Wetland Health and Importance Research Programme

3

Aquatic Invertebrates as Indicators of Human Impacts in South African Wetlands

Author: M Bird
Series Editor: H Malan

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WETLAND HEALTH AND IMPORTANCE RESEARCH PROGRAMME

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Report to the

Water Research Commission

by

Author: M Bird
Series Editor: H Malan

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Front Cover: A seasonal wetland from the Rondevlei Nature Reserve,
Cape Town, South Africa
Inset: A Culicid larva (mosquito)
Photographs: M Bird

PREFACE

This report is one of the outputs of the Wetland Health and Importance (WHI) research programme which was funded by the Water Research Commission. The WHI represents Phase II of the National Wetlands Research Programme and was formerly known as "Wetland Health and *Integrity*". Phase I, under the leadership of Professor Ellery, resulted in the "WET-Management" series of publications. Phase II, the WHI programme, was broadly aimed at assessing wetland environmental condition and socio-economic importance.

The full list of reports from this research programme is given below. All the reports, except one, are published as WRC reports with H. Malan as series editor. The findings of the study on the effect of wetland environmental condition, rehabilitation and creation on disease vectors were published as a review article in the journal Water SA (see under "miscellaneous").

An Excel database was created to house the biological sampling data from the Western Cape and is recorded on a CD provided at the back of Day and Malan (2010). The data were collected from mainly pans and seep wetlands over the period of 2007 to the end of 2008. Descriptions of each of the wetland sites are provided, as well as water quality data, plant and invertebrate species lists where collected.

An overview of the series

Tools and metrics for assessment of wetland environmental condition and socio-economic importance: handbook to the WHI research programme by E. Day and H. Malan. 2010. (This includes "*A critique of currently-available SA wetland assessment tools and recommendations for their future development"* by H. Malan as an appendix to the document).

Assessing wetland environmental condition using biota

Aquatic invertebrates as indicators of human impacts in South African wetlands by M. Bird. 2010.

The assessment of temporary wetlands during dry conditions by J. Day, E. Day, V. Ross-Gillespie and A. Ketley. 2010.

Development of a tool for assessment of the environmental condition of wetlands using macrophytes by F. Corry. 2010.

Broad-scale assessment of impacts and ecosystem services

A method for assessing cumulative impacts on wetland functions at the catchment or landscape scale by W. Ellery, S. Grenfell, M. Grenfell, C. Jaganath, H. Malan and D. Kotze. 2010.

Socio-economic and sustainability studies

Wetland valuation. Vol I: Wetland ecosystem services and their valuation: a review of current understanding and practice by Turpie, K. Lannas, N. Scovronick and A. Louw. 2010.

Wetland valuation. Vol II: Wetland valuation case studies by J. Turpie (Editor). 2010.

Wetland valuation. Vol III: A tool for the assessment of the livelihood value of wetlands by J. Turpie. 2010.

Wetland valuation. Vol IV: A protocol for the quantification and valuation of wetland ecosystem services by J. Turpie and M. Kleynhans. 2010.

WET-SustainableUse: A system for assessing the sustainability of wetland use by D. Kotze. 2010.

Assessment of the environmental condition, ecosystem service provision and sustainability of use of two wetlands in the Kamiesberg uplands by D. Kotze, H. Malan, W. Ellery, I. Samuels and L. Saul. 2010.

Miscellaneous

Wetlands and invertebrate disease hosts: are we asking for trouble? By H. Malan, C. Appleton, J. Day and J. Dini (Published in Water SA 35: (5) 2009 pp 753-768).

EXECUTIVE SUMMARY

RATIONALE

The importance of wetland habitats for various human concerns and as a critical store of biodiversity is now recognised on a worldwide scale. Traditionally, research and conservation attention has been centred on rivers and lakes and only relatively recently has the focus shifted to wetlands. The recent emphasis on wetland protection and management has created an urgent need to develop assessment tools to establish and monitor human impacts in wetland ecosystems so as to prioritise wetlands for conservation and rehabilitation actions and to monitor the effects of these actions. Biological assessment or “bioassessment” is one of the means of investigating wetland condition and involves the evaluation of ‘a wetland’s ability to support and maintain a balanced, adaptive community of organisms having a species composition, diversity and functional organisation comparable with that of minimally disturbed wetlands within a region’ (DWAF, 2004, adapted from Karr and Dudley, 1981). Potential indicator groups for bioassessment purposes include macrophytes, algae and diatoms, aquatic invertebrates, birds and fish. Macrophytes emerge as the most popular biotic assemblage for use in wetland bioassessment worldwide and the ecology and functioning of wetland plants is relatively well understood in comparison to other biotic assemblages inhabiting wetlands (Adamus *et al*., 2001; DWAF, 2004). Aquatic invertebrates are regarded as the second most useful group for wetland bioassessment worldwide (Adamus and Brandt, 1990; Butcher, 2003; DWAF, 2004), although their ecological and functional roles in wetland ecosystems are not well understood.

