hicks (2000) 1= intolerant taxa 10=most tolerant taxa	SASS5 Dickens and Graham (2002) 15= intolerant taxa 1=most tolerant taxa	SWAMP Chessman *et al.* (2002) 100= intolerant taxa 1=most tolerant taxa
TURBELLARIA	Dugesiidae	GC	4	3	58
ANNELIDA	Oligochaeta	GC	8.5	1	43
HIRUDINEA	Hirudinidae	PR	7	3	-
GASTROPODA	Viviparidae	SC	6	5	-
	Ancylidae	SC	10	6	53
	Hydrobiidae	SC	7	3	100
	Lymnaeidae	SC	6	3	23
	Physidae	SC	8	3	38
	Planorbidae	SC	8	3	62
PELECYPODA	Sphaeriidae	FC	8	3	27
	Unionidae	FC	2	6	41
AMPHIPODA	Crangonyctidae	GC	9	13	-
	Gammaridae	GC	4	13	-
	Talitridae	GC	6	13	-
DECAPODA	Potamonautidae	-	-	3	62
HYDRACARINA	All mites	PR	2	8	1
EPHEMEROPTERA	Baetidae	GC/SC	4	4	57
	Caenidae	GC	7	6	56
	Leptophlebiidae	GC	2	9	-
	Tricorythidae	GC	4	9	-
ODONATA	Aeshnidae	PR	3	8	58
	Calopterygidae	PR	6	10	-
	Coenagrionidae	PR	9	4	55
	Corduliidae	PR	5	8	61
	Gomphidae	PR	2	6	-
	Lestidae	PR	9	8	50
	Libellulidae	PR	5	4	69
HEMIPTERA	Belostomatidae	PR	8	3	-
	Corixidae	PI-H	8	3	20
	Gerridae	PR	8	5	-
	Naucoridae	PR	8	7	-
	Nepidae	PR	8	3	-
	Notonectidae	PR	8	3	39
	Pleidae	-	-	4	31
	Veliidae	-	-	5	68
	Unknown/Other	PR	8	-	-
COLEOPTERA	Dytiscidae	PR	8	5	49
	Elmidae	GC/SH	4	8	-
	Gyrinidae	PR	4	5	-
	Haliplidae	SH/PI-H	8	5	60
	Hydraenidae	PR	8	8	-
	Hydrophilidae	GC	8	5	55
MEGALOPTERA	Corydalidae	PR	2	8	-
	Sialidae	PR	4	6	-

TRICHOPTERA	Hydroptilidae	GC/SC	6	6	66
	Leptoceridae	OM	4	6	47
	Polycentropodidae	FC/PR	6	12	-
LEPIDOPTERA	Pyralidae	SH	2	12	69
DIPTERA	Ceratopogonidae	PR	6	5	60
	Chironomidae (red)	OM	8	2	43
	Chironomidae (other)	OM	6	2	-
	Culicidae	PR	10	1	66
	Dixidae	GC	1	10	-
	Empididae	PR	6	6	-
	Ephydridae	OM	10	3	60
	Simuliidae	-	-	5	70
	Tabanidae	PR	6	5	58
	Tipulidae	OM	5	5	1
	Unknown/Other	-	7	-	-

Key:

* 0 given to intolerant taxa, 10 given to most tolerant taxa

Trophic Group		**Trophic Group**	
Scrapers	SC	Gathering Collectors	GC
Filtering Collectors	FC	Predators	PR
Shredders	SH	Omnivores	OM
Piercers-Herbivores	PI-H		

APPENDIX 4. Pilot Study Activity Trap Data Sheet

Karkloof Nature Reserve			
Wetland Name: *Melmoth*		**Date**: / /2003	
GPS co-ordinates: **Longitude**: *30°16'E*	**Latitude**: *29°18'S*	**Altitude**: *1595m*	
Weather: Rain ☐	Showers ☐	Overcast ☐	Clear/ Sunny ☐

SAMPLE			
Sample Number:	**Depth:** At surface ☐ 15cm/20cm below surface ☐ Just below surface ☐ On substrate ☐		
Electrical conductivity: mS/m	**Total Dissolved Solids:** ppm	**Temperature:** °C	**pH:**
Time in:	**Time out:**	**Comments:**	

SAMPLE			
Sample Number:	**Depth:** At surface ☐ 15cm/20cm below surface ☐ Just below surface ☐ On substrate ☐		
Electrical conductivity: mS/m	**Total Dissolved Solids:** ppm	**Temperature:** °C	**pH:**
Time in:	**Time out:**	**Comments:**	

SAMPLE			
Sample Number:	**Depth:** At surface ☐ 15cm/20cm below surface ☐ Just below surface ☐ On substrate ☐		
Electrical conductivity: mS/m	**Total Dissolved Solids:** ppm	**Temperature:** °C	**pH:**
Time in:	**Time out:**	**Comments:**	

APPENDIX 5. Macrohabitat Score Sheet

MACROHABITAT SCORE SHEET			
Wetland Name:	Date:	Photographs taken N S E W sample point ☐	Est. area of wetland:
Weather: Rain ☐ Showers ☐ Overcast ☐ Clear/ Sunny ☐		Additional comments:	
% of different cover: Sedge/ Grass % Phragmites % Typha % Algae % Open water % Other _____ %			

MACROHABITAT SCORING		
1) HABITAT ALTERATION- IMMEDIATE LANDSCAPE (within the wetland-area estimated by vegetation distribution) Severity and extent of alteration		SCORE:
BEST	As expected for reference, no evidence of disturbance	0
MODERATE	Low intensity alteration or past alteration that is not currently affecting wetland	7
FAIR	Highly altered, but some recovery if previously altered	14
POOR	Almost no natural habitat present, highly altered habitat	21

Checklist			
Vegetation Removal Disturbance			
Grazed ☐		Tree plantation ☐	
Substrate/ Soil Disturbances and Sedimentation			
Vehicle Use ☐		Sediments inputs (from inflow or erosion) ☐	
Other:			

2) HYDROLOGICAL ALTERATION Severity and degree of alteration		SCORE:
BEST	As expected for reference, no evidence of disturbance	0
MODERATE	Low intensity alteration or past alteration that is not currently affecting wetland	7
FAIR	Less intense than 'poor', but current or active alteration	14
POOR	Currently active and major disturbance to natural hydrology	21

Checklist		
Inlet restriction present Weir ☐ Dam ☐ Other:	Outlet restriction present Weir ☐ Dam ☐ Other:	
Drainage ☐	Impounding ☐	Canalisation ☐

3) BUFFER LANDSCAPE DISTURBANCE (< 100m from wetland) Extent and intensity		SCORE:
BEST	As expected for reference site, no evidence of disturbance	0
MODERATE	Predominately undisturbed, some human influence	7
FAIR	Buffer significantly modified	14
POOR	Nearly all or all of the buffer modified, intensive land use surrounding wetland	21

Checklist		
Intensive cropping ☐	Presence of exotic veg. ☐	Human disturbance ☐

4) LANDSCAPE (IMMEDIATE) INFLUENCE (100m – 500m from the wetland) Extent and intensity		SCORE:
BEST	As expected for reference site, no evidence of disturbance	0
MODERATE	Predominately undisturbed, some human influence	7
FAIR	Landscape significantly modified	14
POOR	Nearly all or all of the landscape modified, intensive land use surrounding wetland	21

Checklist		
Intensive cropping ☐	Presence of exotic veg. ☐	Human disturbance ☐

5) IMPAIRMENT FROM DAIRY Extent and intensity		SCORE:
BEST	No evidence of impairment from dairy	0
MODERATE	Moderate impairment attributed to dairy	7
FAIR	Significant impairment attributed to dairy	14
POOR	Server impairment attributed to dairy	21

Checklist

Slope and distance from dairy to wetland ☐

QUESTIONS TO DAIRY:
Details of any mitigation structures, present or past (what, when established):
When was dairy established?
How many cows are milked at dairy each day?
Has this number of cows always been milked at this dairy?
During a year, does the land use between the wetland and the dairy change (how)?

6) PHYSICAL CHARACTERISTICS		SCORE:
BEST	As expected for reference and no evidence of change in natural characteristics	0
MODERATE	Little or no evidence of a change in natural physical characteristics	7
FAIR	Significant change in natural physical characteristics	14
POOR	Severe change in natural physical characteristics	21

Checklist					
Odour:	Sewage ☐	Sulphur ☐	Normal ☐	Petroleum ☐	Colour:
Observations:	Dead fish ☐	Algae ☐			

TOTAL ENVIRONMENTAL SCORE FOR WETLAND	**SCORE:**

OTHER COMMENTS:

Draw a map of the wetland and up to 200m into upland
Show: approximate scale, wetland shape, wetland dimensions, location of major vegetation classes, inlets, outlets, surrounding land use, roads, foot paths, storm drains, north etc.

APPENDIX 6. Physico-chemical Data Sheet

PHYSICO-CHEMICAL DATA SHEET			
Wetland Name:	Sample Number:	Impacted ☐ Reference ☐ Photograph taken ☐	
Time of Sample:	GPS co-ordinates- ALT. m	Longitude: . . .	Latitude: . . .
Distance from dairy to sample point: m	Depth: cm	Flow (circle): Very low Low Medium High	If impacted, B/A/D of point source (circle)
pH:		Phosphorus:	µg/L
TDS:	ppm	Ammonia:	mg/L
Electrical conductivity:	mS/m	Temperature:	°C
Nitrogen:	mg/L	Turbidity:	cm
OTHER COMMENTS:			

APPENDIX 7. Sample Biotope Detection Assessment (SBDA) Index

Metric		Criteria	Score
1	Area of sedge-dominated vegetation standing in water >0.5m deep within wetland	>25m^2	5
		12-25m^2	3
		<12m^2	1
2	% density of vegetation in a 1m^2 quadrat at sample point	>60%	5
		60-30%	3
		<30%	1
3	% of sediment covering base of white tray	<30%	5
		30-60%	3
		>60%	1
4	% of duckweed sp. covering water surface when sample placed in white tray	<30%	5
		30-60%	3
		>60%	1
5	% of vegetation occupying sample when placed in white tray	<30%	5
		30-60%	3
		>60%	1

Component B1

Developing a macroinvertebrate sampling technique for palustrine wetlands in South Africa: a pilot investigation in the KwaZulu-Natal midlands

Towards the development of a macroinvertebrate sampling technique for palustrine wetlands in South Africa: a pilot investigation in the KwaZulu-Natal midlands

Abstract

This paper details the investigation undertaken to derive a suitable sampling technique for collecting a representative sample of aquatic macroinvertebrates from a palustrine wetland's macroinvertebrate community in South Africa. The study took place at Melmoth Vlei, in KwaZulu-Natal, South Africa during November 2003. The aim of this study was to undertake a preliminary investigation on the development and testing of a macroinvertebrate sampling technique for use in palustrine wetlands (sensu Cowardin *et al.* 1979), which could contribute to the development of a South African wetland health biomonitoring programme. Sweep nets and activity traps were evaluated for their effectiveness at macroinvertebrate collection. Sweep net sampling was tested over a range of sweep intensities to determine the minimum number of sweeps required to collect a representative sample of a wetland's macroinvertebrate community. Sampling efficiency of activity traps placed at four depths was tested, and taxon diversity and composition of sweep net and activity trap samples were compared to determine whether activity traps are required to supplement sweep net data. A total of 32 taxa (identified to family level) were identified in the samples collected. Taxon diversity and composition did not differ in the activity traps placed at the four depth locations. Taxon diversity did not differ significantly between different sweep intensities. This maybe as a result of high variability of macroinvertebrate distribution within a wetland, however there is evidence to suggest this result is due to an insufficient sample size. There was a significant difference in taxon composition between the different sweep intensities ($p<0.05$) and between activity trap and sweep net samples ($p<0.05$). Sixty-eight percent of taxa appeared more

frequently in sweep net sampling compared to activity trap sampling. Two taxa were found exclusively in activity traps, however, the number of these taxa collected was not significant and they do not represent any unique trophic group. Based on these findings, activity traps are not required to supplement sweep net data, and a technique using a sweep net with a sweep intensity of five would be suitable to collect a representative sample of macroinvertebrates from a palustrine wetland.

Introduction

Wetlands are valuable resources that supply many products, functions and values to people (Finlayson 1996), as well as being areas of high biodiversity (Hails 1996). Many of South Africa's wetlands have been degraded, largely due to agricultural development, such as drainage and poor land use practices (Kotze *et al.* 1995). To date more than half of all wetlands in KwaZulu-Natal have been modified to the extent that their functioning has been impaired (Begg 1990). In order for South Africa to comply with national legislation and international conventions, and cater for human and environmental needs, methods for monitoring water quality need to be developed for all water resources, including wetlands. To date there has been little work done on the assessment of wetland health in South Africa. In the U.S.A., the U.S. Environmental Protection Agency (EPA) has undertaken extensive work since 1989, favouring the use of aquatic macroinvertebrate assemblages for rapid bioassessment (Sutherland and Stribling 1995). Research on wetland biomonitoring has also been performed in Australia (Chessman *et al.* 2002, Butcher 2003).

Internationally, macroinvertebrates in streams and rivers have been used extensively as water quality biomonitoring tools (Wissinger 1999, Hicks 2000, Butcher 2003). However, limited work has been performed on their use in wetlands (Butcher 2003). South Africa has a standard macroinvertebrate biomonitoring water quality and river health tool developed for streams and rivers, called SASS5 (South African Scoring System Version 5). SASS5 is specifically aimed at detecting changes within the macroinvertebrate community of streams and rivers (Dallas *et al.* 1999, Dickens and Graham 2002). SASS was originally derived from the British Monitoring Working Party system (BMWP) by Chutter (1994, 1998). In many cases where wetland biomonitoring programmes using aquatic macroinvertebrates have been developed, they have

largely been derived from local river monitoring methods (Hicks 2000, Teels and Adamus 2002, Butcher 2003).

The sampling technique used to collect biota is an integral component of any bioassessment protocol. Sweep net sampling is the most commonly used method for collecting aquatic macroinvertebrates from a shallow water body (Helgen 2002), and is the standard sampling technique used with SASS5. Activity traps were tested to determine if they are required to be used in conjunction with sweep net sampling, as previous studies show that activity traps are more efficient at collecting motile taxa compared to sweep nets (Hilsenhoff 1987, Helgen 2002).

The aim of this study was to undertake a preliminary investigation on the development and testing of a macroinvertebrate sampling technique for use in palustrine wetlands (sensu Cowardin *et al*. 1979), which could contribute to the development of a South African wetland health biomonitoring programme.

To achieve this aim, this study had the following objectives:

1. Evaluate the effectiveness of using i) sweep net, and ii) activity trap sampling for collecting a representative sample of aquatic macroinvertebrates from sedge-dominated palustrine wetland macroinvertebrate communities
2. Derive a potential aquatic macroinvertebrate sampling technique for use in sedge-dominated palustrine wetlands
3. Identify areas for further research concerning the development of a wetland aquatic macroinvertebrate biomonitoring protocol

Study area

Melmoth Vlei (29°18'S, 30°16'E) is situated in the Karkloof area of the KwaZulu-Natal midlands, South Africa. The wetland is 104ha in extent, at an altitude of 1595m. Melmoth Vlei forms part of the Myamvubu Vlei system, which is situated in the Thukela Catchment (Figure 1). It is located near to the source of the Myamvubu River which rises close to Mt. Gilboa (Begg 1989). The wetland has a mean daily maximum temperature of 21.5°C, a mean daily minimum temperate of 7.5°C and receives an average of 1100mm of precipitation annually (Schulze 1982). In 1989, the wetland became part of the Karkloof Nature Reserve and thus formally protected from degradation and modification. The catchment surrounding the Myamvubu Vlei system is mainly moist midlands mistbelt grassland (Camp 1997). The wetland is fenced to exclude cattle, however, a small number of cattle were observed grazing in the wetland during the study period. There is afforestation within the catchment, but it is likely to have minimal effect on Melmoth Vlei, as it does not occur immediately adjacent to or above the wetland, and occupies a small proportion of the wetlands catchment. Sampling for this study took place in emergent vegetation bordering an open water area of the wetland.

Melmoth Vlei is dominated by hygrophilous plant communities and grasslands which are regularly burnt during the dry season (Begg 1989). There is a distinct zonation of plant communities present. In the saturated central areas, *Carex acutiformis* is the dominant species, while other hygrophilous species such as *Cyperus* spp., *Kyllinga melanosperma* and *Mariscus* spp. also occur. In the dryer fringes, grass dominated communities, characterised by species such as *Aristida junciformis, Monocymbium ceresiiforme* as well as various herbs, occur (Begg 1989).

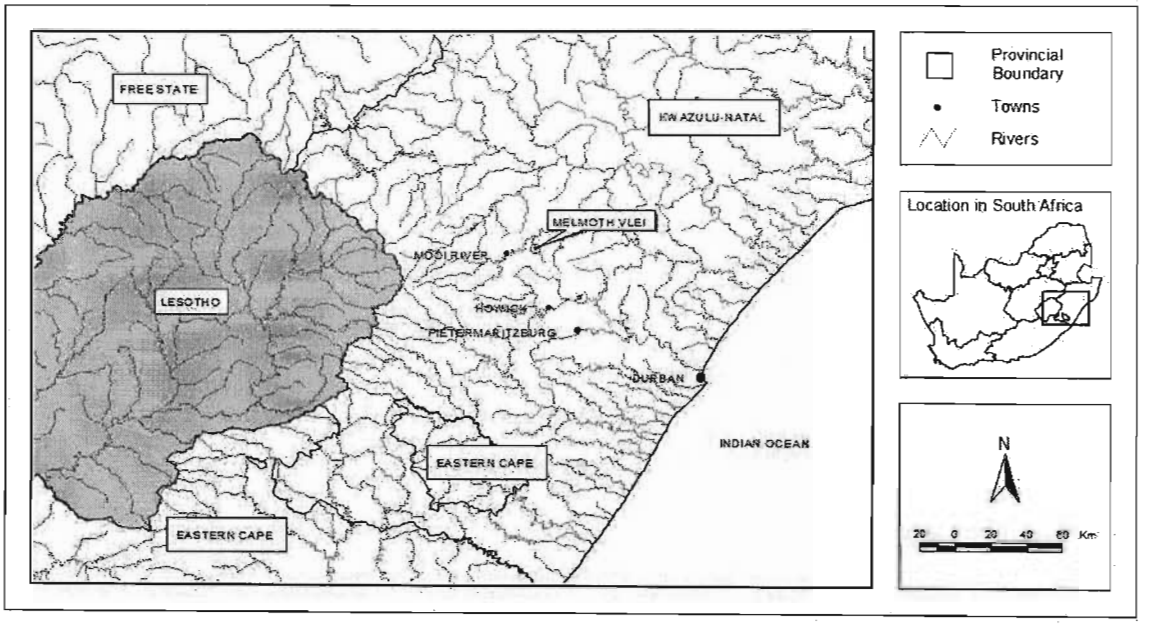


Figure 1: Map showing location of Melmoth Vlei in KwaZulu-Natal, South Africa

Materials and Methods

Macroinvertebrate and physico-chemical sampling was conducted between 5th and 11th November 2003. All sampling was carried out within one week as recommended by Hicks (2000).

Site selection

Melmoth Vlei was identified as being one of the least impacted wetlands in the KwaZulu-Natal midlands area, through examination of 1:50 000 maps of the area and consultation with local environmental organisations. A site visit was conducted to verify that there were no land use activities or alterations present that would have a negative impact on the wetland.

Physico-chemical tests

Physico-chemical tests were carried out to confirm that the water throughout the wetland was of a similar quality (and of a near-pristine standard), as variation in water quality could affect the results obtained. Temperature, pH, electrical conductivity and total dissolved solids (TDS) were measured using a HANA HI 991 330 Portable pH/EC/TDS/temperature meter. Physico-chemical sampling was conducted 24 hours after the traps were set and immediately before the traps were emptied. This was done to allow the water to settle overnight, as disturbance from installing the traps could have influenced the physico-chemical results. For each of the four activity trap depth placements, the minimum and maximum results were derived for each physico-chemical parameter, together with the overall minimum and maximum result of each test. For the sweep net samples, physico-chemical measurements were taken at three separate locations, covering the areas in which sweep net sampling was performed. Minimum and maximum results for each parameter for each of the three locations were collected. These results were compared against DWAF guidelines (1996).

Biotope sampled

Samples were taken from emergent vegetated areas, dominated by sedges, in water less than one metre in depth. The vegetation sampled bordered open water within the wetland. Vegetated areas were selected as they are recorded to have a higher taxon diversity in comparison to open water areas that lack submerged vegetation (Olson *et al.* 1995). Sedge-dominated vegetation provides a dense biotope and large surface area which supports a greater abundance of macroinvertebrates, and is an easier vegetation type in which to drag a sweep net through in comparison to *Typha* and *Phragmites* beds (C. Dickens, Leader: Environmental Assessment and Reporting, INR, pers. comm.).

Sweep net macroinvertebrate collection

There are three sweep net techniques commonly employed to collect macroinvertebrate samples from a wetland:

- Fixed number of sweeps (Difranco and Stevenson 2000, Hicks 2000, Battle *et al.* 2001, Chessman et al. 2002, Gernes and Helgen 2000)
- Fixed length of time (Apfelbeck 1999, Chessman *et al.* 2002)
- Fixed number of organisms required to be collected (Apfelbeck 1999)

For this study, the 'fixed number of sweeps' method was employed, as the majority of wetland bioassessment protocols which use a sweep net to collect macroinvertebrates favour this method (Helgen 2002).

The sweep net used for macroinvertebrate sampling in this study has a pore size of 1mm (as used in the SASS5 protocol). The net is mounted on a square aluminium frame 300mm by 300mm, and a one metre handle is attached centrally to one side of the frame (Chutter 1998). An intensity range (number of sweeps) of two to six sweeps was tested using the sweep net (see *Sweep technique*). This range was chosen as a number of authors recommend sweep intensities ranging between two and five (Friday 1987, Gernes and Helgen

2000, Reber *et al.* 2000). In addition, a six sweep intensity series was performed. The Gernes and Helgen (2000) sweep net method was chosen for this study, as this protocol incorporates the collection of macroinvertebrates from the water surface, water column and substrate. Several other wetland bioassessment studies also incorporate the sampling of these three locations when conducting sweep net sampling (Gernes and Helgen 2000, Hicks 2000, Burton 2001, Chessman *et al.* 2002).

Sweep technique

The sweep net is dragged strongly from the surface, down through the water column at a 45 degree angle, until it touches the bottom and then brought to the surface with the mouth facing the surface, ensuring that macroinvertebrates are not lost. This constitutes a single sweep and an intensity of one (Gernes and Helgen 2000). For higher sweep intensities, this technique is repeated for the appropriate number of times. The net is only cleared after the specified number of sweeps has been completed.

A sample is the collection of macroinvertebrates by means of a specified sweep intensity. Sampling at each intensity (two-six sweeps) was repeated ten times. Each sample was collected from suitable habitat (see *Biotope sampled*) at different locations within the wetland, ensuring that sample locations were not disturbed by previous sample collections. In moving to each sampling point, care was taken to keep disturbance to a minimum. For each sample, all sweeps were conducted at the same location within a $1m^2$ area. Following the collection of each sample, the contents of the net were deposited in a white photographic tray. The net was then inverted and additional water was passed through the net to wash out any remaining organisms into the tray. The net was then checked for any missed organisms. Large pieces of vegetation were discarded after ensuring the majority of invertebrates had been removed. The contents of the tray were then washed though a sieve (mesh size 1mm), and placed in a

labelled one litre plastic container with clean water. The collected invertebrates were stored in a cooler box and transported to the laboratory.

Activity trap macroinvertebrate collection

The Helgen (2002) technique for activity trap construction was followed for this study. This particular trapping technique was chosen because it has been shown to be effective (Brinkman and Duffy 1996), and very inexpensive and lightweight (Hanson *et al.* 2000). Activity traps were constructed using two-litre soft drink bottles. A funnel was cut from the top end of a bottle, and four grooves of 3mm x 55mm were cut into the rim of the funnel to attach it to the open end of the bottle. The trap was attached to a solid metal rod (10mm by 1000mm) by a sliding PVC bracket, also constructed from a section of a soft drink bottle (Figure 2). The metal rod was then pressed into the substrate.

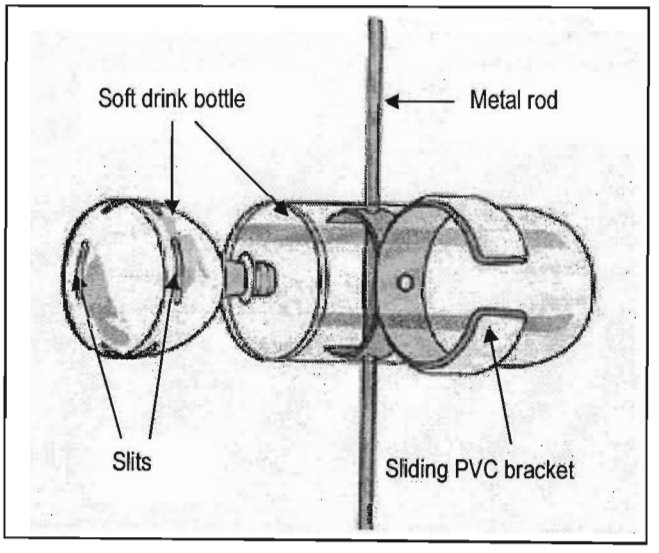


Figure 2: Activity trap made from a soft drink bottle (after Helgen 2002)

A total of 80 activity traps were placed at four different depths. Twenty were situated at the surface with the trap half-filled with water (this position was tested as Hanson *et al.* (2000) note concerns about activity traps not trapping surface-associated taxa). Twenty traps were placed just below the water surface; twenty were positioned between 150mm and 200mm below the

surface, and a further 20 were placed resting on the substrate (Gernes and Helgen 2000, Helgen 2002). Traps were set approximately 100mm apart.

Those traps placed below the water's surface were back-filled with water before being set, in order to decrease the oxygen level in the traps, and thus reduce the activity of predators, which might devour trapped organisms and thus influence sampling results (Helgen 2002). However, as a number of trapped adult frogs and tadpoles drowned during the first trapping session, a small amount of air was left in each trap during the second trapping session.

Activity traps were left in place for 24 hours, to allow both nocturnal and diurnal invertebrates to be collected (Helgen 2002). Care was taken to create as little disturbance as possible when installing each trap. After 24 hours, the contents of each of the activity traps were stored in a labelled one litre plastic jar, and transferred to a cooler box until transported to the laboratory. Activity trapping took place over two days due to the time required to set-up the traps, collect the samples, and identify the macroinvertebrates.

Macroinvertebrate identification

Identification of organisms took place under laboratory conditions (as recommended by Barbour *et al.* 1996 and Helgen 2002). Each sample was emptied into a white photographic tray. Large pieces of vegetation were discarded after ensuring that all organisms had been removed. The sample was left to stand for five minutes. This was done to allow vegetation and sediment to settle, and invertebrates to emerge from the substrate. All observed invertebrates in the samples were then removed using a pipette or tweezers and placed in a Petri dish for closer examination. Invertebrates were predominately identified to family level with the help of two identification books: Gerber and Gabriel (2002a, b). A hand lens (Triplet 10x) was used to identify specimens. Each sample was examined until no new macroinvertebrates were

detected after five minutes. Samples were processed as soon as possible, as it was easier to locate and identify macroinvertebrates when they were alive and mobile. Every sample collected was processed on the same day it was collected from the wetland. This was done to reduce bias as different taxa vary in their ability to survive confined in containers, and collected predatory taxa (e.g. Odonata and some Hemiptera) could eat other macroinvertebrates within the sample. If a macroinvertebrate could not be identified, it was placed in a vial with a 70% ethanol solution for later identification by a specialist entomologist. A selection of identified macroinvertebrates was also shown to an entomologist for auditing purposes as recommended by Hicks (2000) and Chessman *et al.* (2002).

Statistical Analysis

Analysis of Variance (ANOVA) was used to investigate the effect of sweep intensity on taxon diversity. To determine the effect of sweep intensity on taxon composition, non-metric multi-dimensional scaling (NMDS) ordination of compositional data using WinKyst (Šmilauer 2003) was employed. This was followed by projection of the site ordination scores onto the first two axes of a principle component analysis, using CANOCO 4.51 (Ter Braak and Šmilauer 2003). Multi-Response Permutation Procedures (MRPP) (PC-ORD, version 4.25), Indicator Species Analysis (ISA) (McCune and Mefford 1999) and the Monte Carlo Test of Significance were also performed. The indicator values were calculated using the method of Dufrene and Legendre (1997). Activity trap macroinvertebrate diversity was analysed using ANOVA. Activity trap macroinvertebrate composition was investigated using NMDS, and activity trap and sweep net macroinvertebrate composition were compared using MRPP, ISA and the Monte Carlo Test of significance.

Results

Physico-chemical variables

The physico-chemical results collected during activity trap and sweep net sampling (Tables 1 and 2) support the assumption that Melmoth Vlei is a wetland with near-pristine water quality, as defined in the South African water quality guidelines (DWAF 1996). Except for pH, there was little variability among the physico-chemical measurements throughout the area sampled (Table 3).

Table 1: Selected physico-chemical results for sites where activity traps were placed

Water chemistry	At surface		Just below surface		Edge of substrate		150 - 200mm below surface		Overall	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Temperature (°C)	15.8	20.5	16.5	19	15.1	20.5	16.8	18.5	15.1	20.5
pH	3.46	5.48	4.06	5.61	3.25	5.48	4.3	4.59	3.25	5.61
Conductivity (mS/m)	28	40	30	36	16	34	30	35	16	40
TDS (ppm)	14	21	15	17	9	21	14	17	9	21

Table 2: Selected physico-chemical results for sites where sweep net sampling was undertaken

Water chemistry	Area 1	Area 2	Area 3	Overall	
				Min.	Max.
Temperature (°C)	18.9	19.1	18.9	18.9	19.1
pH	3.6	4.6	4.41	3.6	4.6
Conductivity (mS/m)	47	40	33	33	47
TDS (ppm)	23	20	15	15	23

Table 3: Physico-chemical results range

Water chemistry	Overall		Range
	Min.	Max.	
Temperature (°C)	15.1	20.5	5.4
pH	3.25	5.61	2.36
Conductivity (mS/m)	16	47	31
TDS (ppm)	9	23	14

Sweep net and activity trap macroinvertebrate results

Thirty-two taxa were identified in the samples collected from Melmoth Vlei (Table 4). Activity trap and sweep net sample results are displayed in Appendix 1 and 2 respectively. Although each sample was sorted and identified separately, results have been pooled for ease of comparison.

Table 4: Macroinvertebrates collected in sweep nets and activity traps in Melmoth Vlei

Order	Family	Sampling method	
		Sweep net	Activity trap
TURBELLARIA	Dugesiidae	✓	✓
ANNELIDA	Hirudinea	✓	✓
CRUSTACEA	Potamonautidae	✓	
HYDRACARINA	-	✓	✓
LEPIDOPTERA	Pyralidae	✓	
ODONATA	Coenagrionidae	✓	✓
	Lestidae	✓	
	Aeshnidae	✓	✓
	Libellulidae	✓	✓
EPHEMEROPTERA	Baetidae	✓	✓
	Caenidae	✓	
	Leptophlebiidae		✓
HEMIPTERA	Belostomatidae	✓	✓
	Corixidae	✓	✓
	Hydrometridae	✓	✓
	Naucoridae	✓	✓
	Nepidae	✓	✓
	Notonectidae	✓	✓
	Pleidae	✓	✓
	Veliidae	✓	
TRICHOPTERA	Ecnomidae	✓	
	Leptoceridae	✓	✓
COLEOPTERA	Dytiscidae	✓	✓
	Gyrinidae	✓	✓
	Hydrophilidae	✓	✓
DIPTERA	Ceratopogonidae	✓	✓
	Chironomidae	✓	✓
	Culicidae	✓	✓
	Dixidae		✓
	Tipulidae	✓	✓
GASTROPODA	Lymnaeidae	✓	✓
	Physidae	✓	✓

Statistical analysis

The effect of sweep intensity on taxon diversity

Although sweep intensities five and six had the highest mean taxon diversity, the mean number of taxa did not differ significantly between sweep intensities ($p<0.05$) ($F_{4, 45df}=2.21$, $p=0.083$). Sweep intensities five and six values are very similar. Sweep intensity four had the lowest mean taxon diversity (Table 5). Standard deviations were larger with higher sweep intensities.

Table 5: Sweep intensity summary statistics for diversity showing mean number of taxa, standard deviation and upper and lower values for tested sweep intensities (n=10)

Sweeps	Mean	Standard deviation	Upper value	Lower value
2	9.7	1.83	13	8
3	9.0	0.94	11	7
4	8.4	2.01	11	5
5	10.6	2.95	15	6
6	10.7	2.36	15	7
All intensities	9.68	2.27	15	5

The effect of sweep intensity on taxon composition

There is a significant difference in taxon composition between different sweep intensities ($p<0.05$) (Table 6). The NMDS ordination graph confirms the pattern shown in the MRPP results (Figure 3). There is an overlap in taxon composition of samples between sweep intensities five and six, and three and four, and a general separation (different taxon composition) of sweep intensity two from other intensities; it is especially distinct from sweep intensity six (Table 7). Other sweep intensities, notably five, are not very distinct from sweep intensity six.

Table 6: Multi-Response Permutation Procedures (MRPP) for sweep intensity composition ($p<0.05$)

Sweep intensity (group)	Average distance	Test statistic (T)*	p value	Significance
2	0.42287669			
3	0.44889463			
4	0.44601546	-5.1010966	0.00001475	significant
5	0.44708827			
6	0.40147313			

* More negative indicates stronger group (intensity) separation
Distance measured by Sorensen (Bray-Curtis)

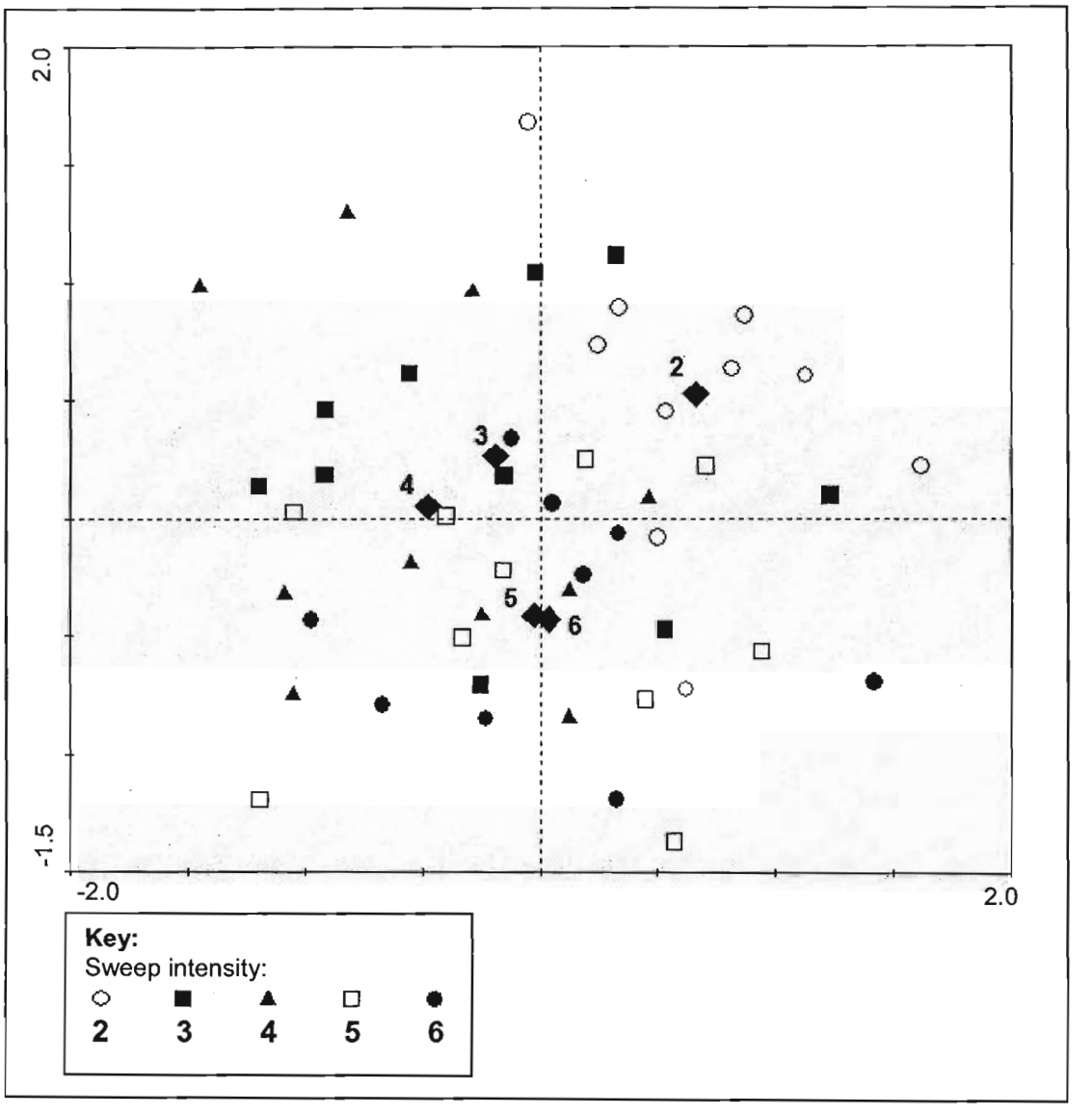


Figure 3: Nonmetric multi-dimensional scaling (NMDS) ordination of taxon composition for different sweep intensities

Table 7: Results of individual sweep intensities (2-5) Multi-Response Permutation Procedures (MRPP) comparisons with sweep intensity six ($p<0.05$)

Sweep intensity	Test statistic	p value	Significance
2	-5.6152519	0.00004901	significant
3	-1.7039034	0.05789053	nearly significant
4	-1.7037356	0.05534845	nearly significant
5	0.75250483	0.76184093	not significant

Distance measured by Sorensen (Bray-Curtis)

Dugesiidae was collected significantly more often with sweep intensity two and Dytiscidae was significantly more common in sweep intensity six. Aeshnidae and Veliidae were collected more frequently in sweep intensities two and three respectively, although this was not significant (Table 8). The remaining taxa were all equally likely to occur in any of the tested sweep intensities.