The topic of this study centres on the use of aquatic invertebrates as a bioassessment tool for inland wetlands in South Africa. Marine (open ocean) and estuarine wetlands (connected to the sea) are not covered in this report. Successful wetland bioassessment programmes using aquatic macro-invertebrates have been developed and implemented in parts of the USA (Helgen, 2002), suggesting their beneficial use for bioassessment in other parts of the world. In South Africa, a method of assessing and monitoring wetland condition is required in order to meet national legislative requirements (National Water Act No. 36 of 1998). The Wetland Health and Importance (WHI) Research Programme was launched in April 2006 by the Water Research Commission (WRC) under Phase II of the National Wetland Research Programme. This study investigates the feasibility of using invertebrates in the bioassessment of wetlands and forms one of the components of the WHI.

STUDY OBJECTIVES

The objectives of this study were to:

- collate and review both local and international literature relating to wetland biological assessment using aquatic invertebrates;
- conduct an investigation into the response of aquatic invertebrates (including micro-crustaceans) to anthropogenic disturbances in isolated depression wetlands of the Western Cape, South Africa;
- identify candidate invertebrate taxa or metrics for assessing human impacts on isolated depression wetlands in the Western Cape; and if useful indicator taxa and/or metrics are established, to provide a protocol for developing an assessment method using aquatic invertebrates; and
- investigate the applicability of the SASS river index to wetlands; in this regard, both lentic (e.g. isolated depressions) and lotic (e.g. valley bottom) wetland types will be investigated.

DOCUMENT STRUCTURE

This report is divided into two major components:

Component 1 is the literature review, which provides an overview of biological assessment techniques in rivers and wetlands. The focus is on collating state-of-the-art information on biological assessment of wetlands using invertebrates worldwide and presents the various potential options for use in South Africa; and

Component 2 is the empirical research undertaken in this study. The key facets of this component are:

a) an exploratory analysis of quantitative relationships between aquatic invertebrates and human disturbance variables in Western Cape isolated depression wetlands;

b) investigation of potential index options for future use in this wetland type and discussion of applicability in other areas and wetland types;

c) investigation of SASS index applicability in isolated depression wetlands of the Western Cape; and

d) investigation of SASS index applicability in valley bottom wetlands of the Western Cape in order to clarify whether SASS is a valid protocol for slow flowing wetlands.

STUDY APPROACH

The empirical research component of this study involved sampling 125 isolated depression wetlands spread across the Western Cape winter rainfall region as well as a set of valley bottom wetlands in the greater Cape Town area. These two wetland types were chosen based on their abundance in the region and suitability for addressing the objectives of this study. The isolated depression wetlands were sampled for aquatic invertebrates and various human disturbance variables (water column nutrient levels and a rapid assessment index of human landscape disturbance) in order to relate human impairment with invertebrate assemblage composition and abundance patterns. The collected data were used to:

- develop a multi-metric Index of Biological Integrity (IBI) and to investigate feasibility of such an index for the given wetland type;
- test the SASS index in terms of its ability to distinguish differential levels of wetland impairment; and
- develop a numerical biotic index approach similar to SASS, but with modifications for use in this specific wetland type.

The indices developed for isolated depression wetlands during the empirical research component of this report were further tested using a dataset provided by De Roeck (2008) in order to validate their ability to classify an independent set of wetlands in terms of landscape disturbance and trophic status (proxied by nutrient levels).

Fifteen valley bottom wetlands were sampled using a modified SASS sampling protocol and SASS ASPT scores were compared among nutrient enrichment and landscape disturbance categories so as to test correspondence between observed impairment and SASS scoring for this wetland type.

MAJOR FINDINGS

Literature review

The body of literature on wetland bioassessment protocols using aquatic invertebrates is small and centred almost entirely on research conducted in the USA and Australia in the last 10-15 years. Wetland invertebrates possess a number of advantageous attributes as biological indicators of disturbance. The majority of published findings in the literature suggest aquatic macro-invertebrates as a beneficial tool for the biological assessment of wetlands, but indices need to be modified, sometimes significantly so, in order to be used in different eco-regions of the same country. With the latter point in mind, river macro-invertebrate indices appear to hold an advantage of often being applicable over broad spatial areas with little or no modification to indices. Certain studies (e.g. Tangen *et al*., 2003) indicate that aquatic macro-invertebrates are not a feasible tool for wetland bioassessment in areas where the influence of natural environmental disturbances outweigh anthropogenic-induced disturbances (e.g. areas with extreme climatic fluctuations between seasons). Other potential pitfalls in developing macro-invertebrate indices for wetland bioassessment include the lack of empirical information on responses of invertebrates to human stressors and the dearth of taxonomic information for making correct identifications of wetland taxa. The use of micro-crustacean taxa could add an important complement of information to the more traditional macro-invertebrate assessment techniques, but is likely to be significantly hindered by the difficulties involved in identification and enumeration of such taxa, which may preclude rapid assessment methods from being developed.