Table 8: Indicator values and Monte Carlo test of significance for sweep intensity taxon composition ($p<0.05$) of samples. s=significant, ns=not significant, nes=nearly significant ($0.05<p<0.08$)

Taxa	Sweep intensity					p*	Significance
	2	3	4	5	6		
Dugesiidae	40	2	2	5	8	0.0020	s
Hirudinea	18	9	3	18	9	0.7670	ns
Potamonautidae	0	0	0	0	10	1.0000	ns
Hydracarina	12	8	5	16	26	0.2060	ns
Baetidae	15	0	7	0	2	0.4210	ns
Caenidae	13	3	9	13	18	0.7700	ns
Coenagrionidae	23	18	23	15	11	0.8550	ns
Lestidae	0	0	5	0	5	1.0000	ns
Aeshnidae	26	13	5	5	1	0.0570	nes
Libellulidae	1	3	6	23	10	0.2090	ns
Pyralidae	3	0	3	0	3	1.0000	ns
Belostomatidae	16	12	20	16	16	0.9640	ns
Corixidae	7	1	1	19	7	0.1500	ns
Hydrometridae	3	0	0	3	3	1.0000	ns
Naucoridae	4	26	10	6	10	1.1510	ns
Nepidae	0	8	0	2	8	0.9670	ns
Notonectidae	11	1	1	1	5	0.8040	ns
Pleidae	22	22	11	18	18	0.9510	ns
Veliidae	0	27	0	7	0	0.0680	nes
Ecnomidae	1	4	0	18	4	0.2600	ns
Leptoceridae	3	0	3	3	0	1.0000	ns
Dytiscidae	0	15	15	15	31	0.0280	s
Gyrinidae	8	3	8	8	1	1.0000	ns
Hydrophilidae	0	5	5	0	0	1.0000	ns
Ceratopogonidae	1	0	8	23	4	0.1540	ns
Chironomidae	6	9	25	20	25	0.3870	ns
Culicidae	4	8	0	4	15	0.5760	ns
Tipulidae	10	0	0	0	0	1.0000	ns
Lymnaeidae	8	0	2	0	8	0.9490	ns
Physidae	2	15	1	9	15	0.8140	ns
Average	8	6	5	8	8	-	-

* Proportion of randomized trials with indicator value equal to or exceeding the observed indicator value

p=(1 + number of runs >= observed)/(1 + number of randomized runs)

Maximum group = Group identifier for group with maximum observed indicator value

The effect of activity trap depth location on taxon diversity

The mean number of taxa does not differ significantly ($p<0.05$) ($F_{3,\ 76df} = 2.37$, $p= 0.077$) among depth positions (Table 9).

Table 9: Activity trap summary statistics for taxon diversity showing mean number of taxa, standard deviation and upper and lower values for tested trap locations (n=20)

Trap location	Mean no. of taxa	Standard deviation	Upper value	Lower value
On Substrate (S)	2.45	1.28	4	0
At the surface (A)	3.55	1.93	7	1
Just below surface (J)	3.7	1.53	6	1
150-200mm below surface (B)	3.0	1.81	5	0
All groups	3.175	1.70	7	0

The effect of activity trap depth location on taxon composition

The overlap in distribution of the samples from the different locations suggests that there is no consistent difference in the taxon composition collected at the four different depths (Figure 4).

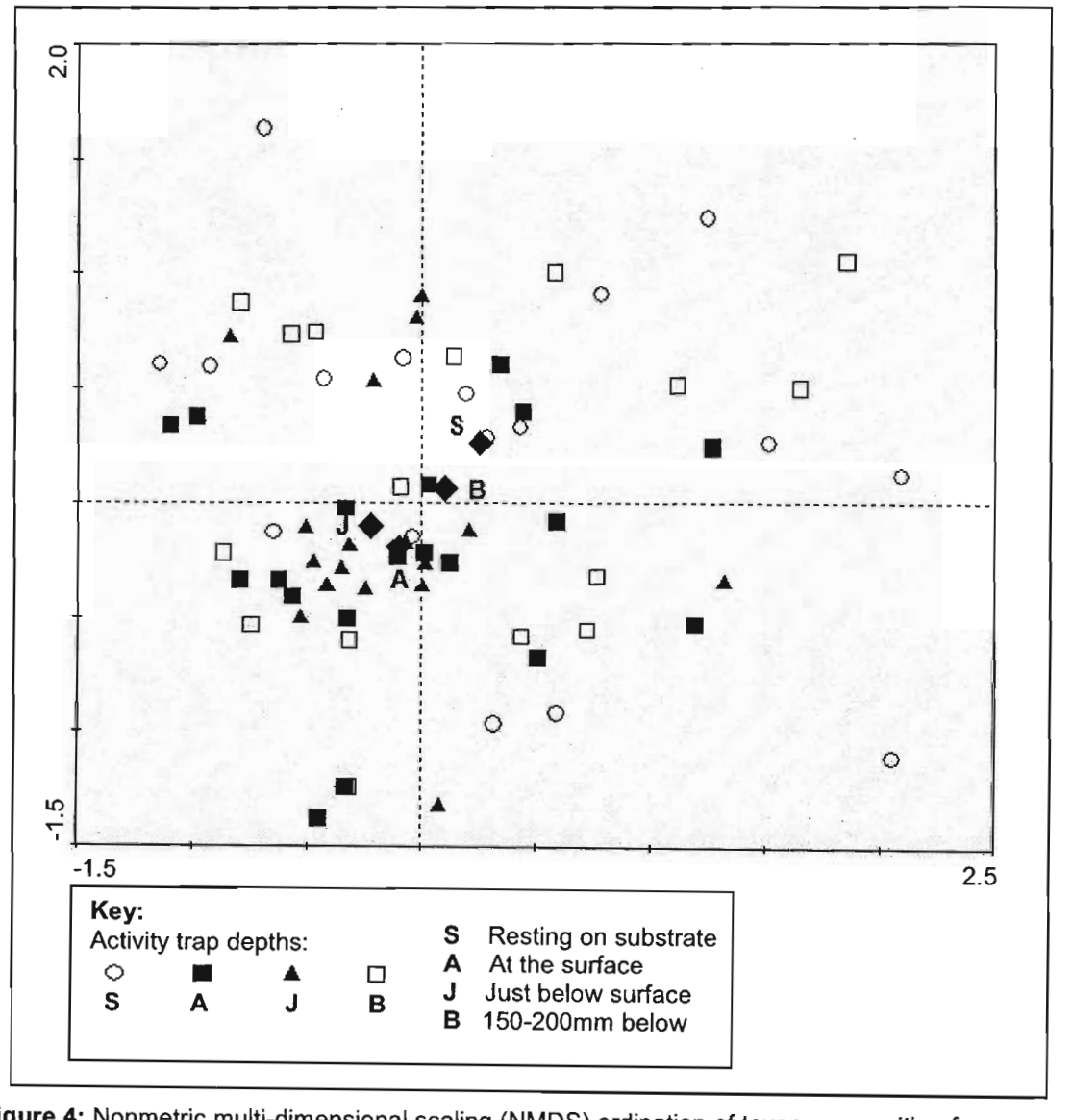


Figure 4: Nonmetric multi-dimensional scaling (NMDS) ordination of taxon composition for different activity trap depth locations

Comparing activity trap and sweep net taxon composition

There is a highly significant difference in taxon composition between the sweep net and activity trap samples ($p<0.05$) (Table 10). Twenty-five taxa (78%) were found more frequently in sweep net samples compared to activity traps. Seven taxa were collected more frequently in activity traps, however only one taxon, Corixidae (Hemiptera) was collected significantly more frequently in activity traps. Two other taxa, Leptophlebiidae (Ephemeroptera) and Dixidae (Diptera) occurred exclusively in activity trap samples but at a low frequency, and consequently were not identified as significant indicators for activity traps (table 11). Six taxa were found exclusively in sweep net samples (Table 4), however only four were significant: Caenidae (Ephemeroptera), Pyralidae (Lepidoptera), Veliidae (Hemiptera) and Ecnomidae (Trichoptera) (Table 11).

Table 10: Multi-Response Permutation Procedures (MRPP) comparison of sweep net and activity trap sample taxon composition ($p<0.05$)

Sampling type	Average distance	Test statistic (T)*	p value	Significance
Activity Trap **Sweep Net**	0.71103110 0.46271797	- 46.627382	0.00000000	significant

* More negative indicates stronger group (intensity) separation
Distance measured by Sorensen (Bray-Curtis)

Table 11: Indicator values and Monte Carlo test of significance for sweep net and activity trap taxon composition ($p<0.05$) of samples. s=significant, ns=not significant, nes=nearly significant ($0.05<p<0.08$)

Taxa	Relative abundance (%)		Relative frequency (%)		Indicator values		p*	Significance
	Activity trap	Sweep net	Activity trap	Sweep net	Activity trap	Sweep net		
Dugesiidae	12	88	5	40	1	35	0.0010	s
Hirudinea	20	80	13	54	3	43	0.0010	s
Potamonautidae	0	100	0	2	0	2	0.3510	ns
Hydracarina	46	54	53	62	24	34	0.2870	ns
Baetidae	35	65	7	12	2	8	0.3650	ns
Caenidae	0	100	0	54	0	54	0.0010	s
Leptophlebiidae	100	0	7	0	7	0	0.1650	ns
Coenagrionidae	14	86	14	88	2	76	0.0010	s
Lestidae	0	100	0	4	0	4	0.1420	ns
Aeshnidae	6	94	3	38	0	36	0.0010	s
Libellulidae	20	80	8	32	2	26	0.0010	s
Pyralidae	0	100	0	6	0	6	0.0710	nes
Belostomatidae	10	90	9	80	1	72	0.0010	s
Corixidae	72	28	67	26	48	7	0.0010	s
Hydrometridae	18	82	1	6	0	5	0.3300	ns
Naucoridae	12	88	7	50	1	44	0.0010	s
Nepidae	12	88	1	10	0	9	0.0380	s
Notonectidae	60	40	24	16	14	6	0.3650	ns
Pleidae	27	73	33	90	9	66	0.0010	s
Veliidae	0	100	0	12	0	12	0.0020	s
Ecnomidae	0	100	0	18	0	18	0.0010	s
Leptoceridae	52	48	7	6	3	3	1.0000	ns
Dytiscidae	24	76	20	64	5	49	0.0010	s
Gyrinidae	22	78	7	24	1	19	0.0110	s
Hydrophilidae	72	28	11	4	8	1	0.3260	ns
Ceratopogonidae	26	74	8	22	2	16	0.0330	s
Chironomidae	13	87	12	80	2	70	0.0010	s
Culicidae	19	81	5	22	1	18	0.0050	s
Dixidae	100	0	5	0	5	0	0.1770	ns
Tipulidae	57	43	3	2	1	1	1.0000	ns
Lymnaeidae	21	79	3	10	1	8	0.1110	ns
Physidae	10	90	4	34	0	30	0.0010	s
Average	26	68	10	28	4	23	-	-

Relative abundance is calculated as the proportional (%) abundance of a particular taxon in a particular group relative to the abundance of that taxon in all groups, i.e. the concentration of abundance in one particular group (McCune and Grace 2002)

Relative frequency is calculated as the percentage of sample units in each group that contain that taxon (McCune and Grace 2002)

* Proportion of randomized trials with indicator value equal to or exceeding the observes indicator value

p=(1 + number of runs >= observed)/(1 + number of randomized runs)

Maximum group = Group identifier for group with maximum observed indicator value

Discussion

A significantly greater number of taxa were collected by sweep net sampling in comparison to activity trap sampling. Brinkman and Duffy (1996) considered the use of activity traps alone to be adequate for collecting a representative sample from a wetland, yet this study's findings show that the average number of taxa collected by activity traps was less than one third of the number collected using a sweep net. Thus, it would appear that relying exclusively on activity trap results could lead to a major underestimation of local taxon diversity. Both techniques collected some taxa exclusively; however sweep net sampling did this to a far greater degree. Only one taxon (Corixidae) was collected significantly more often using activity traps. Activity traps collected two taxa (Leptophlebiidae and Dixidae) which sweep net sampling missed. Although these taxa are considered indicators of good water quality for rivers in SASS5, there is evidence that SASS5 tolerance scores are not applicable to wetlands (Bowd in prep). Both of these taxa belong to the same functional trophic group (gathering collectors) (Hicks 2000); this group was adequately represented in sweep net samples (Dugesiidae, Baetidae, Caenidae and Hydrophilidae). Some authors consider sweep net sampling to be inefficient at collecting motile taxa, such as predatory Coleoptera and Hemiptera (Burton 2001, Helgen 2002), and the addition of activity traps within a sampling protocol to compensate for this has been recommended (Hilsenhoff 1987, Helgen 2002). However, during the current study, with the notable exception of Corixidae, sweep net sampling proved to be more effective at collecting these taxa than activity traps.

Hanson *et al.* (2000) considered activity traps to be inadequate as they typically sampled surface associated taxa poorly. Sampling at the surface was specifically included in this study in an attempt to account for this, and, although traps at the surface collected a slightly larger number of taxa, there was no significant difference between the sampling depths.

Although the time required to collect and process a single activity trap sample was roughly equivalent to that needed for sweep net sampling, the need for multiple samples to be collected requires a much larger investment of time and effort than sweep net sampling. Overall, with the need for multiple activity traps to be installed in a wetland, the need to revisit a site after 24 hours, and the time required to identify invertebrates in several samples, much less effort is required for sweep net sampling, and, at least in this study, better results were obtained.

When setting the activity traps, the change from back-filling the traps in order to remove all air, to the addition of a small amount of air, did not appear to affect the number and type of macroinvertebrates collected. However, due to the limited sampling effort, further research is required to determine if the addition of air within a trap significantly affects macroinvertebrate predation.

In this study, the different sweep intensities did not yield a significantly different number of taxa. One explanation for this could be due to the high variability of macroinvertebrate distribution within the wetland. There is evidence to suggest that sample size was insufficient as: Dugesiidae, Aeshnidae and Veliidae appear more frequently in sweep intensity two and three respectively in comparison to sweep intensity six, as taxon diversity of sweep intensity four was anomalously low, and as the standard deviation did not decrease with increased sweep intensities. One other explanation for a lack of statistical difference between sweep intensities could be due to sweep intensities greater than six not being tested. As sweep intensity six samples collected excessive amounts of vegetation and sediment, sampling intensities greater than six does not appear to be a practical option. It was considered that increased number of sweeps may also increase the chance of more motile organisms escaping, however both Notonectidae and Corixidae, both highly motile macroinvertebrates, were well represented in the higher sweep intensity

samples (Appendix 2). From the results of this study, it is suggested that samples consisting of a five sweep intensity (which has a very similar mean taxon diversity and composition to that of six sweeps) should be used. This would minimise the risk of underestimating diversity, while limiting the collection of excess vegetation and sediment (which can lead to prolonged and less effective sample sorting and analysis).

The overall taxon diversity collected at Melmoth Vlei was low compared to samples collected from rivers in good condition in the KwaZulu-Natal midlands using the SASS5 protocol (pers. obs, Chutter 1998). This can be explained in part due to the fact that only a single biotope type was sampled during this study (SASS5 samples multiple biotopes), and the fact that wetlands typically have fewer biotopes than rivers. Wetlands also lack some of the biotopes characteristic of rivers, which contain many taxa, for example riffles, which provide an environment for several taxa that require high levels of dissolved oxygen to survive (Gerber and Gabriel 2002a). Low taxon diversity could also have been 'perceived' as a result of the large amounts of sediment and vegetation in some of the samples, which may have prevented the detection of all taxa present. Lastly, the sampling of rivers using the SASS5 protocol allows the researcher to conduct sampling for a greater length of time (Dickens and Graham 2002) which may result in a higher taxa count, compared to that collected by a fixed number of sweeps.

For this study there were no time restrictions for sample examination. Although time is a variable which can be kept constant, time limits were considered unsuitable as it was important that the largest possible proportion of macroinvertebrates within a sample be detected and identified.

There are a number of factors which could have contributed to organism detection bias during sample examination. The likelihood of bias from these

factors would have increased if a time restriction had been implemented. These factors include:

- Varying amounts of sediment and vegetation within the samples
- Larger more active and more visible organisms are more easily located than sedentary organisms, and
- Certain invertebrates are less easily detected as a result of their cryptic colouration/shape (e.g. Leptoceridae and Caenidae)

Sweep net sampling for one minute, two minutes and three minutes was initially considered for this study, as sampling under timed conditions is in keeping with a sampling method practiced in the U.S. (Burton 2001) and Australia (Chessman *et al.* 2002). However, it was concluded that collecting samples in vegetated areas for longer than 30 seconds (the average time taken to collect a six sweep intensity sample) would not be practical, due to the large amount of vegetation and sediment that would be collected. Excess vegetation also hinders the movement of the net, thus allowing trapped organisms to escape.

In the field excess vegetation was removed from a sample before it was placed in a container (notably sweep intensity samples five and six), as recommended by Ell (2000). Before this vegetation was discarded it was searched for organisms, which were then returned to the sample. As many organisms were found attached to vegetation, it was beneficial to keep some vegetation in the sample for later examination. Sediment rich samples required the longest search times, and fewer organisms were found within them in comparison to samples containing only vegetation. It is therefore recommended that sediment rich samples should be re-sieved with clean water, or/and (depending on the amount of sediment in a sample) subdivided and additional clean water added to disperse sediment.

Although the level of taxonomic identity that is sufficient for the biomonitoring of wetland water quality is debated (Fredrickson and Reid 1988, Adamus and Brandt 1990, Chessman *et al.* 2002, Helgen 2002), family level identification was selected due to: the previous success of SASS5 using this taxonomic level to monitor water quality; the limited entomological experience of researchers involved with this study, and other users likely to employ this technique; and the drive towards a rapid method that provides a high level of repeatability among users. However, relatively few taxa were collected in a single sample compared to an average SASS5 single biotope sample collected from a stream with similar water quality as Melmoth Vlei (pers. obs.). Thus, it is recommended that further research be conducted on selected taxa that are well represented within wetlands, notably Coleoptera and Diptera. Work at a higher taxonomic resolution within such groups may produce results that have potential for biomonitoring applications.

Recommended sampling technique

Based on the findings of this study, the following protocol for collecting macroinvertebrates from palustrine wetlands is recommended. To ensure adequate representivity of a wetland, the technique should be performed a minimum of twice (Ell 2000) but preferably up to six times throughout the study wetland (DiFranco and Stevenson 2000, Battle *et al.* 2001, Chessman *et al.* 2002, Helgen 2002).

1. Using a 300mm by 300mm aluminium framed sweep net, with a pore size of 1mm, and a handle length of one metre, five sweeps using the Gernes and Helgen (2000) method are performed in quick succession at a single location within the wetland. The Gernes and Helgen (2000) method (which constitutes a single sweep) requires a sweep net to be dragged strongly from the surface, down through the water column at a 45 degree angle, until it touches the bottom and then brought to the surface with the mouth facing

the surface. All sweeping for a single sample must be conducted at the same location within a $1m^2$ area

2. Sampling must take place in water 300-1000mm in depth, and within a vegetated area dominated by sedges
3. Contents of sweep net are placed in white photographic tray. Net is inverted and rinsed into tray to ensure all organisms are collected. Net is then examined for missed organisms
4. Large pieces of vegetation are inspected for macroinvertebrates, and then discarded from sample. All vegetation must not be removed from the sample because vegetation holds a high proportion of macroinvertebrates, and should be examined during step 8. If vegetation is removed and when collecting qualitative data, the percentage of both retained and discarded vegetation from a sampled must be recorded to avoid human error
5. Sample is sieved (using a sieve with mesh size of 1mm) to remove excess water and sediment
6. Sample is stored in a container with clean water and transported to the laboratory*
7. In the laboratory, sample is placed in white photographic tray with clean water and allowed to settle for five minutes
8. Sample is searched while macroinvertebrates are still alive. Searching is discontinued when no new taxa have been found after five minutes
9. Taxa that cannot be certainly identified should be retained and preserved in a 70% ethanol solution for expert identification

*Sample identification can be performed in the field or a laboratory; however it is important that for a study, sample identification is conducted consistently under well lit conditions

The technique described above has a number of limitations. Firstly it requires the presence of standing water, ideally 500mm or more in depth. This means that it may not be suitable for sampling many wetlands during the dry season when water levels are low. Furthermore, this technique is inappropriate for wetlands where there is little or no surface water present. The technique was

derived for sedge-dominated palustrine wetlands. This technique may not be practical in wetlands dominated by other vegetation types (e.g. *Typha* and *Phragmites* beds), nor in wetlands that have large amounts of woody material below the water surface, due to the difficulty in moving the net freely. In such cases (and in wetlands with shallow standing water) activity traps may prove more effective (Adamus and Brandt 1990) than was the case in this study, and further research should be done in this area. It would also be of value to test the technique in other regions of the country, as results may vary nationally.

Conclusion and Recommendations

Based on the findings of this study, sweep net sampling collected a significantly greater proportion of taxa than did activity trap sampling. Both techniques collected taxa that were not collected by the other technique. However, although activity traps collected some taxa that were poorly represented in sweep net samples, these were the exception, and did not represent any unique trophic group. Thus, based on these findings, the use of activity traps in addition to sweep net sampling does not justify the extra effort required.

There was no significant difference in taxon diversity collected using different sweep intensities. This result may have been due to an insufficient sample size. It can be concluded that it would be possible to use sweep intensities two to six for the sampling technique. However, using a sweep intensity of five was considered most appropriate, as samples collected using this sweep intensity were most similar in taxon diversity and composition to sweep intensity six, and greater sweep intensities collected an unmanageable amount of vegetation and sediment.

The aim of this study was to undertake a preliminary investigation on the development and testing of a macroinvertebrate sampling technique for use in palustrine wetlands. In order to develop a reliable, repeatable and scientifically proven protocol for use in a national wetland health biomonitoring programme, further research is required. Recommended areas for further research are:

- The testing of the proposed technique with a larger sample size, and over different wetland types and geographic regions
- Sampling of all wetland biotopes
- Sampling during different seasons

- Research into the applicability of certain taxa groups (notably Coleoptera, Diptera and Hemiptera) identified to genus or species level for wetland biomonitoring

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Appendix 1: Combined number of macroinvertebrates collected from activity traps. Twenty traps were set at each of the four depths. Although each sample was sorted and identified separately, results have been pooled

Family	Dugesiidae	Hirudinea	Hydracarina	Baetidae	Leptophlebiidae	Coenagrionidae	Aeshnidae	Libellulidae	Belostomatidae	Corixidae	Hydrometridae	Naucoridae	Nepidae	Notonectidae	Pleidae	Leptoceridae	Dytiscidae	Gyrinidae	Hydrophilidae	Ceratopogonidae	Chironomidae	Culicidae	Dixidae	Tipulidae	Lymnaeidae	Physidae
Placed on substrate	1	2	4	0	2	6	0	0	0	10	0	0	0	2	5	1	3	1	2	1	4	1	1	0	0	1
Placed at water surface	1	2	13	1	0	1	0	1	3	14	1	0	1	5	10	1	3	0	3	3	1	0	2	0	0	1
Placed just below surface	1	4	13	2	1	0	0	2	2	18	0	4	0	10	5	1	4	0	2	1	0	0	1	0	1	0
Placed 150mm-200mm below surface	1	2	9	2	1	4	2	1	2	9	0	1	0	1	5	2	5	0	1	1	4	4	0	2	1	1

Appendix 2: Combined number of macroinvertebrates collected from sweep net intensities. Ten sets of sweeps were carried out at each intensity. Although each sample was sorted and identified separately results have been pooled

Family / Sweep intensity	Dugesiidae	Hirudinea	Potamonautidae	Hydracarina	Baetidae	Caenidae	Coenagrionidae	Lestidae	Aeshnidae	Libellulidae	Pyralidae	Belostomatidae	Corixidae	Hydrometridae	Naucoridae	Nepidae	Notonectidae	Pleidae	Veliidae	Ecnomidae	Leptoceridae	Dytiscidae	Gyrinidae	Hydrophilidae	Ceratopogonidae	Chironomidae	Culicidae	Tipulidae	Lymnaeidae	Physidae
Two	9	6	0	6	3	6	10	0	7	1	1	8	3	1	3	0	3	10	0	1	1	1	3	0	1	6	2	1	2	2
Three	1	5	0	5	0	3	9	0	5	2	0	7	1	0	8	2	1	10	4	2	0	8	2	1	0	6	3	0	0	5
Four	2	3	0	4	2	5	10	1	3	3	1	9	1	0	5	0	1	7	0	0	1	7	3	1	3	10	0	0	1	1
Five	3	7	0	7	0	6	8	0	3	6	0	8	5	1	4	1	1	9	2	4	1	7	3	0	5	9	2	0	0	4
Six	4	5	1	9	1	6	7	1	1	4	1	8	3	1	5	2	2	9	0	2	0	10	1	0	2	10	4	0	2	5

Component B2

Testing the applicability of the SASS5 scoring procedure for assessing wetland health: a case study in the KwaZulu-Natal midlands, South Africa

Testing the applicability of the SASS5 scoring procedure for assessing wetland health: a case study in the KwaZulu-Natal midlands, South Africa

Abstract

This paper documents the procedure undertaken to test the applicability of the South African Scoring System Version 5 (SASS5) scoring and calculation procedure in nutrient enriched palustrine wetlands in the midlands of KwaZulu-Natal, South Africa. Four reference wetlands and three dairy effluent impacted wetlands were sampled. Six macroinvertebrate samples were collected with a sweep net from each wetland. For each sample, the macroinvertebrates were identified and assigned a predetermined SASS5 tolerance score between 1 and 15, with higher scores indicating increased sensitivity to poor water quality. The SASS5 scores for each sample were then summed (total SASS5 score) and divided by the total number of taxa identified in the sample. Data for selected physico-chemical variables, macrohabitat condition, biotope suitability and organism detectability were collected to assist in interpretation of results. Thirty-nine taxa, identified to family level, were collected during sampling. SASS5 scores ranged from 15 to 82. Five of the wetlands had mean SASS5 scores of between 46 and 59. Five of the wetlands had an intra-wetland SASS5 score range of greater than 30. Average score per taxa (ASPT) values ranged from 3.3 to 5.5, and few high scoring (≥8) taxa were collected. There was no significant difference in SASS5 scores between samples collected above, at and downstream of an effluent discharge point within the same impacted wetland. SASS5 scores for reference wetlands were also not significantly higher than those recorded for impacted wetlands. Comparison of ranked SASS5 scores and environmental data did suggest a relationship between the variables, but this was not significant. Based on the SASS5 score water quality guidelines, all sampled wetlands were considered to have impacted water quality. However, this was not supported by the macrohabitat and physico-chemical results. Possible reasons for the low SASS5 scores include: the lack of biotopes present in wetlands

compared to rivers, limited habitat availability due to low rainfall during the sampling season, lower levels of dissolved oxygen present compared to rivers, and the limited detectability of organisms due to large amounts of substrate in the samples. Without modification, SASS5 appears unsuitable for assessing wetlands. However, variation in taxon composition within impacted wetlands and between reference and impacted wetlands, revealed through multivariate analysis, suggests that macroinvertebrates are responsive to changes in wetland condition, and thus show potential as indicators of wetland water quality. Further research should focus on the testing of SASS5 throughout the year, in a range of wetland types, and in wetlands moderately to severely impacted by pollutants. It is recommended that a habitat or biotope index be further developed and used in conjunction with any future wetland macroinvertebrate bioassessment protocols.

Introduction

Globally, wetlands are considered to be one of the most endangered habitat types (Maltby 1991). In South Africa, there has been considerable loss of wetlands and their associated benefits to humanity, such as water quality enhancement (Kotze *et al.* 1995). The degradation of South African wetlands is primarily due to agricultural development, such as drainage and poor land use practices (Kotze *et al.* 1995). This loss is a concern now recognised by national government as requiring urgent action (DEAT 1997). The protection of wetlands is considered essential in sustaining South Africa's water resources (Whyte 1995). There are several statutes applicable to wetlands including the South African National Water Act 36 of 1998, the Environment Conservation Act 73 of 1989, and the National Environmental Management Act 107 of 1998. For South Africa to comply with both national and international legislation and conventions, and provide for both environmental and human needs, water quality monitoring techniques need to be developed and implemented for all water resources, including wetlands (DWAF 2004).

South Africa already has a well established biomonitoring protocol for streams and rivers called SASS5 (South African Scoring System Version 5). This system uses macroinvertebrates as biomonitoring water quality tools, and has been specifically designed to detect changes within aquatic macroinvertebrate communities (Dallas *et al.* 1999, Dickens and Graham 2002). In SASS5, tolerance values which range between 1 and 15, with higher scores indicating increased sensitivity to poor water quality, are assigned to chosen aquatic invertebrates at family level. These values are processed through simple calculations to determine river health (see *Derivation of SASS5 scores*).

SASS was originally derived from the British Monitoring Working Party system (BMWP) by Chutter (1994). Since its original derivation, the method has been modified four times. The latest modification of this method, SASS5, has been

carried out by Dickens and Graham (2002). Recently, a simplified biomonitoring method based on SASS5, called mini-SASS, has been developed. Mini-SASS has been designed as an environmental education tool for use by communities to monitor the health of their local rivers and streams (Graham *et al.* 2004).

SASS5 is currently considered as the industry standard for biomonitoring in South Africa (de la Rey *et al.* 2004) and plays a dominant part in the South African National Rivers Health Programme (Uys *et al.* 1996). It has also been employed to assist with the determination of the Ecological Reserve as required by the South African National Water Act 36 of 1998 (Dickens and Graham 2002). SASS5 has been used extensively throughout South Africa by institutions including irrigation boards (e.g. Umlaas Irrigation Board), water boards (e.g. Umgeni Water), the CSIR, DWAF, universities and consultants (Graham *et al.* 2004).

Extensive work on wetland biomonitoring using macroinvertebrates has taken place in the U.S.A. and Australia. Within the U.S.A, the U.S. Environmental Protection Agency (EPA) has developed wetland biomonitoring in many states, and much of this work has been well documented. Hicks (2000) has developed a wetland macroinvertebrate sampling protocol for New England, and has published a comprehensive manual called NEFWIBP (New England Freshwater Wetland Invertebrate Biomonitoring Protocol). In Australia Chessman *et al.* (2002) have developed SWAMPS (Swan Wetland Aquatic Macroinvertebrate Sensitivity), specifically for wetlands in the Perth region.

In many cases where wetland biomonitoring programmes using aquatic macroinvertebrates have been developed, they have largely been derived from local river monitoring methods (Teels and Adamus 2002). The first step towards developing a macroinvertebrate biomonitoring protocol for wetlands in South Africa therefore is to investigate to what extent SASS5 is applicable to wetlands.

To date, no attempt at testing the applicability of SASS5 in wetlands has been published.

The aim of the study was to test whether SASS5 could be used to determine the health of nutrient enriched palustrine wetlands in South Africa. The study was also intended to contribute to baseline data on macroinvertebrates in palustrine wetlands. Due to wetlands and rivers having different biotopes, the SASS5 sampling protocol could not be used, thus an appropriate sampling technique, derived during a previous study, has been employed (Bowd *et al.* in prep). For this study, sampling of both reference (RWs) and impacted wetlands (IWs) was conducted. RWs are wetlands that are minimally impacted by human activities, and are representative of the natural conditions of the area (Hicks 2000, Parker 2002). For the purpose of this study, IWs are wetlands that receive dairy effluent as their main source of nutrient enrichment. The pollution type (dairy effluent) and wetland type (palustrine) were restricted to minimise variability of results, as pollution and wetland type can both affect taxon composition (Waterwatch, Water and Rivers Commission 1996, Batzer *et al*. 1999).

Several authors recommend assessing wetlands within the same landscape context in order to gain an understanding of a wetland's condition and its stressors (Hicks 2000, Teels and Adamus 2002, van der Valk 2002). Therefore, a macrohabitat score sheet was derived, and used in conjunction with physico-chemical information, to compare with the macroinvertebrate samples.

As wetland biotopes influence the composition and diversity of macroinvertebrate communities (Davis *et al.* 1993, Mitsch and Gosselink 2000, Chessman *et al.* 2002), and the presence of vegetation and sediment in a sample can affect the detection of macroinvertebrates (Helgen 2002), a sample biotope and detection assessment (SBDA) index was developed (USFWS 1980, Chessman *et al.* 2002, DWAF 2004). Chutter (1998) identified a need for a biotope assessment technique to be used in conjunction with SASS when key biotopes were absent

from a sampling site (van Staden 2003). The technique used was originally referred to as the Invertebrate Habitat Assessment Index (IHAI) (McMillan 1998), however it was renamed as the Invertebrate Habitat Assessment System (IHAS) in 1999 (McMillan 2002). The purpose of the SBDA index is thus to assist in discriminating as to whether results might be due to SASS5 being inapplicable/applicable in palustrine wetlands, or alternatively due to poor quality of sampling biotope and/or the detectability of macroinvertebrates.

The aim of this study was to determine whether SASS5 could be used to assess the health of nutrient enriched palustrine wetlands in the midlands of KwaZulu-Natal, South African.

To achieve this aim, this study had the following objectives:

1. Identify and measure a range of parameters which can indicate palustrine wetland health
2. Establish if any aspect of the SASS5 scoring procedure (total SASS5 score, ASPT or taxon diversity) is applicable to the determination of the health of nutrient enriched palustrine wetlands
3. Make recommendations for further testing of SASS5, as well as other derived wetland bioassessment methods

Study area

Sampling took place at three IWs and three RWs in the KwaZulu-Natal midlands of South Africa. Data from a fourth RW (RW 1), derived from a previous study, have been included (Figure 1).

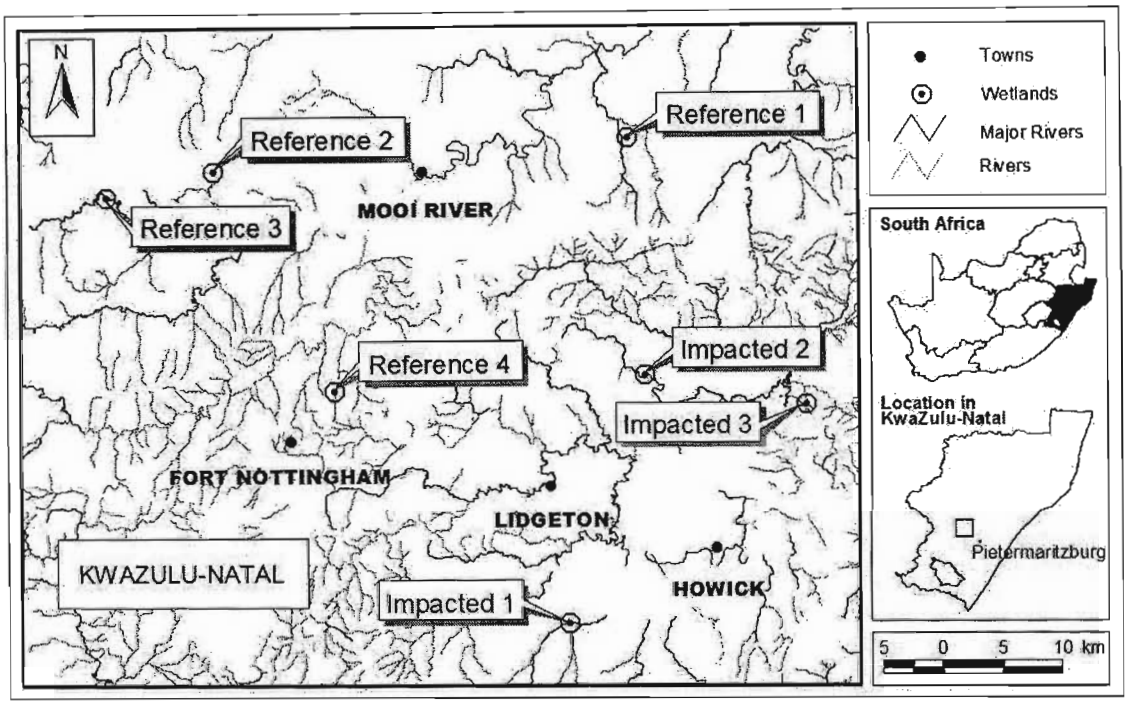

Figure 1: Map showing location of sampled wetlands in KwaZulu-Natal, South Africa

General information for all wetlands sampled is summarised in Table 1. Physical and climatic information were derived from Camp (1995) and Begg (1989). Catchment size was derived using 1:50 000 topographical maps of the area.