Empirical component: isolated depression wetlands

Indicator taxa – macro-invertebrates

The majority of macro-invertebrate families sampled during this study showed a generalist pattern of response to the human disturbance variables, in that these families seem to tolerate a wide range of human-imposed disturbance conditions. Fourteen families were described in this manner as ‘generalists’, whereas 11 families showed some observable response to human impairment. A considerable number of families appear to be very localized in their distributions (15 families were present in <5% of sites) and were too rare for the purpose of deducing patterns. Those families for which relationships to human disturbance variables were found, tended to have weak patterns of association in relation to comparable studies in the literature.

Indicator taxa – micro-crustaceans

Only 7 of the 50 micro-crustacean taxa identified from this study showed potential as indicators of human disturbance. Of these, only 3 taxa (*Metadiaptomus purcelli*, *Zonocypris cordata* and *Daphnia pulex/obtusa*) showed good patterns with reliable sample sizes. The majority of taxa analyzed against human disturbance variables showed a typically generalist-type response and would not be of any particular use for bioassessment purposes. Almost half the taxa (22) were too rare for analysis (present in less than 5% of sites), indicating that their distributions are most likely too localized for use in a bioassessment index. An important point to stress when it came to micro-crustaceans is that the difficulties encountered in getting reliable identifications considerably outweighed the usefulness of the results obtained for bioassessment purposes.

Testing metrics – macro-invertebrates

As observed with macro-invertebrate families, relationships between metrics (summary measures of macro-invertebrate community composition) and human disturbance variables were not particularly strong and the power to infer wetland condition was low in comparison to published metrics. A multi-metric IBI (Index of Biological Integrity) was developed using a set of the most optimal metric results from this study, but regressions of total IBI scores with human disturbance variables proved weak and had low inferential power.

Testing metrics – micro-crustaceans

Thirteen metrics were assessed using micro-crustaceans, but provided little information for bioassessment purposes. Relationships were weak between metrics and human disturbance variables and produced only two feasible metrics (% Copepoda and % Ostracoda), both of which had low inferential power and would be expected to suffer from a reasonably high error rate.

Testing a numerical biotic index approach using macro-invertebrates

A preliminary numerical biotic index similar in design to SASS was developed for use on isolated depression wetlands in the Western Cape region. Tolerance scores on a scale of 1-9 were allocated to family-level taxa based on the correlational output of indicator taxa testing. The allocated scoring range was narrower than stipulated for the SASS index (1-15) due to less clear-cut responses of macro-invertebrate indicator taxa in wetlands. Regression testing of the wetland numerical biotic index ASPT values against human disturbance variables indicated greater inferential power than observed when applying similar regressions using the IBI multi-metric or SASS indices. However, even the numerical biotic index had a reasonably low inferential power in terms of its ability to distinguish levels of wetland impairment.

SASS testing

SASS ASPT scores did show a certain degree of correspondence with impairment conditions in wetlands both in terms of nutrient levels and landscape disturbance, but unlike for rivers, the differences were not clear-cut. Regression tests revealed that the SASS index scores had very low inferential power in terms of determining wetland condition and IBI and numerical biotic index approaches performed considerably better.

Valley bottom wetlands

SASS was able to detect impairment (in terms of landscape disturbance and nutrient levels) among valley bottom wetlands to some degree, but results were not significant. Thus, any inferences of wetland condition based on SASS ASPT scores would be unreliable. Compared to the accuracy of SASS in rivers, the distinction between least impaired and disturbed sites becomes blurry in valley bottom wetlands and the reliability of SASS appears to decrease considerably.

A posteriori index testing with an independent dataset

The IBI and numerical biotic index developed during the empirical component of this study (‘training’ dataset) were validated using an independent test dataset provided by the study of De Roeck (2008), who sampled 58 isolated depression wetlands in the winter rainfall region of the Western Cape for aquatic macro-invertebrates and environmental factors during the period July-September 2004. The IBI and numerical biotic index performed poorly in the test dataset in terms of relationships with nutrient values and land use categories. There were some differences in the sampling protocols among the training and test datasets, which did not make the two datasets completely comparable. However, the majority of evidence in this report (from both training and test datasets) points towards a generalist-type response of aquatic invertebrates to human disturbance in isolated depression wetlands of the Western Cape. This suggests that instead of pursuing index development for this wetland type, a more useful avenue would be to test the numerical biotic index on other wetland types and regions (incorporating suitable modifications where necessary), because for isolated depression wetlands in the Western Cape, aquatic invertebrates appear to give reasonably poor and inconsistent bioassessment results.

CONCLUSIONS AND RECOMMENDATIONS

Isolated depression wetlands

- The macro-invertebrate families sampled in this study did not show clear relationships with human disturbance variables as proxied by landscape use (HDS) and nutrient levels (PO_4 and NH_4) among wetlands. The majority of families showed a generalist response to human disturbances and results do not provide encouragement for establishment of an invertebrate index for this wetland type.