Table 1: General information for study wetlands. RW=reference wetland, IW=impacted wetland, B=before impact, A=at impact, D=down stream of impact, *=partially attributable to impoundment

General information	RW 1	RW 2	RW 3	RW 4	IW 1	IW 2	IW 3
Longitude Latitude	30.09.36.0 29.10.48.0	29.50.54.6 29.12.15.7	29.46.05.0 29.13.24.0	29.56.21.0 29.22.09.0	30.06.55.4 29.32.34.1	30.10.19.3 29.21.29.2	30.17.37.4 29.22.49.0
Altitude (m)	1595	1583	1534	1471	1100	1308	1102
Size (ha)	104	47.3	162	39.01	38.2	19.33	44
Width (m) Average Maximum	280 650	92 256	266 528	102 294	147 253	239 280	98 205
Catchment size (km^2)	3.5	5.3	40.7	3.1	14.6	4.7	0.7
Mean annual precipitation (mm)	1100	947	947	777	794	986	986
Mean annual temperature (^oC)	15	14.2	14.2	14.6	16.6	15.9	15.9
Plant communities within wetland	Sedge/grass *Typha*	Sedge/grass	Sedge/grass *Typha*	Sedge/grass *Phragmites*	Sedge/grass *Phragmites Typha* Algae	Sedge/grass *Phragmites Typha* Algae	Sedge/grass *Phragmites Typha*
Estimated average distance between sample points (m)	10	15	15	10	50 between B, A and D 10 between two samples taken at each location	50 between B, A and D 10 between two samples taken at each location	20 between B, A and D 10 between two samples taken at each location
Area of surface water in wetland (%)	2	1.5	4	30	60*	20*	2*

Methods

Sampling of the six main wetlands was conducted between 29th January 2004 and 17th February 2004. Sampling at RW 1 took place on 10th November 2003.

Wetland selection

Three IWs and four RWs were sampled for this study. Hicks (2000) and Parker (2002) recommend sampling a minimum of three RWs, and Apfelbeck (1999) recommends that 75% of samples in a study should be collected from RWs, with the remaining 25% being extracted from IWs.

Potential RWs and IWs in the KwaZulu-Natal midlands were identified through examination of 1:50 000 topographical maps of the area. Site visits and consultations with local environmental organisations and land owners were conducted in order to select suitable wetlands for the study. The main features considered were: the availability of suitable sampling biotope (see *suitable sampling biotope*) including depth of water; the present surrounding land use (<700m from the wetland's saturated edge); and the absence of any manmade structures (such as dams and weirs) upstream of the wetland. When selecting IWs, wetlands that were least impacted by activities that could affect water quality (other than dairy farming), were preferred (Karr and Chu 1999).

When selecting the wetlands, an attempt was made to choose those whose physical and climatic attributes (Hicks 2000, Parker 2002) and wetland classification (Hicks 2000) were as similar as possible (Karr and Chu 1999). If a RW differs from an IW in more than one way, any differences in the biological communities cannot be attributed to a single factor (Hicks 2000). Only one wetland type (palustrine) was sampled in this study as different wetland types (e.g. palustrine, lacustrine) have different invertebrate assemblages (Batzer *et al.* 1999).

Field data collection procedure

At each wetland, samples were taken from six sample points (as recommended by DiFranco and Stevenson (2000), Battle *et al.* (2001), Chessman *et al.* (2002), Helgen (2002)). Suitable sampling points were selected throughout each RW (see below). At IWs, two sampling points adjacent to the effluent discharge point, two below, and two above the effluent discharge point were selected (Hicks 2000).

Suitable sampling biotope

Sampling points were located in emergent vegetation dominated by sedges, as this biotope contains greater taxon diversity in comparison to open water areas that lack submerged vegetation (Olson *et al.* 1995). Sampling took place in water between 0.3-1m in depth, as 0.3m is the minimum depth in which a sweep net can effectively collect organisms, and a depth of less than 1m is recommended by Apfelbeck (1999), Gernes and Helgen (2000), Helgen (2002) and DiFanco and Stevenson (2000). Only wetlands with permanent water were sampled, as a wetland's hydroperiod is likely to affect aquatic macroinvertebrate composition (Detenbeck 2002). Samples were taken from permanently inundated locations.

At each sampling point, four types of data were collected:

1. *Physico-chemical data*

A physico-chemical data sheet was completed for each sample point (Appendix 1). Temperature, pH, electrical conductivity and total dissolved solids (TDS) were measured using a calibrated HANA HI 991 330 Portable pH/EC/TDS/temperature meter. Turbidity, odour, colour of water and current velocity were also noted. Although many sampling protocols require the measuring of dissolved oxygen, this parameter was not measured, as oxygen levels in wetlands naturally fluctuate over the course of a day, and thus dissolved oxygen is not a good indicator of wetland condition (Hicks 2000).

Nitrogen (nitrate + nitrite), ammonia and phosphorus testing was carried out to obtain an indication of the level of dairy effluent reaching a wetland, as they are among the main pollutants released from a dairy (DWAF 1996). Other activities that can cause increased levels of these three compounds are fertilizer application, ineffective wastewater treatment systems, fossil fuel and urban runoff (Adamus and Brandt 1990, DWAF 1996). Thus, the presence of these activities/sources was taken into account during the analysis of results. Water samples were collected using sterile plastic containers. Samples were stored in a refrigerator overnight and processed within 24 hours.

2. *Macroinvertebrate collection*

A standard SASS5 sweep net was used to collect macroinvertebrate samples. The net has a pore size of 1mm and is mounted on a square aluminium frame 300mm by 300mm (Chutter 1998). A one metre handle is attached centrally to one side of the aluminium frame. A sampling protocol derived for palustrine wetlands (Bowd *et al.* in prep) was used in this study. The protocol uses the Gernes and Helgen (2000) sweep method, and incorporates samples from the water surface, water column and substrate. The sampling of these three locations is a common feature of several wetland bioassessment protocols (Gernes and Helgen 2000, Hicks 2000, Burton 2001, Chessman *et al.* 2002). One sample comprises of five sweeps carried out in quick succession. A description of one sweep is given below.

Sweep technique

The sweep net is drawn strongly from the surface, through the water column (at a 45 degree angle), until the net reaches the bottom of the wetland. The net is then lifted to the surface with the opening of the net parallel to the surface, ensuring that macroinvertebrates do not escape.

Post extraction from a wetland, the contents of the net were placed in a white tray (250mm by 350mm). The net was then inverted and additional water was passed

through the net to wash out any remaining organisms into the tray. Following this the net was checked for any macroinvertebrates clinging to the mesh. Larger pieces of vegetation were discarded after ensuring the majority of macroinvertebrates had been removed. Not all vegetation was removed, as vegetation holds a large proportion of macroinvertebrates within a sample (DiFranco and Stevenson 2000, Bowd *et al.* in prep). The contents of the white tray were then placed on a sieve (mesh size 1mm) and rinsed. The contents of the sieve were placed in a container with clean water.

3. Macrohabitat score sheet

A single macrohabitat score sheet was completed for each RW, and three macrohabitat score sheets were completed for each IW (one each for above, at and below the effluent discharge point) (Appendix 2). The macrohabitat score sheet was derived from Hicks (2000) and Gernes and Helgen (2002).

The macrohabitat score sheet has six key aspects which cover a range of disturbances within both the landscape and the wetland (as suggested by Teels and Adamus 2002):

1 Habitat alteration within the wetland*
2 Hydrological alteration affecting flow of water into/out of wetland- severity and degree of alteration
3 Buffer landscape disturbance from wetland edge* to 100m from wetland edge- extent and intensity
4 Landscape (immediate) influence from 100m from wetland edge* to 500m from wetland edge- extent and intensity
5 Impairment from dairy (for IW sample sites)- extent and intensity
6 Physical characteristics

* Wetland area estimated by vegetation distribution

For each aspect there is a choice of four answers that reflect wetland condition, range in degree of disturbance, and have relative scores:

0 Best- no evidence of disturbance

7 Moderate- predominantly undisturbed

14 Fair- significant disturbance

21 Poor- major disturbance

The scores for each aspect were combined to derive the degree of impairment of each wetland (as recommended by Teels and Adamus 2002).

Land owners of each of the wetlands identified were questioned about what impacts the wetland may receive, how the land use surrounding the wetland changes throughout the year, land use in recent weeks, the permanence of water in the wetland, and the last rainfall event. These questions were recommended by Parker (2002) and Butcher (2003) who stress that the time lag between stressor occurrence and macroinvertebrates responses is a major factor which must be investigated.

When scoring the aspects, distance between disturbance and wetland, type of alteration, land use and severity of impact were considered. Also considered was the time period (current, recent or distant past) at which alteration/land use change occurred. Greater weight was given to alterations/land use in areas upstream or upslope of the site (as recommended by Teels and Adamus 2002 and van der Valk 2002).

4. Sample biotope and detection assessment (SBDA)

The scoring of five metrics (Table 2) was conducted once for each RW, and three times for each of the IW (one each for above, at and below the effluent discharge point). Aspects of the metrics and scoring procedure was derived from DeKeyser *et al.* (2003), Fennessy *et al.* (2002), McMillan (2002) and Gernes and Helgen (1999).

Table 2: Sample biotope and detection assessment (SBDA) index

Metric	Acronym	Criteria	Score
Area of sedge-dominated vegetation standing in water >0.5m deep within wetland	Sedge m^2	$>25m^2$ $12\text{-}25m^2$ $<12m^2$	5 3 1
% density of vegetation in a $1m^2$ quadrat at sample point	% veg.$1m^2$	>60% 60-30% <30%	5 3 1
% of sediment covering base of white tray	% sed.	<30% 30-60% >60%	5 3 1
% of *Lemna minor* covering water surface when sample is placed in white tray	% *L. minor*	<30% 30-60% >60%	5 3 1
% of vegetation occupying sample when placed in white tray	% veg.	<30% 30-60% >60%	5 3 1

For each sample site, the score of each metric was summed (total metric score), and then classed into one of three SBDA classifications:

5-11 Poor biotope / detectability

12-18 Fair biotope / detectability

19-25 Good biotope / detectability

The availability of suitable sampling biotope has been assessed because, if a wetland has a less than adequate area ($<12m^2$) where sampling can take place, disturbance from the collection of other samples could affect the macroinvertebrates collected, and consequently affect results. A greater choice of suitable sampling biotope is likely to have a positive influence on results.

It was considered important to assess vegetation density, as vegetation provides a critical habitat structure for macroinvertebrates, and vegetation density is likely to affect macroinvertebrate community (Mitsch and Gosselink 2000).

The collection of vegetation, *Lemna minor* and sediment in a sample can hinder the detection of macroinvertebrates during sample examination. Although the collection of vegetation can accommodate some invertebrates and thus improve sample results (Bowd *et al.* in prep), excessive amounts can cause invertebrates to go unnoticed (Helgen 2002).

Data analysis

Macroinvertebrate identification

For each sample, macroinvertebrates were identified to family level, under laboratory conditions (as recommended by Barbour *et al.* 1996 and Helgen 2002) and recorded on a SASS5 score sheet. Each sample was placed into a white tray (see previous section). Samples were left to stand for five minutes to allow vegetation and sediment to settle and invertebrates to emerge (Dickens and Graham 2002). A hand lens (Triplet 10x) was used to assist in macroinvertebrate identification. Invertebrates were identified using Gerber and Gabriel (2002a, b). Each sample was examined until no new macroinvertebrates were detected after five minutes (Dickens and Graham 2002). As it was easier to locate and identify live macroinvertebrates (Fong and Nou 2001, Bowd *et al.* in prep), all samples were analysed on the same day they were collected. This was done to reduce any bias as different taxa vary in their ability to survive confined in containers (pers. obs.). Macroinvertebrates that could not be identified were preserved with a 70% ethanol solution for later identification by an entomologist. A selection of identified macroinvertebrates was also shown to an entomologist for auditing purposes (as recommended by Hicks (2000) and Chessman *et al.* (2002)).

Derivation of SASS5 scores

To determine water quality for each of the sample points using the SASS5 scoring procedure, the predetermined taxa tolerance values of all the invertebrates found in a sample were added together (the SASS5 score), and the taxon diversity was recorded. The ASPT (average score per taxa) was then calculated by dividing the SASS5 score by the number of taxa found in the

sample. Finally, reference was made to Table 3, and the water quality value predicted by SASS5 for a sample point was determined.

Table 3: The SASS5 score water quality guidelines (Chutter 1998). WQ=water quality, HD=habitat diversity

SASS5 score	ASPT	Water quality and habitat diversity
>100	>6	WQ Natural HD High
<100	>6	WQ Natural HD Reduced
>100	<6	Borderline case between WQ natural and some deterioration in WQ
50-100	<6	Some deterioration in WQ
<50	variable	Major deterioration in WQ

Phosphorus, ammonia and nitrogen testing

Phosphorus levels in the samples were measured using a calibrated UV-Visible Spectrophotometer (Varian, model Cary 1E). Ammonia and nitrogen levels were measured using an Autoanalizador TRAAC 2000 (Bran and Lubbe). The proportion of total ammonia that comprised un-ionised ammonia (NH_3) was calculated as a function of pH and water temperature (DWAF 1996). Outliers are given in the results, but were excluded from calculations of average values for each site. In such cases, the next highest value is provided for comparison. All chemical data were interpreted according to guidelines provided by DWAF (1996).

Statistical analysis

Correspondence analysis (CA), using the programme CANOCO 4.51 (Ter Braak and Šmilauer 2003), was used to reveal patterns in the variation of taxon composition among and within the wetlands sampled. Analysis of Variance (ANOVA) was used to identify if there was a significant variation in SASS5 scores, ASPT and taxon diversity between the wetlands sampled. To identify how wetland SASS5 score, ASPT and taxon diversity means differed to one another,

the Tukey *Post hoc* honest statistical difference (HSD) test, using Statistica 5.1 (version 5, 1998 edition, StarSoft Inc, Tulsa, Oklahoma, U.S.A) was conducted. Significance was defined at a level of 0.05. The Tukey HSD test was used to rank SASS5 score, ASPT and taxon diversity for all wetlands. Spearmen's rank correlation coefficient was used to identify if SASS5 score, ASPT, physico-chemical and macrohabitat score were statistically related to one another. Canonical correspondence analysis (CCA), using the programme CANOCO 4.51 (Ter Braak and Šmilauer 2003), was used to examine the direct effect of the physical and chemical (pollutant) characteristics of the water on variation in taxon composition among wetlands as a means of identifying possible indicator taxa that were sensitive to water pollution. The effects of the selected physico-chemical variables on composition was assessed with a Monte Carlo Permutation Test (n=499).

Results and discussion

Physico-chemical, macrohabitat and macroinvertebrate results

Physico-chemical results

In South Africa, the inorganic nitrogen concentrations of unimpacted aerobic surface waters are typically <0.5mgN/L, but these concentrations may increase to >5-10mgN/L in highly enriched systems. All samples tested had low nitrogen levels, with values of <0.5mgN/L being collected for both RWs and IWs and thus were oligotrophic (Tables 4 and 5 respectively). The exceptions were the 'down stream' samples collected from IW 1 and the 'at' impact samples taken from IW 3, which were slightly mesotrophic.

The RWs that were tested for phosphorus, were all mesotrophic (between 5 and 25μg/L), with the exception of RW 4, which was slightly eutrophic. The IWs (including the 'before' impact samples) were all eutrophic (between 25 and 250μg/L). Unimpacted waters commonly have a phosphorus concentration of between 1 and 50μg/L, and all measured RWs closely conform to this. All IW samples, with the exception of the 'down stream' IW 3, had phosphorus levels in excess of 100μg/L. Impacted systems usually have a nitrogen: phosphorus ratio of <10:1, and unimpacted systems generally have a ratio of 25-40:1 (DWAF 1996). No ratios for RWs could be determined due to the lack of measurable nitrogen. The ratio of nitrogen to phosphorus for all IW samples was <10:1; this is a result of high phosphorus levels and relatively low inorganic nitrogen levels.

All samples tested for un-ionised ammonia collected from both RWs and IWs were within an acceptable range (Target Water Quality Range (TWQR)) according to DWAF (1996). All un-ionised ammonia results were low, as pH and temperature for the measured samples were generally low, and the proportion of un-ionised ammonia present is a function of temperature and pH.

As electrical conductivity and TDS are related (TDS ≈ electrical conductivity x 6.5), electrical conductivity has been omitted from all statistical procedures following Table 5. TDS is known to be a good indicator of water quality, however interpretations of one-off samples are of limited value (DWAF 1996). Although TDS levels at the IWs were lower at the 'before' impact sample points compared to the 'at' and 'down stream' sample points, there was no trend in the RWs having less total dissolved solids in comparison to the IWs.

According to chemical data collected from the wetlands, none of the wetland sample sites, including the 'at' and 'down stream' samples collected from the IWs, were severely polluted. Most notable were the elevated levels of phosphorus at the IWs.

Table 4: Physico-chemical information for reference wetlands (RW). B=before impact, A=at impact, D=down stream of impact, NT=not taken, *=next highest value

Aspect	RW 1		RW 2		RW 3		RW 4	
	mean	outlier	mean	outlier	mean	outlier	mean	outlier
Physico- chemical								
pH	4.2		4.7		5.8		5.6	
Conductivity (mS/m)	40		65		51		91	
TDS (ppm)	19		32		26		46	
Phosphorus (µg/L)	NT		6	360 (30*)	6	200 (30*)	64	1000 (130*)
Ammonia (mg/L)	NT		0.05		0.07		0.3	
Nitrogen (mg/L)	NT		0		0		0	
Physical measurements								
Temperature (°C)	19		20.55		23.93		19.88	
Depth (cm)	70		52		50		77	
Turbidity (cm)	45		33		48		23	

Table 5: Physico-chemical information for impacted wetlands (IW). B=before impact, A=at impact, D=down stream of impact, *=next highest value

Aspect	IW 1					IW 2					IW 3				
	B	A	D	mean	outlier	B	A	D	mean	outlier	B	A	D	mean	outlier
Physico- chemical															
pH	6	6.2	5.9	6.03		4.6	4.5	4.5	4.53		4.6	4.8	5	4.8	
Conductivity (mS/m)	87	118	120	108		36	98	75	69		25	60	64	49	
TDS (ppm)	43.5	58.5	59.5	53.83		18.5	48.5	31	32.7		12.5	29.5	32	24.7	
Phosphorus (µg/L)	100	165	100	122		150	450	150	166	670 (230*)	150	150	30	110	
Ammonia (mg/L)	1.1	1.7	1.7	1.6	22.9 (2.3*)	0.08	0.27	0.08	0.14		0	0	0	0	
Nitrogen (mg/L)	0.1	0.19	0.58	0.29		0.2	0.27	0.19	0.22		0.5	0.98	0.26	0.58	
Physical measurements															
Temperature (°C)	24.5	23.7	24.3	24.2		24.7	24.3	23.3	24.1		22.5	19.7	22.5	21.6	
Depth (cm)	68	81	78	75		59	64	66	63		58	60	53	57	
Turbidity (cm)	34	37	30	34		30	26	24	27		29	34	36	33	

Macrohabitat score results

With the exception of RW 1, grazing, afforestation, sediment input, or a combination of these impacts, were present at all wetlands (Table 6). Three of the RWs had minor habitat alteration, while all IWs were highly altered. Draining and impounding had occurred at all of the IWs, and canalisation was present in two of these wetlands. IW 3 was severely canalised and thus scored highest.

Of the four RWs, only RW 3 had some cropping located within the wetland's 100m buffer. All IWs had intensive cropping within their buffer areas and, although IWs 2 and 3 also featured human disturbance (dirt roads) and exotic vegetation, IW 1 scored the highest, as intensive cropping within this wetland's buffer was particularly severe. RW 3 and 4 had minimally modified landscapes (100-500m from wetland edge), whereas all the IWs were highly impacted by intensive cropping, exotic vegetation, human disturbance or a combination of these impacts.

As no odour or colour of water indicative of impacted conditions was recorded, only the presence of algae influenced the scoring. Algae was only recorded at the 'at' and 'downstream' sample points at IWs 1 and 2.

The distance between the IWs and the dairies ranged between 100 to 300 metres. All dairies had slurry pits to reduce dairy effluent from entering the wetland. All dairies were assigned the same score for their effect on their respective wetland (Table 6). Although the dairy adjacent to IW 3 had only been established for one year (whereas the other two were much older) this wetland scored the same as the other two IWs, as more cows were milked daily and it was located only 100m from the dairy.

The wetlands were ranked in order of impairment according to the derived macrohabitat scores (Table 10). The 'at' and 'down stream' sites of the IWs scored highest, with IW 1 and 2 being considered the most degraded. As

expected, all RWs were less impaired than any of the IWs. All 'before' sites were less impaired than any of the 'at' and 'down stream' IW sites, but more impaired than the RW sites.

Table 6: Macrohabitat scores for all wetlands. RW=reference wetland, IW=impacted wetland, Alt.=alteration, G=grazed, PL=plantation, SI=sediment input, D=drained, I=impounded, C=canalised, IC=intensive cropping, EX=exotic vegetation, HD=human disturbance, Br=brown, Re=red, Al=algae, B=before impact, A=at impact, D=down stream of impact

Aspect	RW 1	RW 2	RW 3	RW 4	IW 1	IW 2	IW 3
Habitat alteration immediate landscape (within wetland)	No alt.	G	SI	G,SI	PL,SI	G,PL	G,PL,SI
					B A D	B A D	B A D
Habitat score	0	7	7	7	14 14 14	14 14 14	14 14 14
Hydrological alteration	No alt.	No alt.	Alt.	Alt.	D,I	D,I,C	D,I,C
Inlet restriction					Dam	-	Dam
Outlet restriction					Dam	Dam	Dam
					B A D	B A D	B A D
Habitat score	0	0	0	0	14 14 14	14 14 14	7 21 21
Buffer landscape disturbance <100m	No alt.	No alt.	IC	No alt.	IC	IC,EX,HD	IC,EX,HD
					B A D	B A D	B A D
Habitat score	0	0	7	0	14 14 14	7 14 14	7 7 7
Landscape influence 100-500m	No alt.	No alt.	IC	HD	IC,HD	IC,EX,HD	IC,EX,HD
					B A D	B A D	B A D
Habitat score	0	0	7	7	14 14 14	14 14 14	14 21 21
Degree of impairment from dairy	None	None	None	None	Moderate –significant	Moderate – significant	Moderate – significant
					B A D	B A D	B A D
Habitat score	0	0	0	0	0 14 7	0 14 7	0 14 7
Physical characteristics					B A D	B A D	B A D
Colour/Observations	Cl	Oil,Cl	Oil,Cl	Br	Br Al Al	Br Al Al,Re	Cl Br Br
Odour	Odourless	Sulphur	Sulphur	Odourless	Sulphur	Sulphur	Odourless
Habitat score	0	0	0	0	0 14 14	0 14 14	0 0 0
Total Habitat scores	**0**	**7**	**21**	**14**	**56 84 77**	**49 84 77**	**42 77 70**

Macroinvertebrate results

Collectively, 38 SASS5 used taxa were identified in the samples. Pyraustidae (Lepidoptera) was the only aquatic taxon collected which is not included in SASS5. Both RWs and IWs had five taxa not collected in the other type of wetland (Table 7). Results for individual RWs and IWs are displayed in Appendix 3 and Appendix 4 respectively.

Of the 17 orders used by SASS5, 12 orders were collected from the sampled wetlands (Table 7). The orders not recorded in the wetlands were Porifera, Coelenterata, Pelecypoda, Plecoptera and Megaloptera. Porifera, although they inhabit slow moving streams, live under pebbles, rocks or any solid substrate (Gerber and Gabriel 2002a). Pelecypoda, Megaloptera and Plecoptera prefer fast flowing water. Megaloptera prefer rocks at the waters edge, and Plecoptera (rarely found in KwaZulu-Natal, Picker (*et al.* 2003)) and Pelecypoda favour gravel or rocky beds (Gerber and Gabriel 2002a, Picker *et al.* 2003). Coelenterata live attached to solid surfaces. It is evident that the biotopes inhabited by these five families are not commonly present in palustrine wetlands, thus it is not unexpected that these macroinvertebrates were absent (see Appendix 5 for a breakdown of which SASS5 families inhabit stones in/out of current, fast/slow moving water).

The macroinvertebrates collected from the wetlands were mostly from the predator and gathering collector functional feeding groups, based on Hicks (2000). Barbour *et al.* (1999) considered piercers, shredders and scrapers to be sensitive organisms, and thus well represented in healthy streams. In this study, piercers (Corixidae) and scrapers (Lymnaeidae, Physidae and Baetidae) were predominantly collected from RWs and sample sites located above the effluent discharge point at the IWs (Table 7, Appendices 3 and 4). Shredders were rare and equally distributed between IWs and RWs.

Table 7: Macroinvertebrates found in reference and impacted wetlands. SASS5 tolerance value-15=intolerant taxa, 1=most tolerant taxa, GC=gathering collectors, PR=predators, SC=scrapers, PI-H=piercers-herbivores, SH=shredders, OM=omnivores (Hicks 2000)

Order	Family	SASS5 tolerance value	Functional feeding group	Found in which type of wetland	
				Reference	Impacted
TURBELLARIA	Dugesiidae	3	GC	✓	✓
ANNELIDA	Hirudinea	3	PR	✓	✓
CRUSTACEA	Potamonautidae	3	-	✓	✓
	Atyidae	8	-		✓
HYDRACARINA	-	8	PR	✓	✓
EPHEMEROPTERA	Baetidae	4	GC/SC	✓	✓
	Caenidae	6	GC	✓	✓
	Leptophlebiidae	9	GC	✓	
ODONATA	Coenagrionidae	4	PR	✓	✓
	Aeshnidae	8	PR	✓	✓
	Libellulidae	4	PR	✓	✓
	Chlorolestidae	8	-		✓
	Platycnemidae	10	-		✓
LEPIDOPTERA	Pyraustidae	-	-	✓	✓
HEMIPTERA	Belostomatidae	3	PR	✓	✓
	Corixidae	3	PI-H	✓	✓
	Hydrometridae	6	-	✓	
	Naucoridae	7	PR	✓	✓
	Notonectidae	3	PR	✓	✓
	Pleidae	4	-	✓	✓
	Veliidae	5	-	✓	✓
	Gerridae	5	PR	✓	✓
TRICHOPTERA	Ecnomidae	8	-	✓	
	Leptoceridae	6	OM	✓	✓
	Hydropsychidae	4	-	✓	
COLEOPTERA	Dytiscidae	5	PR	✓	✓
	Gyrinidae	5	PR	✓	✓
	Hydrophilidae	5	GC	✓	✓
	Elmidae	8	GC/SH	✓	✓
	Haliplidae	5	SH/PI-H		✓
	Helodidae	12	-	✓	✓
DIPTERA	Ceratopogonidae	5	PR	✓	✓
	Chironomidae	2	OM	✓	✓
	Culicidae	1	PR	✓	✓
	Psychodidae	1	-	✓	
	Tabanidae	5	PR		✓
GASTROPODA	Lymnaeidae	3	SC	✓	✓
	Physidae	3	SC	✓	✓

CA analysis of taxon composition of the seven wetlands sampled, showed no separation of RW and IW taxon composition along CA1 (Figure 2). However, axis 1 does show that the taxon composition of samples D1, A3, and A4 taken from IW 2, differed from that of samples D2, B5 and B6 taken from the same wetland, and that of the other six wetlands. Axis 2 shows a clear separation of RW and IW taxon composition. There was a large variation in taxon composition within IW 2 and IW 3 (along both axes), while the RWs had relatively little variation. The composition of the samples taken upstream of the effluent discharge point at all IWs (samples B5 and B6) are generally more similar to the RW samples. This distribution indicates that minimally impacted sites have different macroinvertebrate communities to those that are impacted. This would suggest that there is potential to use macroinvertebrates as indicators of wetland condition.

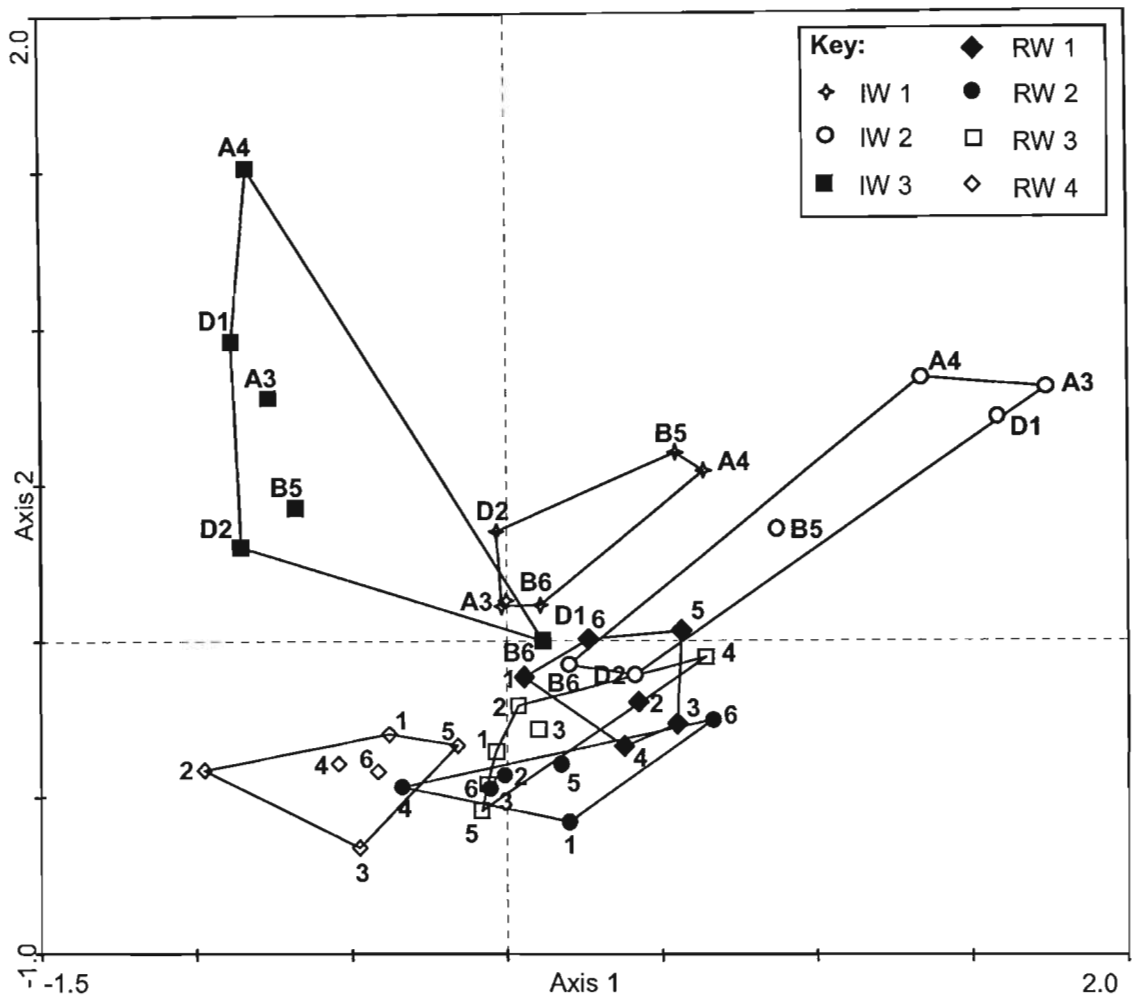

Figure 2: A plot of sites from seven wetlands along the first two axes of a correspondence analysis (CA) of presence/absence macroinvertebrate data. For the impacted wetlands- D=sample taken down stream of dairy impact, A=sample taken at dairy effluent discharge point, B=sample taken before (upstream) dairy effluent discharge point. RW=reference wetland, IW=impacted wetland

Application of SASS5

Mean SASS5 scores differed significantly ($p<0.05$) ($F_{6,\ 35df}=3.81$, $p=0.005$) between the seven sampled wetlands, with SASS5 scores of between 15 and 82 being recorded (Appendix 6 and Table 8). Five of the seven wetlands had mean SASS5 scores of between 46 and 59, the remaining two wetlands, RW 2 and IW 2, had much lower (34.17 and 27.17 respectively) SASS5 scores. There was a significant difference between IW 1 and IW 2 (0.0022), RW 3 and IW 2 (0.006), and a near significant variation between RW 3 and RW 2 (0.057). The range in

SASS5 score at each wetland was generally large, with five of the seven wetlands having a SASS5 score range greater than 30. RW 1 had the largest variation in SASS5 score (18), and RW 4 had by far the smallest variation (6) (half the next smallest variation) (Table 8). There was no apparent trend in the 'before' impact samples having higher SASS5 scores compared to the 'at' and 'downstream' samples, of the same wetland. Nor was there any trend with RWs having greater SASS5 scores than the IWs. There was no trend in high mean SASS5 scores having larger variation.

Mean ASPT values differed significantly between wetlands ($p<0.05$) ($F_{6,\ 35df}=4.39$, $p=0.002$). ASPT ranged between 4.02 and 5. When considering all wetlands, there was a significant variation between RW 3 and RW 2 (0.002), RW 3 and RW 4 (0.007), and RW 3 and IW 2 (0.034). Most of the wetlands had an ASPT variation of approximately 1.

The mean number of taxa collected differed significantly ($p<0.05$) ($F_{6,\ 35df}=2.77$, $p=0.026$) between the sampled wetlands. When comparing all wetlands, there was a significant variation ($p<0.05$) in taxon diversity between IW 2 and RW 3 (0.049) and IW 2 and IW 1 (0.038). RW 4 had the smallest variation (1.37) while RW 1 had the largest (3.66) (Table 8). Interestingly, the mean number of taxa collected was very similar for RWs 1, 3 and 4 and IWs 1 and 3 (10.17-11.83) (Table 8).

Table 8: Summary SASS5 results for reference and impacted wetlands. RW=reference wetland, IW=impacted wetland (n=6) (n=42 for 'all wetlands') (extreme values given in brackets)

Wetland	Taxon diversity			SASS5 score			ASPT		
	Mean	Standard deviation	Range	Mean	Standard deviation	Range	Mean	Standard deviation	Range
RW 1	10.17	3.66	7 (6-14)	46.83	17.97	37 (27-64)	4.57	0.34	0.9 (4-4.9)
RW 2	8.50	3.62	10 (5-15)	34.17	15.68	42 (21-63)	4.02	0.40	1.1 (3.3-4.4)
RW 3	11.67	2.16	6 (9-15)	58.50	12.52	34 (44-78)	5.00	0.36	0.9 (4.5-5.4)
RW 4	11.33	1.37	4 (9-13)	46.67	6.19	16 (38-54)	4.13	0.30	0.7 (3.7-4.5)
IW 1	11.83	1.94	6 (9-15)	54.67	14.65	41 (41-82)	4.58	0.52	1.6 (3.9-5.5)
IW 2	6.50	3.56	10 (3-13)	27.17	13.91	37 (15-52)	4.27	0.39	1 (4-5)
IW 3	10.33	2.70	6 (8-14)	46.33	12.21	29 (33-62)	4.48	0.35	1.0 (4.1-5.1)
All wetlands	10.05	3.20	12 (3-15)	44.90	16.32	67 (15-82)	4.44	0.47	2.2 (3.3-5.5)

At IW 1, one of the downstream samples had a SASS5 score of 82, while the next highest SASS5 score was 55, which was taken upstream of the impact (Appendix 6). There is thus a range of 27 between the two scores, which is the largest of all the wetlands sampled. This sample had the highest taxon diversity (15) of all the samples collected at that wetland (with 12 being the next highest taxon diversity at that wetland). Many taxa collected in this sample were also relatively high scoring (notably Helodidae which scores 12) compared to the taxa collected in the other samples from IW 1 (Appendix 4). Although the downstream IW 1 sites had the lowest SBDA total metric score in comparison to the other 'at' and 'before' impact sample sites, there was extremely good availability of suitable sampling biotope (Table 9).

Among the RWs, RW 2 had the greatest range between the top two SASS5 scores. One of the samples had a SASS5 score 63, the next highest SASS5 score at this wetland was 38 (Appendix 6), a range of 25. This sample had a much higher taxon diversity (15) compared to other samples collected from the same wetland, with the next highest taxon diversity being 10 (Appendix 6). Although the SBDA index was only carried out once for each RW, the SBDA results reveal that RW 2 had very limited suitable sampling biotope, sparse vegetation, and high amounts of sediment and vegetation in the samples (Table 9). Thus, the large variation in SASS5 score, and to some extent the relatively low SASS5 score, ASPT and taxon diversity (in comparison to other the RWs), can be attributed to poor biotope availability and quality and/or detectability of macroinvertebrates in the sample.

At IW 2, one of the 'before impact' samples had a SASS5 score of 52, the next highest SASS5 score in that wetland was 35 (Appendix 6), also taken 'before impact'. The sample that scored 52 had a much higher taxon diversity (13) compared to the other samples collected from the same wetland (the next highest taxon diversity was 8, also taken from 'before impact'). The SBDA index results reveal that the 'before impact' site had less vegetation in the samples

compared to the other samples, and a greater area of suitable sampling biotope and density of vegetation compared to the 'downstream' samples taken from the same wetland (Table 9). Thus, the SBDA index can help explain why the before 'impact' samples had a greater SASS5 score compared to the other samples taken from the same wetland.