- Despite relatively poor bioassessment results for isolated depression wetlands in the Western Cape, a prototype framework for a numerical biotic index has been developed during this study (essentially a modification of the SASS river index), which shows potential for testing in other wetland types and regions of South Africa. In this regard, the prescribed approach is to first use a training dataset in order to modify tolerance scoring criteria according to the prevalent taxa for a given wetland type/region; followed by testing of the index with an independent set of data to clarify its inferential power.

- The lack of clear indicator taxa for seasonally inundated wetlands investigated in this study is likely to be a common pattern in seasonal wetlands throughout South Africa due to the ‘generalist-type’ adaptations of taxa to these transient environments. Only more research on seasonal wetlands found in other areas of the country can confirm this prediction. Evidence presented in this study, however, suggests that research effort towards the development of aquatic invertebrate indices in South Africa should rather be concentrated on perennial wetlands, where more specialist invertebrate taxa are likely to be found and are thus more likely to show responses to human disturbance. This recommendation is also relevant in the context of developing wetland indices using other biotic assemblages (e.g. diatoms) in that more specialist taxa are likely to inhabit perennial wetlands and thus bioassessment research for other biotic assemblages is expected to be more fruitful in perennial environments.

- The identification of wetland macro-invertebrate taxa to family level is appropriate for future index testing and development in South Africa.

- The multi-metric IBI approach, although shown to be useful in certain parts of the United States, is not recommended as a way forward for rapid wetland bioassessment in South Africa. This conclusion is reached due to a combination of factors: the need for quantitative data; the often laborious process of calculating metrics; the sometimes required identification of taxa beyond family level; and the relatively poor performance of this approach compared to the numerical biotic index as observed during the empirical component of this study.

- Based on results from this study and those of Bowd *et al*. (2006a), the use of SASS for determining the impairment state of truly lentic wetlands appears unfeasible, however a modified version of this index shows some potential.

- Preliminary evidence from metrics and indicator species testing suggests that micro-crustaceans are not useful for inclusion in wetland bioassessment indices in South Africa. This conclusion is reached partly because of the laborious enumeration and identification procedures involved and partly because of the lack of good indicator patterns observed in this study. More research in other wetland types and regions would offer clarification of this issue.

Valley bottom wetlands

- Although the number of valley bottom wetlands investigated in this study was comparatively low (n=15), SASS appeared unable to reliably distinguish impairment levels among sites in comparison to the precision witnessed when using this index in rivers. It is concluded that a certain degree of inferential power is lost when transferring SASS from rivers to valley bottom wetlands. Bioassessment methods less reliant on surface water (e.g. soil indices, macrophyte indices) may prove more feasible for this wetland type as the SASS sampling protocol requires the presence of a suitable amount of surface water for sweep netting.

- Empirical evidence collected from this study and the literature (Bowd *et al*., 2006a; Vlok *et al*., 2006; Dallas, 2009) reaches a firm conclusion that the SASS river index should not be directly applied in the bioassessment of wetlands (including those with flow) without some degree of modification for the different suite of macro-invertebrate taxa and habitats characterizing wetlands.

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ABBREVIATIONS

ASPT – Average Score per Taxon

DWAF – Department of Water Affairs and Forestry

EC – electrical conductivity

FCI – Functional Capacity Index

HDS – human disturbance scores

IBI – Index of Biological Integrity

N – nitrogen

NTU – nephelometric turbidity units

P – phosphorus

pCCA – partial canonical correspondence analysis

RBP – Rapid Bioassessment Protocol

SANBI – South African National Biodiversity Institute

SASS – South African Scoring System

SD – standard deviation

SE – standard error

SRP – Soluble reactive phosphorus

TSA – total surface area

TSS – total suspended sediments

USACE – United States Army Corps of Engineers

WHI – Wetland Health and Importance (Research Programme)

WRC – Water Research Commission

1. GENERAL INTRODUCTION

1.1 The term ‘wetland’ as used in this document

South Africa is a signatory to the Ramsar convention and has adopted the Ramsar definition of wetlands: “areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six metres” (Davis, 1994). This definition is the most widely used worldwide, but is also one of the broadest definitions in existence and even encompasses shallow open ocean areas. The proposed South African wetland classification system of Ewart-Smith *et al*. (2006) initially splits wetlands into three groups (level 1 primary discriminators) according to connectivity to the sea, namely marine systems (part of the open ocean), estuarine systems (partially enclosed systems connected to the open ocean) and inland systems (no existing connection to the open ocean). For the purposes of this report, the term ‘wetland’ will refer to the subset of wetlands belonging to inland systems and thus does not cover wetland types that have a tidal influence.

1.2 Rationale for the study

The importance of wetland habitats for human society and as a store of biological diversity has become increasingly recognised on a global scale since the late 1960s (Cowan, 1995; Ramsar COP8, 2002). The recent emphasis on wetland protection and management has created an urgent need to develop tools for assessing and monitoring human impacts on wetland ecosystems in order to prioritise conservation and rehabilitation actions and to monitor the effects of these actions. Biological assessment or “bioassessment” is one means of investigating wetland condition and involves the evaluation of ‘a wetland’s ability to support and maintain a balanced, adaptive community of organisms having a species composition, diversity and functional organisation comparable with that of minimally disturbed wetlands within a region’ (DWAF, 2004). Potential indicator groups for bioassessment purposes include macrophytes, algae and diatoms, aquatic invertebrates, birds and fish. Macrophytes emerge as the most popular variable for use in wetland bioassessment worldwide and the ecology and functioning of wetland plants are relatively better understood for plants than for other groups inhabiting wetlands (Adamus *et al*., 2001; DWAF, 2004).

Aquatic invertebrates are regarded as the second most useful group for wetland bioassessment worldwide (Adamus and Brandt, 1990; Butcher, 2003; DWAF, 2004), although their ecological and functional roles in wetland ecosystems are not well understood. This study investigates the use of aquatic invertebrates as a bioassessment tool for inland wetlands in South Africa.

The South African Scoring System version 5 (SASS 5, Dickens and Graham, 2002) provides an effective and relatively easy-to-use means of assessing water quality of rivers in South Africa using macro-invertebrates. Preliminary studies using SASS5 for wetlands suggest that the method is not particularly applicable to this habitat (Bowd *et al.,* 2006a). Reasons for the poor performance of SASS in wetlands have not been directly evaluated in the literature, but have been speculated on by authors such as Bowd (2005) and Bowd *et al.* (2006a, b) who suggest that there are perhaps too few biotopes in wetlands and significantly different types of invertebrate taxa in wetlands compared to rivers (e.g. abundant micro-crustaceans which are too small to see with the naked eye and would be missed in the SASS scoring procedure). Other factors might include the low diversity of invertebrates in wetlands, which could bias wetlands towards receiving low SASS scores, and the very limited amount of information on the tolerances of wetland invertebrates to pollution when compared to riverine macro-invertebrates. The performance of the SASS protocol in wetlands is examined in sections 4.1.3.3 and 4.2 below. SASS is an index tailored for use in flowing water environments, which are characterised by a diversity of macro-invertebrate taxa with adaptations for clinging to the substrate in running water. Lentic environments, on the other hand, are more often characterised by a lower diversity of invertebrates and a numerical dominance by micro-crustacean zooplankton, which are adapted to living in still water. Therefore, based purely on the different assemblages present in lotic and lentic environments due to the different adaptations required for these environments, one might well hypothesise that a single invertebrate index cannot adequately score the condition of both rivers and wetlands. With flow being the primary physical variable distinguishing the invertebrate assemblages characterising wetlands and rivers, one might further hypothesise that the flow characteristics of a wetland would determine the suitability of using a SASS-like macro-invertebrate index or an index specifically tailored for assemblages more common in truly lentic environments. In this regard, while SASS may be appropriate for river channels flowing through wetlands, its suitability in purely lentic wetlands is unlikely. It may be that SASS is inappropriate for assessing truly lentic wetlands, but that it works for wetlands with some flow.

Successful wetland bioassessment programmes using aquatic macro-invertebrates have been developed and implemented in parts of the USA (Helgen, 2002), suggesting their beneficial use for bioassessment in other parts of the world. In South Africa, a method of assessing and monitoring wetland condition is required in order to meet national legislative requirements (National Water Act No. 36 of 1998). The Wetland Health and Importance (WHI) Research Programme was launched in April 2006 by the Water Research Commission (WRC) under Phase II of the National Wetland Research Programme. This study investigates the feasibility of using invertebrates in the bioassessment of wetlands and forms one of the components of the WHI.

With the possible exception of the genus *Daphnia* (Cladocera), very little research has been conducted worldwide on the pollution sensitivities of wetland invertebrate taxa. In contrast, a vast array of eco-toxicology studies has been conducted to ascertain the pollution tolerances of a wide range of riverine taxa. As a starting point towards the formulation of an invertebrate index for wetland condition, one first needs to investigate possible links between invertebrate taxa and varying degrees of human disturbance in wetlands. Furthermore, the responses of invertebrates to different types of disturbance need to be distinguished. This report documents investigations into the responses of invertebrate assemblages, and their taxa, to varying levels and types of human disturbance within the Western Cape region of South Africa.

1.3 Existing approaches to index development

Three major approaches that have been used to assess the influence of human disturbance factors on wetlands using aquatic invertebrates have been extracted from the literature:

- the multi-metric IBI method;
- the multivariate method; and
- the numerical biotic index approach.