Table 9: Sample biotope and detection assessment (SBDA) index results. D=downstream of impact, A=at impact, B=before impact, see Table 2 for definition of metrics

Wetland	D/A/B	Sedge m^2	% veg. $1m^2$	% sed.	% *L. minor*	% veg.	Total metric score	Class
RW 1	-	5	3	3	5	3	19	Good
RW 2	-	1	1	1	5	1	9	Poor
RW 3	-	5	5	5	5	3	23	Good
RW 4	-	5	3	5	5	5	23	Good
IW 1	D	5	3	1	5	1	15	Fair
IW 1	A	5	3	3	5	1	17	Fair
IW 1	B	5	5	3	5	1	19	Good
IW 2	D	1	1	1	1	3	7	Poor
IW 2	A	3	3	1	1	1	9	Poor
IW 2	B	3	3	1	1	5	13	Fair
IW 3	D	5	5	1	5	1	17	Fair
IW 3	A	5	5	1	5	1	17	Fair
IW 3	B	5	5	1	5	1	17	Fair

RW 3 and IW 1 had higher mean SASS5 scores than the other wetlands (Table 8) and were statistically different to that of IW 2 (0.006 and 0.022 respectively). IW 1 also had the highest individual SASS5 score (82) (Appendix 6). However, IW 1 had among the lowest physico-chemical and macrohabitat rankings (Table 10). The SBDA individual metric results reveal that both RW 3 and IW 1 had large areas of dense sedge-dominated vegetation standing in water deeper than 0.5m (Table 9). The quality and diversity of biotope present is known to influence SASS results (Chutter 1998), and it is possible that these optimum conditions contributed to the high scores of these wetlands.

RW 2 and IW 2 had lower mean SASS5 scores (and taxon diversity) than the others (Table 8). There are a number of possible explanations for this. IW 2 had the most impacted physico-chemical measurements (Table 5 and 10) including relatively high levels of phosphorus, and these conditions may have affected the

macroinvertebrate community. According to the SBDA results, both wetlands also had a limited amount of suitable biotope to sample; this was the case for IW 2 at the downstream sites, and at all sites for RW 2. Furthermore, both sets of samples contained large amounts of material; IW 2 samples had *Lemna minor*, and RW 2 samples had much sediment and vegetation (Table 9). It is likely that this would have reduced the number of taxa detected in the samples, thus resulting in lower SASS5 scores. In fact, this factor may have affected the results of a number of sites- IW 1 and 3 in particular had large amounts of sediment and vegetation in the samples (Table 9). As Helgen (2002) notes that samples should not be overloaded with vegetation, it is possible that the SASS5 scores for these sites would have been higher had excess amounts of vegetation, sediment and *Lemna minor* not been collected.

The high variability in SASS5 scores between sites within a single wetland (in both IWs and RWs), perhaps due to biotope availability, suggests that invertebrate distribution within a wetland is patchy. It is suggested that multiple samples and mean values be used even with proven appropriate invertebrate tolerance scores. Further research is required to determine the number of samples needed to collect a representative sample from a wetland.

As macroinvertebrate communities are strongly influenced by vegetation availability, an attempt must be made to sample within vegetation that is relatively homogenous with respect to type, quality and quantity. However, as wetland samples commonly contain large amounts of vegetation/sediment, and the availability of suitable sampling biotope can vary greatly between wetlands, it is recommended that an index similar to the SBDA index be developed, to be used in conjunction with a standard macroinvertebrate bioassessment protocol. The index must be robust and reliable, and take into account varying plant communities. Extensive research should be performed on how macroinvertebrate assemblages vary between vegetation types.

Comparison of SASS5 scores, Physico-chemical and Macrohabitat rankings

Four methods have been employed to assess the water quality of the wetlands sampled. The mean SASS5 scores and ASPT values, physico-chemical measurements and macrohabitat scores were all ranked and compared to assess the degree to which the methods gave consistent results (Table 10).

SASS5 ranking

RW 3 had the highest mean SASS5 score, followed by IW 1. There was minimal difference (0.67) between RW 1, RW 4 and IW 3 SASS5 mean score, and they were considered to be equal. RW 2 had a slightly higher mean SASS5 score than IW 2.

ASPT ranking

Similar to SASS5 ranking, RW 3 had the highest mean ASPT. There was minimal difference (0.1) between IW 1, RW 1 and IW 3, and they were considered to be equal. IW 2 had a higher mean ASPT than RW 4, and RW 2 had the lowest mean ASPT (4.02) (Table 8).

Physico-chemical ranking

Physico-chemical ranking of the wetlands was based on the relative amounts of three determinands, ammonia, nitrogen and phosphorus, recorded at each wetland. TDS was not considered in this analysis, as all values were low, and SASS does not appear to be affected by low TDS counts (Chutter 1998). However, it must be noted that SASS has not been statistically correlated to any specific physico-chemical variable (Vos *et al.* 2002, de la Rey *et al.* 2004), and that is not the purpose of this study. The chemical and physical characteristics of water are defined by several parameters, making it frequently impossible to establish which single parameter or combination of parameters are responsible for a biological response (Chutter 1998). Rather, these parameters were chosen

to represent general water quality, as they are known to be among the primary pollutants present in dairy effluent.

RW 2 and 3 were considered to be least impacted, as they had the lowest measurements of nitrogen and phosphorus, and very low levels of ammonia (Table 4). As ammonia, nitrogen and phosphorus measurements had not been collected at RW 1, this wetland was assumed to have physico-chemical measurements equal to or better than these wetlands, based on the pristine nature of the wetland and its immediate catchment (and absence of any alteration in comparison to other sampled wetlands). As RW 4 had slightly higher ammonia and phosphorus levels recorded, it was ranked fourth. All three IWs were similar to each other, but IW 2 was considered the most impacted due to its slightly higher phosphorus levels.

Macrohabitat ranking

Macrohabitat rankings were derived in Table 6.

Table 10: Ranking of SASS5, ASPT, physico-chemical results and macrohabitat scores for reference and impacted wetlands. RW=reference wetland, IW=impacted wetland (7=least impacted)

Wetland	SASS5	ASPT	Physico-chemical	Macrohabitat
RW 3	7	7	7	4
IW 1	6	6	3	1
RW 1	5	6	7	7
RW 4	5	2	4	5
IW 3	5	6	3	3
RW 2	2	1	7	6
IW 2	1	3	1	2

Overall, the four variables appear to support each other well. The RWs are, for the most part, consistently ranked as being less impacted in comparison to the IWs. It was found that SASS5 score and ASPT were significantly related ($p<0.05$) (Table 11). Physico-chemical rank and macrohabitat were also significantly related ($p<0.05$) (Table 11), although RW 3 was ranked lower for its macrohabitat score relative to its high physico-chemical and SASS5 rankings (Table 10). The main reasons for the lower macrohabitat ranking at RW 3, were the extensive

intrusion of maize cropping into the 100m buffer zone, and the presence of maize and plantations within the surrounding landscape, aspects which were largely absent from the other RWs. As the maize is farmed using the minimum till method, and the macrohabitat score sheet does not allow for farming practicing to be taken into account, it is possible that the wetland does not experience adverse impacts from the nearby cropping. The wetland itself appeared to be in very good condition, and according to the SBDA results, there was a large amount of suitable biotope for sampling (Table 9). It is therefore likely that due to the combination of the minimum till method and the availability of good sampling biotope, the wetland has good physico-chemical results and SASS5 scores.

Table 11: Statistical comparison of SASS5, ASPT, physico-chemical and macrohabitat ranking (*=p<0.05)

Ranking	ASPT		Physico-chemical		Macrohabitat	
	R value	p value	R value	p value	R value	p value
SASS5	0.769	0.0432*	0.311	0.49763	-0.148	0.75108
ASPT	-	-	0.078	0.86853	-0.259	0.57424
Physico-chemical	-	-	-	-	0.823	0.02285*

Two SASS5 rankings did not agree with their respective physico-chemical measurements and macrohabitat scores. RW 2 SASS5 score was ranked second, while it had some of the best physico-chemical measurements and macrohabitat scores. It is likely that the low SASS5 score for RW 2 was a result of limited suitable sampling biotope, and low invertebrate detectability due to large amounts of sediment and *Lemna minor* being present in the samples. The high SASS5 score for IW 1, which was considered the most impacted, is less clear. The occurrence of the dairy and maize cropping close to the edge of the wetland, and the presence of a pit containing decaying livestock corpses close to the wetland edge all attest to the low physico-chemical and macrohabitat rankings. In this case, the presence of much suitable biotope is likely to have influenced the score (Table 9).

Wetland SASS5 scores compared with river SASS experiences

Based on the current SASS5 score water quality guidelines (Table 3), all of the wetlands would be considered to have 'some' to 'major deterioration in water quality' (Chutter 1998). Clearly, this is not supported by the physico-chemical and macrohabitat results, which suggest that all wetlands sampled during this study had good to very good water quality (DWAF 1996). The RWs sampled had physico-chemical measurements that were of a similar standard to that collected from the Sabie River, which is considered to be one of the least impacted rivers in the country (Chutter 1998). Despite this, SASS scores and ASPT values recorded for the Sabie River are typically between 100-200 and 6-7.2 respectively, which is substantially higher than the SASS scores and ASPT values recorded for any of the RW site (which ranged between 21-78 and 3.3-5.4 respectively). SASS scores along the Mgeni River in KwaZulu-Natal are similar to that of the Sabie River (ranging between 70 and 165, with ASPT values of 5.8-7.3), Thus, a river with similar water quality in the same area as the sampled wetlands has much higher SASS5 scores and ASPT values.

A number of factors possibly contribute to these low scores, some of which have already been mentioned. One of the most important factors is likely to be low biotope diversity. Where biotope diversity is low, SASS score is low relative to the ASPT (Chutter 1998). Biotope diversity is considered to be the single most important extraneous factor affecting SASS scores, and SASS scores where water quality is natural are the most sensitive to biotope diversity (Chutter 1998). In rivers that have good water quality but limited biotope diversity, SASS scores are lower, while ASPT scores remain high. Sites on the Crocodile River in Mpumalanga had SASS scores of 40-100 associated with ASPT values of 5-7. Wetlands typically lack the biotope diversity that is present in many rivers. Furthermore, this study intentionally ignored other biotope types such as *Phragmites* beds. It is possible that the sampling of other biotopes could result in an increased SASS5 score, however, it is improbable that the incorporation of further wetland biotopes into a wetland study would dramatically increase either

SASS5 score or ASPT, as *Phragmites* beds and open water biotopes are likely to be relatively taxa poor (C. Dickens, Leader: Environmental Assessment and Reporting, INR, pers. comm., Olson *et al.* 1995).

Sampling of the wetlands took place during a below average rainfall year, with the result that suitable sampling biotope was often very limited. It did appear that the presence of good quality biotope affected the scores obtained. Therefore, it is possible that during years of higher rainfall, when more suitable habitat is present, higher SASS5 scores would be recorded.

As previously mentioned, the collection of large amounts of sediment and vegetation within the sample also appears to have an effect on the number of taxa collected and therefore the SASS5 score. The amounts of these materials are typically much lower in rivers.

Most of the above reasons are likely to influence SASS5 scores, but are not likely to substantially affect ASPT values. Samples typically had only 1-3 organisms with a SASS5 tolerance rating of 8 or above. Using Gerber and Gabriel (2002a) and Picker *et al.* (2003), the SASS5 scores of macroinvertebrates that exclusively inhabit biotopes not commonly found in palustrine wetlands (stones in/out of current, moderate to fast flowing water) were summed, and the ASPT calculated (Appendix 5). The same process was followed for those macroinvertebrates that have the ability to survive in biotopes found in palustrine wetlands. The ASPT for taxa unlikely to inhabit palustrine wetlands was 9.5, compared to an ASPT of 5.5 for the expected taxa. It appears that, as many 'intolerant' SASS5 taxa are unable to survive in palustrine wetland biotopes, it is unlikely that samples collected from wetlands will ever achieve the high ASPT values typical of unimpacted rivers if the current SASS5 scores are used.

The distribution of taxa in these wetlands suggests that SASS5 tolerance scores are not entirely appropriate for use in palustrine wetlands in South Africa. One

explanation for this could be that the macroinvertebrates that inhabit wetlands are adapted to the natural, diurnal fluctuations of oxygen levels characteristic of wetlands (Helgen 2002), and therefore do not respond in the same way as river macroinvertebrates to lowered oxygen levels caused by pollution (Gernes and Helgen 2000). Another explanation could be that the families recorded during this study are represented by different species to those that are collected from streams.

It is believed that the RWs used in this study are among the least impacted wetlands in the province, yet the SASS5 scores that are recorded for them would imply that deterioration of water quality has taken place. When considering the SASS5 site scores without referring to the water quality guidelines, there appears to be a loose relationship between SASS5 score, recorded physico-chemical measurements and macrohabitat assessment of the wetlands studied (Tables 10 and 11). This suggests that macroinvertebrate communities have potential as wetland biomonitoring tools. A stronger relationship between SASS5 score and physico-chemical measurements may have been observed, if water quality had been more variable between wetlands.

Potential indicator taxa

In the CCA analysis using the constrained effects of the selected chemicals measured in this study, nitrogen accounted for the majority of the variation in macroinvertebrate composition (Figure 3). The effects of ammonia, TDS and phosphorus on taxon composition were much smaller in comparison to that of nitrogen. It was unexpected that phosphorus would account for much less variation compared to nitrogen (as indicated by the shorter arrow), given that nitrogen was low for all samples tested, and phosphorous varied from mesotrophic in most of the RWs to eutrophic in all of the IWs.

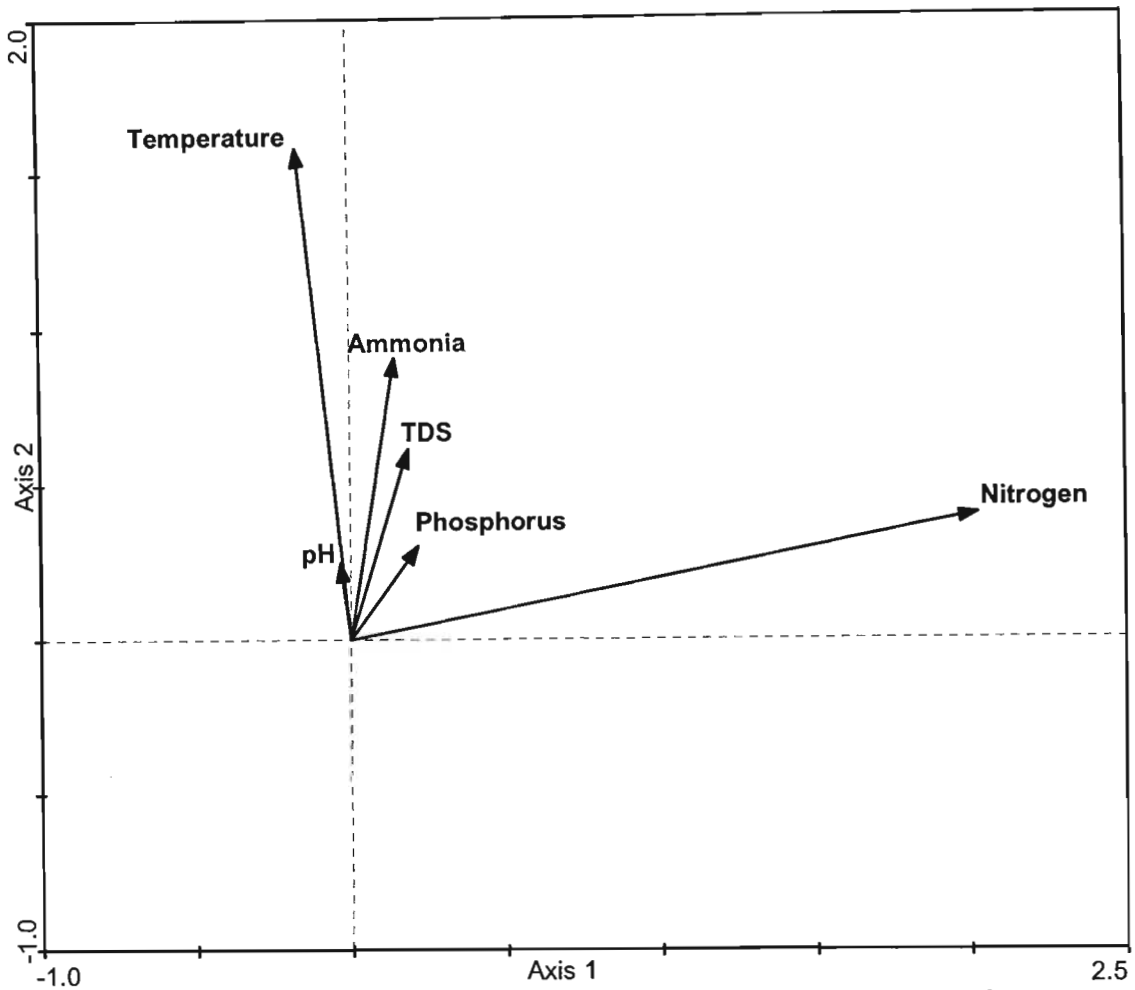

Figure 3: A plot of physico-chemical attributes taken from seven wetlands along the first two axes of a canonical correspondence analysis (CCA) of presence/absence macroinvertebrate data. Environmental variables with the longest arrow relative to an axis have the greatest effect in constraining that axis. If the arrows of two variables subtend a small angle they are closely correlated, if they subtend an angle of 90° there is no relationship, if they subtend >90° they are negatively correlated.

SASS5 has not been statistically correlated to any specific physico-chemical variable (Vos *et al.* 2002, de la Rey *et al.* 2004), however, in this study, a number of taxa appeared to be responsive to varying nitrogen levels. In the CCA analysis of variability of taxon composition between the seven sampled wetlands, only taxa with at least 10 percent of their variance accounted for in the ordination have been shown; to avoid clutter and to indicate only those taxa most responsive along the gradients. A total of nine taxa appeared along the main gradient (CCA1) (Figure 4).