The **multi-metric IBI method** is employed by the United States Environmental Protection Agency (US EPA) wetland bio-monitoring programme (Helgen, 2002) and aims to produce an Index of Biological Integrity (IBI, Karr, 1981). The approach involves sampling wetlands across a gradient of disturbance and assessing which attributes of invertebrate community composition correlate with the gradient of disturbance. Invertebrate attributes which correlate well with degree of impact are incorporated into a set of metrics, which contribute to an overall index for the wetland type being studied. Attributes/metrics in this context are simple summary measures of invertebrate samples such as ‘total number of taxa’, ‘dytiscidae abundance’ or ‘chironomidae abundance’. The multi-metric approach is based on the premise that in order to single out human disturbance or impact as the causal factor explaining differences in invertebrate community composition, one must control for possible natural causal factors between wetlands being analysed. These environmental factors may include physico-chemical properties of the water and sediments, wetland type and geographic region. Wetlands are highly variable in terms of environmental variables and controlling for every single influence on invertebrate assemblage composition is virtually never possible. In this regard, the US EPA multi-metric approach recommends that major factors such as wetland type and geographic region should be controlled for by comparing wetlands across a gradient of disturbance within the same eco-region and for one wetland type only. The multi-metric approach employs the sampling of ‘reference’ and ‘project’ wetlands. Reference sites are those sites that are minimally impacted by human disturbance and that reflect the natural condition of the wetland type under study. Wetlands in a completely natural state may not exist for certain wetland types, depending on the region, and thus the term ‘least impaired’ is employed henceforth in this study. Project wetlands are those that make up the remainder of the gradient of disturbance and may vary from moderately to severely impaired. The major strength of the multi-metric approach lies in its simplicity and the fact that results are relatively easy to interpret. Difficulties may involve finding a set of wetlands that meet the appropriate criteria for this approach (e.g. a gradient of disturbance, controlling for major natural forcing factors).

Another approach towards developing an invertebrate index uses **multivariate methods,** which generally involve the simultaneous statistical analysis of an array of environmental variables together with invertebrate data for a set of wetlands within a specified region. These methods aim to identify individual or reduced sets of environmental variables or impact types that best explain variations in invertebrate assemblage composition between wetlands. This method may yield more powerful results than the multi-metric approach (i.e. better estimates of accuracy and precision), but setting up the data analysis is complicated and often requires specialised practitioners (Reynoldson *et al*., 1997). Multivariate analysis methods are perhaps best suited to assessment of the relative influence of a suite of environmental factors on wetland invertebrate communities, where one is unable to control all these factors. However, as the number of environmental factors analysed simultaneously increases, so the sample size (number of wetland sites) should correspondingly increase. Therefore, it is best to control for the maximum number of factors between the wetland sites being studied so as to increase reliability of multivariate results. Despite the potential power of instituting a multivariate biological assessment index, a large database of information on the response of wetland invertebrate taxa to different anthropogenic stressors is required in order to set up a reliable predictive model. Because of this, the decision was taken to omit multivariate analyses from empirical investigations in the present study. The complexity of such an approach suggests it may, however, become feasible in the longer term once larger databases of information on responses of wetland invertebrates to human stressors become available in South Africa. In the shorter term, more user-friendly and rapid biological assessment approaches are required.

The third approach to index development, extracted from the river bioassessment literature, is the **numerical biotic index approach**. This involves calculation of index scores by assigning sensitivity weightings to individual taxa (generally at the family level) based on their known tolerances to pollution. Final index scores are generated by summing or averaging the values for all taxa or individuals in a sample. Numerical indices are rapid and effective and are perhaps the most popular choice of rapid bioassessment method for rivers worldwide (Armitage *et al*., 1983; Camargo, 1993; Stark, 1985; Chessman, 1995; Chessman and McEvoy, 1998; Dallas, 2002). The SASS index for rivers in South Africa is an example of a numerical biotic index. Although a very useful index approach, numerical biotic indices have received scant attention in the wetland bioassessment literature to date. This is perhaps attributable to the worldwide dearth of information on pollution sensitivities of wetland taxa compared to river taxa. It seems that the only case study testing this approach on wetlands is that of Chessman *et al*. (2002), who developed such an index for the wetlands of the Swan Coastal Plain near Perth, Australia. They assigned tolerance values to invertebrate families based on responses to anthropogenic disturbance (primarily eutrophication) and suggested that such an index could be easily adapted for use in other parts of Australia and on other continents. Their findings indicate the potential of such an index in South African wetlands and the approach warrants further investigation. A potential factor hindering development of such an index is the complete lack of pollution sensitivity information from which to derive tolerance scores. Without direct eco-toxicology results, the only feasible way to develop such an index in South African wetlands is to use a correlative approach whereby invertebrate tolerance scores are inferred from their presence-absence and/or abundances in wetlands with varying degrees of anthropogenic impairment.

1.4 Broad study approach

This study comprises two components, which together will aid in determining the feasibility of creating biotic indices for assessment of human impacts on wetlands in South Africa using aquatic invertebrates. The first component comprises an extensive literature review of the knowledge and research on wetland invertebrates as a biological assessment tool both worldwide and in South Africa. The second is an empirical investigation of factors shaping wetland invertebrate assemblages in the Western Cape province of South Africa. The empirical research component involves an assessment of relationships between anthropogenic factors and invertebrate assemblages and tests various index approaches (SASS, multi-metric IBI, numerical biotic index) on isolated depression wetlands in the Western Cape, using data collected from wetlands in this study and also by analysing an independent dataset provided by De Roeck (2008). Individual invertebrate taxa are tested against human disturbance factors to see if specific taxa are responding to impairment. This will allow a preliminary assessment of the feasibility of developing a numerical biotic index such as SASS, but specifically for wetlands. If invertebrate taxa or metrics are established that respond predictably to disturbance factors in Western Cape wetlands, then these results can be tested for wetlands in other regions of South Africa. This testing in other regions/wetland types will not be conducted, but is a recommended option if a successful approach is established. Another important aspect of the empirical research component of this study is to validate where the use of SASS5 is appropriate in terms of flow. In this regard, both lentic (isolated depressions) and slow-flowing (valley bottom) wetlands will be assessed using SASS5 protocol to determine its applicability in these types of environments.

1.5 Description of isolated depression and valley bottom wetlands

For the purposes of this report, appropriate description of an isolated depression wetland is given by Ewart-Smith *et al*. (2006): “A basin-shaped area with a closed elevation contour that allows for the accumulation of water and is not connected via a surface inlet or outlet to the drainage network. For example, it receives water by direct precipitation, groundwater or as limited runoff from the surrounding catchment but no channelled surface inflows or outflows are evident.” Isolated depression wetlands have a basin-shaped morphometry, increasing in depth from the perimeter to the centre, and are hydrologically isolated from other water sources in terms of surface flows.

Valley bottom wetlands are described by Ewart-Smith *et al*. (2006): “A valley bottom is a functional unit at the bottom of a valley that receives water from an upstream channel and/or from adjacent hill slopes. The area is not subject to periodic over-bank flooding by a river channel.” By ‘functional unit’, these authors are referring to a level 3 discriminator in the wetland classification system hierarchy. Valley bottom wetlands occur in low-lying, gently sloped areas and are not hydrologically isolated systems in that they receive water from an upstream channel and/or adjacent hill slopes. Because valley bottom wetlands occur in low gradient landscapes, they generally contain flowing surface water during the wet season, but unlike rivers do not have a single clearly defined channel. Instead, this wetland type usually has braided or undefined channels and is characterised by having slow, diffuse flows across the landscape (except during times of heavy flooding). The reader is referred to Ewart-Smith *et al*. (2006) and SANBI (2009) for further details on the classification of South African wetlands. Although SANBI (2009) incorporates an updated version (draft form – March 2009) of the classification system of Ewart-Smith *et al*. (2006), wetland type-descriptions used in this study were drawn from the latter authors as it was the document available during the planning and execution of this study.

1.6 Study objectives

The objectives of this study were to:

- collate and review both local and international literature relating to wetland biological assessment using aquatic invertebrates;

- conduct an investigation into the response of aquatic invertebrates (including micro-crustaceans) to anthropogenic disturbances in isolated depression wetlands of the Western Cape, South Africa;

- identify candidate invertebrate taxa or metrics for assessing human impacts on isolated depression wetlands in the Western Cape; and if useful indicator taxa and/or metrics are established, to provide a protocol for developing an assessment method using aquatic invertebrates; and

- investigate the applicability of the SASS river index to wetlands; in this regard, both lentic (e.g. isolated depressions) and lotic (e.g. valley bottom) wetland types will be investigated.

2. LITERATURE REVIEW

2.1 Introduction

Wetlands are conspicuous features in the landscape and are now well recognized for their ecological importance and services they provide to human society. They may perform various hydrological functions such as purification of catchment surface water, floodwater attenuation, groundwater recharge and erosion control (Richardson, 1994; Costanza *et al*., 1998; Mitsch and Gosselink, 2000; Mitsch *et al*., 2005; Zedler and Kercher, 2005; Brauman *et al*., 2007). Wetlands are a critical store of biological diversity and present unique habitats within terrestrial landscapes (Ramsar COP7, 1999; Williams *et al.,* 2004; Dudgeon *et al*., 2006; Verhoeven *et al*., 2006). Furthermore, wetlands are regarded as highly productive systems and often have economic and social values (Thibodeau, 1981; Leitch and Shabman, 1988; Turner, 1991; Gren *et al.,* 1994; Costanza *et al*., 1998; Woodward and Wui, 2001; Schuyt, 2005; Brander *et al*., 2006).

Until relatively recently (late 1960s) wetlands did not enjoy this kind of positive recognition and draining, infilling or other forms of destruction of wetlands were considered accepted practices worldwide (Cowan, 1995; Danielson, 2002; DWAF, 2004). Wetlands were often perceived as impediments to development and progress or as productive lands suitable for agriculture and were not afforded protection by law. Public policies may even have supported wetland degradation, as was the case in the USA whereby the Federal Swamp Land Act (1850) deeded wetland acreage from federal land for conversion to agriculture (Danielson, 2002). Besides direct destruction of wetland habitat, human-induced stressors on wetlands such as pollution, habitat and hydrological alterations have significantly changed the biotic integrity and functional ability of a vast number of wetland ecosystems worldwide, particularly in urban and agricultural areas (Karr, 1991; Ehrenfeld, 2000; Danielson, 2002; Zedler and Kercher, 2005; Verhoeven *et al*., 2006).