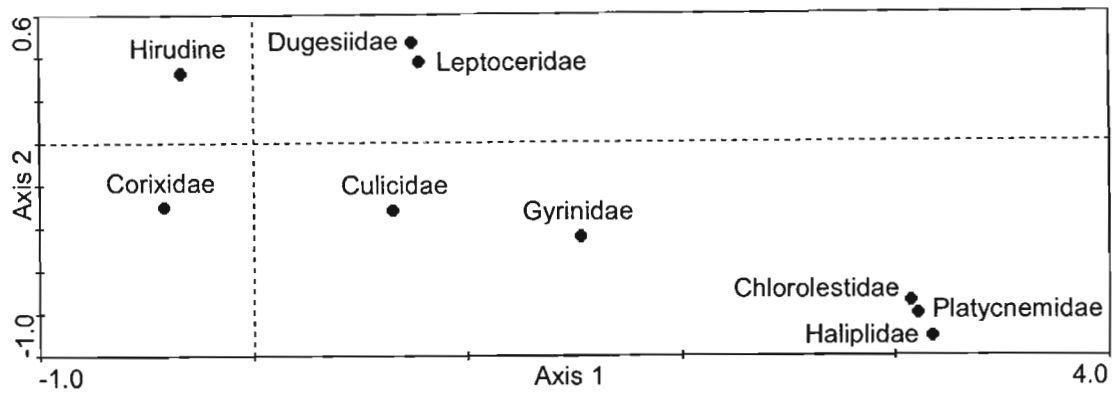

Figure 4: A plot of taxa with 10% or more of their variance accounted for along the first two axes of a canonical correspondence analysis (CCA) of presence/absence macroinvertebrate data.

The nine taxa along with their respective SASS5 tolerance scores are displayed in Table 12. The results are a reverse trend to what might have been expected, as generally, those taxa regarded as 'intolerant' by SASS5 appear to prefer higher nitrogen conditions, compared to taxa considered 'tolerant' by the same scoring system. One explanation for this result could be due to all sampled wetlands having low levels of nitrogen at the time of sampling (Table 4 and 5). Thus, the variation in the level of nitrogen between wetlands was not great enough to have any noticeable effect on invertebrate composition.

These nine identified taxa make potentially good candidates for a wetland bioassessment tool, as nitrogen is one of two main components of eutrophication, and a common pollutant in wetlands. However, further investigation is required into the applicability of these taxa.

Table 12: Taxa sensitive to the presence of nitrogen

Taxa	SASS5 score	Sensitivity to nitrogen
Corixidae	3	Most
Hirudinea	3	
Culicidae	1	
Dugesiidae	3	
Leptoceridae	6	
Gyrinidae	5	
Chlorolestidae	8	
Platycnemidae	10	
Haliplidae	5	Least

Conclusion and Recommendations

Although the distribution of taxa in these wetlands suggests that macroinvertebrates are responsive to changes in wetland condition, SASS5 tolerance scores do not appear to be appropriate for use in palustrine wetlands in South Africa. The water quality in the sampled wetlands was of good quality, yet taxon diversity, SASS5 scores and ASPT values were very low compared to that of rivers with similar water quality. Few high scoring taxa were recorded. However, although not all statistically related, comparisons of SASS5 scores, measured physico-chemical variables and macrohabitat scores between wetlands suggest that there is a relationship between SASS5 and wetland condition.

The availability of suitable sampling biotope influences the composition of macroinvertebrates greatly. The collection of large amounts of material, such as vegetation and sediment, in a sample affects macroinvertebrate detectability, and thus also affects results.

Recommendations

Based on the findings of this study, it is suggested that a wetland adaptation of SASS5 would require the reassignment of modified scores to certain taxa based on their distribution in wetlands of varying water quality. The SASS5 score level of 100 (as specified in the SASS5 score water quality guidelines) is inappropriate for wetlands. Either the range of taxa tolerance scores should be increased (1 to >15), or the score level of 100 be lowered. To accommodate the lower diversity of wetland taxa, the ASPT value of six should also be reduced.

Further testing of SASS5 as well as other derived wetland bioassessment methods should address the following:

- Testing throughout the year to identify how wetland macroinvertebrate communities (and thus SASS5 scores) fluctuate seasonally

- Sampling wetlands whose water quality is severely impacted, to ascertain the responses of macroinvertebrates to highly degraded conditions
- Sampling across a wider range of impacted wetlands (e.g. peat mining, residential) and types (e.g. lacustrine, forested) to identify taxa which are responsive to a range of impacts
- Conducting more detailed work on biotope description
- Developing a robust and reliable standard index featuring biotope, notably vegetation metrics, and detectability assessment metrics to be used in conjunction with a standard wetland macroinvertebrate bioassessment protocol
- Investigating the optimum number of samples required to account for the patchiness of wetland invertebrate distribution, by collecting more baseline data across a variety of different biotopes within a wetland
- Further research on certain taxa, notably those responsive to nitrogen, concerning their potential as bioindicators

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Appendix 1: Physico-chemical data sheet

PHYSICO-CHEMICAL DATA SHEET			
Wetland Name:	Sample Number:	Impacted ☐ Reference ☐ Photograph taken ☐	
Time of Sample:	GPS co-ordinates- ALT. m	Longitude: . . .	Latitude: . . .
Distance from dairy to sample point: m	Depth: cm	Flow (circle): Very low Low Medium High	If impacted, B/A/D of point source (circle)
pH:		Phosphorus:	µg/L
TDS:	ppm	Ammonia:	mg/L
Electrical conductivity:	mS/m	Temperature:	°C
Nitrogen:	mg/L	Turbidity:	cm
OTHER COMMENTS:			

Appendix 2: Macrohabitat score sheet

MACROHABITAT SCORE SHEET

Wetland Name:	Date:	Photographs taken N S E W sample point ☐	Est. area of wetland:

Weather:	Additional comments:
Rain ☐ Showers ☐ Overcast ☐ Clear/ Sunny ☐	
% of different cover: Sedge/ Grass % Phragmites % Typha % Algae % Open water % Other _____ %	

MACROHABITAT SCORING

1) HABITAT ALTERATION- IMMEDIATE LANDSCAPE (within the wetland-area estimated by vegetation distribution) Severity and extent of alteration		SCORE:
BEST	As expected for reference, no evidence of disturbance	0
MODERATE	Low intensity alteration or past alteration that is not currently affecting wetland	7
FAIR	Highly altered, but some recovery if previously altered	14
POOR	Almost no natural habitat present, highly altered habitat	21

Checklist

Vegetation Removal Disturbance

Grazed ☐	Tree plantation ☐

Substrate/ Soil Disturbances and Sedimentation

Vehicle Use ☐	Sediments inputs (from inflow or erosion) ☐

Other:

2) HYDROLOGICAL ALTERATION Severity and degree of alteration		SCORE:
BEST	As expected for reference, no evidence of disturbance	0
MODERATE	Low intensity alteration or past alteration that is not currently affecting wetland	7
FAIR	Less intense than 'poor', but current or active alteration	14
POOR	Currently active and major disturbance to natural hydrology	21

Checklist

Inlet restriction present Weir ☐ Dam ☐ Other:	Outlet restriction present Weir ☐ Dam ☐ Other:

Drainage ☐	Impounding ☐	Canalisation ☐

3) BUFFER LANDSCAPE DISTURBANCE (< 100m from wetland) Extent and intensity		SCORE:
BEST	As expected for reference site, no evidence of disturbance	0
MODERATE	Predominately undisturbed, some human influence	7
FAIR	Buffer significantly modified	14
POOR	Nearly all or all of the buffer modified, intensive land use surrounding wetland	21
Checklist		
Intensive cropping ☐	Presence of exotic veg. ☐	Human disturbance ☐

4) LANDSCAPE (IMMEDIATE) INFLUENCE (100m – 500m from the wetland) Extent and intensity		SCORE:
BEST	As expected for reference site, no evidence of disturbance	0
MODERATE	Predominately undisturbed, some human influence	7
FAIR	Landscape significantly modified	14
POOR	Nearly all or all of the landscape modified, intensive land use surrounding wetland	21
Checklist		
Intensive cropping ☐	Presence of exotic veg. ☐	Human disturbance ☐

5) IMPAIRMENT FROM DAIRY Extent and intensity		SCORE:
BEST	No evidence of impairment from dairy	0
MODERATE	Moderate impairment attributed to dairy	7
FAIR	Significant impairment attributed to dairy	14
POOR	Server impairment attributed to dairy	21
Checklist		
Slope and distance from dairy to wetland ☐		
QUESTIONS TO DAIRY: Details of any mitigation structures, present or past (what, when established): When was dairy established? How many cows are milked at dairy each day? Has this number of cows always been milked at this dairy? During a year, does the land use between the wetland and the dairy change (how)?		

6) PHYSICAL CHARACTERISTICS		SCORE:
BEST	As expected for reference and no evidence of change in natural characteristics	0
MODERATE	Little or no evidence of a change in natural physical characteristics	7
FAIR	Significant change in natural physical characteristics	14
POOR	Severe change in natural physical characteristics	21
Checklist		
Odour: Sewage ☐ Sulphur ☐ Normal ☐ Petroleum ☐ Observations: Dead fish ☐ Algae ☐		Colour:
TOTAL ENVIRONMENTAL SCORE FOR WETLAND		**SCORE:**

OTHER COMMENTS:

Draw a map of the wetland and up to 200m into upland
Show: approximate scale, wetland shape, wetland dimensions, location of major vegetation classes, inlets, outlets, surrounding land use, roads, foot paths, storm drains, north etc.

Appendix 3: Reference wetland macroinvertebrates collected (SASS5 tolerance score shown in bold). ^: 1species=4, 2species=6, >2species=12

Family / order	Dugesiidae	Hirudinea	Potamonautidae	Hydracarina	Baetidae	Caenidae	Leptophlebiidae	Coenagrionidae	Aeshnidae	Libellulidae	Pyraustidae	Belostomatidae	Corixidae	Gerridae	Hydrometridae	Naucoridae	Notonectidae	Pleidae	Veliidae	Ecnomidae	Hydropsychidae	Leptoceridae	Dytiscidae	Elmidae	Gyrinidae	Helodidae	Hydrophilidae	Ceratopogonidae	Chironomidae	Culicidae	Psychodidae	Lymnaeidae	Physidae
Wetland	3	3	3	8	4^	6	9	4	8	4	-	3	3	5	6	7	3	4	5	8	4^	6	5	8	5	12	5	5	2	1	1	3	3
RW 1	•	•		•		•		•	•			•	•			•		•	•				•						•	•			
RW 1		•						•		•		•						•	•	•									•				•
RW 1		•		•		•				•								•											•				
RW 1		•		•		•		•				•											•						•				
RW 1		•		•		•		•	•			•	•					•		•		•						•	•				
RW 1				•		•		•		•		•				•		•		•			•	•			•	•	•				•
RW 2				•								•	•			•	•	•					•						•				
RW 2		•	•										•				•	•	•										•				
RW 2			•	•	•			•	•			•	•			•	•	•	•				•						•		•		•
RW 2			•										•			•			•				•						•				
RW 2		•	•	•	•								•				•						•						•				
RW 2									•			•					•	•					•						•				
RW 3				•	•				•	•			•				•		•				•			•			•				
RW 3		•		•					•			•	•		•	•		•	•					•		•			•	•		•	•
RW 3		•	•	•									•						•				•			•			•				•
RW 3		•		•				•					•					•					•			•	•		•			•	•
RW 3				•				•	•	•	•		•				•	•	•	•	•					•						•	•
RW 3		•	•	•		•			•	•		•	•				•		•				•			•							•
RW 4			•		•	•		•					•	•			•		•				•						•			•	
RW 4		•	•		•	•	•	•					•	•			•		•		•									•		•	
RW 4			•		•	•				•			•			•	•						•							•		•	
RW 4					•	•		•		•			•			•	•		•		•		•						•	•		•	
RW 4				•	•	•		•		•						•	•		•				•						•			•	•
RW 4			•			•							•			•	•		•			•	•									•	•

Appendix 4: Impacted wetland macroinvertebrates collected (SASS5 tolerance score shown in Bold). * D=downstream of impact, A=at impact, B=before impact. ^: 1species=4, 2species=6, >2species=12

Family / order		Dugesiidae	Hirudinea	Potamonautidae	Atyidae	Hydracarina	Baetidae	Caenidae	Chlorolestidae	Coenagrionidae	Platycnemidae	Aeshnidae	Libellulidae	Pyraustidae	Belostomatidae	Corixidae	Gerridae	Naucoridae	Notonectidae	Pleidae	Veliidae	Leptoceridae	Dytiscidae	Elmidae	Gyrinidae	Haliplidae	Helodidae	Hydrophilidae	Ceratopogonidae	Chironomidae	Culicidae	Tabanidae	Lymnaeidae	Physidae
Wetland	*	3	3	3	8	8	4^	6	8	4	10	8	4	-	3	3	5	7	3	4	5	6	5	8	5	5	12	5	5	2	1	5	3	3
IW 1	D	•				•	•			•			•		•					•		•	•	•							•			•	
IW 1	D	•	•		•	•	•					•			•		•	•			•	•					•				•		•	•	
IW 1	A	•	•			•		•		•			•				•				•									•			•	
IW 1	A	•	•				•			•				•			•			•	•							•	•	•			•	
IW 1	B	•	•				•			•			•	•			•			•	•	•	•					•	•					
IW 1	B	•	•	•	•	•	•			•		•	•	•	•					•													•	
IW 2	D		•																				•					•	•	•				
IW 2	D		•			•							•																	•			•	
IW 2	A					•																						•		•				
IW 2	A	•	•			•																						•		•				
IW 2	B		•			•											•			•			•					•		•				•
IW 2	B		•				•			•		•	•		•	•	•		•				•							•	•				•
IW 3	D				•		•						•				•				•				•					•	•			
IW 3	D			•	•					•							•	•					•								•		•		
IW 3	A	•		•	•	•	•		•				•				•		•		•				•					•	•			•
IW 3	A	•		•						•	•	•									•	•	•		•	•				•	•				•
IW 3	B	•				•	•			•						•	•		•		•		•		•						•				
IW 3	B	•				•	•			•									•				•				•			•					

Appendix 5: SASS5 macroinvertebrates not expected/expected to inhabit biotopes commonly found in palustrine wetlands. ^: when a single specie is found in a sample

Family / order	Require	Only inhabit biotopes not commonly found in palustrine wetlands			Inhabit biotopes commonly found in palustrine wetlands		
		Stones in / out of current	Moderate / fast flowing water	SASS5 Score	Vegetation / muddy areas / sediment	Still / slow moving water	SASS5 Score
Baetidae					✓		4^
Caenidae					✓	✓	6
Heptageniidae			✓	13			
Leptophlebiidae					✓	✓	9
Oligoneuridae			✓	15			
Polymitarcyidae			✓	10			
Prosopistomatidae			✓	15			
Tricorythidae		✓	✓	9			
Ecnomidae						✓	8
Polycentropodidae		✓	✓	12			
Hydropsychidae					✓		4^
Philopotamidae		✓	✓	10			
Psychomyiidae		✓		8			
Hydroptilidae		✓		6			
Pisuliidae					✓	✓	10
Leptoceridae					✓		6
Dytiscidae					✓	✓	5
Elmidae					✓		8
Gyrinidae						✓	5
Helodidae					✓	✓	12
Haliplidae					✓		5
Hydraenidae					✓	✓	8
Hydrophilidae					✓	✓	5
Psephenidae			✓	10			
Belostomatidae					✓	✓	3
Corixidae					✓	✓	3
Gerridae						✓	5
Hydrometridae					✓	✓	6
Nepidae					✓	✓	3
Naucoridae					✓	✓	7
Notonectidae						✓	3
Pleidae					✓		4
Veliidae						✓	5
Aeshnidae						✓	8
Gomphidae					✓	✓	6
Corduliidae		✓		8			
Libellulidae					✓	✓	4
Calopterygidae					✓	✓	10
Chlorocyphidae		✓		10			
Chlorolestidae					✓	✓	8
Coenagrionidae					✓	✓	4
Lestidae					✓	✓	8
Platycnemidae					✓	✓	10
Protoneuridae					✓	✓	8
Athericidae			✓	10			

Blepharoceridae	✓	✓	15			
Ceratopogonidae				✓	✓	5
Chironomidae				✓		2
Culicidae					✓	1
Dixidae					✓	10
Empididae				✓		6
Ephydridae					✓	3
Muscidae					✓	1
Psychodidae					✓	1
Simuliidae		✓	5			
Syrphidae				✓	✓	1
Tabanidae				✓	✓	5
Tipulidae				✓		5
Notomemouridae		✓	14			
Perlidae		✓	12			
Pyralidae				✓		12
Corydalidae	✓	✓	8			
Sialidae					✓	6
Dipseudopsidae					✓	10
Hydracarina				✓	✓	8
Dugesiidae				✓		3
Potamonautidae				✓		3
Atyidae				✓	✓	8
Palaemonidae	✓	✓	10			
Oligochaeta				✓	✓	1
Hirudinae				✓	✓	3
Poritera	✓		5			
Ancylidae	✓		6			
Lymnaeidae				✓		3
Physidae				✓		3
Planorbidae		✓	3			
Thiaridae				✓		3
Corbiculidae		✓	5			
Sphaeriidae				✓		3
Unionidae				✓		6
Total			219			312
Taxon diversity			23			57
ASPT			9.5			5.5

This is not an exhaustive list of SASS5 macroinvertebrates, only taxa featured in Gerber and Gabriel (2002a) and those whose distribution is not restricted to rivers of the southern and western Cape are listed

Appendix 6: SASS5 samples site scores for both reference and impacted wetlands. RW=reference wetland, IW=impacted wetland, D=downstream of impact, A=at impact, B=before impact

Wetland	D/A/B	Taxon diversity	SASS5 score	ASPT
RW 1	-	14	62	4.4
RW 1	-	9	36	4
RW 1	-	6	27	4.5
RW 1	-	6	29	4.8
RW 1	-	13	63	4.8
RW 1	-	13	64	4.9
RW 2	-	8	35	4.4
RW 2	-	7	23	3.3
RW 2	-	15	63	4.2
RW 2	-	6	25	4.2
RW 2	-	10	38	3.8
RW 2	-	5	21	4.2
RW 3	-	10	54	5.4
RW 3	-	15	78	5.2
RW 3	-	9	44	4.9
RW 3	-	11	52	4.7
RW 3	-	13	69	5.3
RW 3	-	12	54	4.5
RW 4	-	11	43	3.9
RW 4	-	13	53	4.1
RW 4	-	9	38	4.2
RW 4	-	12	44	3.7
RW 4	-	12	54	4.5
RW 4	-	11	48	4.4
IW 1	D	12	54	4.5
IW 1	D	15	82	5.5
IW 1	A	9	41	4.6
IW 1	A	11	43	3.9
IW 1	B	12	53	4.4
IW 1	B	12	55	4.6
IW 2	D	5	20	4
IW 2	D	5	20	4
IW 2	A	3	15	5
IW 2	A	5	21	4.2
IW 2	B	8	35	4.4
IW 2	B	13	52	4
IW 3	D	8	33	4.1
IW 3	D	8	36	4.5
IW 3	A	14	62	4.4
IW 3	A	13	60	4.6
IW 3	B	11	46	4.2
IW 3	B	8	41	5.1