A major turning point for wetland conservation worldwide was *The Convention on Wetlands of International Importance especially as Waterfowl Habitat* held in Ramsar, Iran, in 1971 (now commonly referred to as the 'Ramsar Convention'). The broad aims of the Ramsar Convention are to halt the worldwide loss of wetlands and to ensure effective conservation of those that remain through wise use and management. Signatories are bound to incorporate wetland conservation into state policy and to ensure active measures are taken to meet the requirements of both the convention and the various COP (‘Convention of the Parties’) reports since then (DWAF, 2004). Currently (March 2008) there are 158 contracting parties to the Convention; South Africa was the fifth signatory. Furthermore, certain countries (e.g. South Africa: National Water Act, 1998, USA: Clean Water Act, 1977, Australia: National Water Quality Management Strategy, 1992) are actively addressing Ramsar obligations and their own need to sustain water resources through revolutionary water laws that aim to ensure availability of good quality fresh water with emphasis on aquatic ecosystems remaining intact.

2.2 Why wetland assessment?

The recent emphasis on wetland protection and management has created an urgent need to develop assessment tools to establish and monitor human impacts on wetland ecosystems in order to prioritise wetlands for conservation and rehabilitation actions and to monitor the effects of these actions (Danielson, 2002; Findlay *et al*., 2002; DWAF, 2004; Cole, 2006). In comparison to rivers and lakes, wetland assessment techniques are poorly developed and the field of wetland assessment and monitoring is in its infancy (Grayson *et al.,* 1999; Rader and Shiozawa, 2001; Brooks *et al.,* 2004). Despite a general neglect of wetlands in the past, various governments and funding agencies worldwide have now set in motion research programmes to fill the knowledge gaps that hinder meaningful progress in wetland assessment. The worldwide literature base on wetland assessment frameworks and techniques is centred on studies in Australia and North America and from reports and guidelines emanating from the Ramsar Convention. However, various countries throughout Europe and Asia, as well as New Zealand and South Africa have more recently started government programmes to develop applicable techniques for assessing and monitoring wetlands within their boundaries, in many cases as a consequence of their Ramsar obligations (for worldwide reviews see Butcher, 2003; DWAF, 2004).

2.3 The early days of aquatic resource assessment

Traditionally (early- to mid-20th century) the condition of aquatic systems was, in general, gauged by measuring various physico-chemical endpoints in the system in order to make judgements on water quality as defined by human requirements (Karr and Dudley, 1981; Huber, 1989; Karr, 1991). A more elaborate contemporary approach was the Saprobic Index developed in Germany in the early 1900s (Kolkwitz and Marsson ,1908; 1909), which incorporated macro-invertebrates (mostly insects) and macrophytes to assess biological oxygen demand in running waters affected by point source pollution (Sladecek,1973). However, both Saprobic and physico-chemical approaches concentrated on human health (through estimation of organic pollution) rather than a broader array of natural resource issues and the shortfalls of such approaches became apparent as the 20th century progressed and significant declines in the quantity and quality of water resources worldwide were observed (Karr, 1987; 1991; US EPA, 1987; Day, 1989; Carpenter *et al*., 1998). With regards the Saprobic system, widespread criticism of the method (see Hynes, 1963; Sladecek, 1965; Goodnight, 1973) centred on its rigidity and inapplicability in all but the most specific of conditions (i.e. heavy sewage pollution in slow and evenly flowing rivers). In terms of physico-chemical approaches to assessing water resources, measuring the multitude of physico-chemical stressors that could possibly affect aquatic ecosystems was realised as ecologically and economically unfeasible (Karr, 1991; Yoder and Rankin, 1995; Karr and Chu, 1999; Danielson, 2002). Even if researchers were attempting to detect a chemical, it was often completely missed unless sampling was carried at exactly the right time (Danielson, 2002).

Wetlands in particular act as natural biochemical ‘cleansers’ in the hydrological landscape and are able to break down a variety of chemical pollutants (Mitsch and Gosselink, 2000). This biogeochemical functionality is often reflected in the short duration-time of certain chemical stressors in wetlands and makes detection of certain human-derived chemicals very difficult. However, these pollutants may still damage the natural ecosystem structure and/or functioning during their time spent in the wetland (Danielson, 2002). The inadequacy of measuring only chemical or physical endpoints in order to determine ecosystem condition became further apparent as scientists realised just how little was understood about the interactions between individual physico-chemical components and between physico-chemical and biotic components within aquatic ecosystems (Karr, 1981; Danielson, 2002). Scepticism toward traditional assessment methods grew steadily towards the latter part of the 20th century and more holistic methods that incorporated assessment of various aspects of the structure and function of physico-chemical and biotic components of aquatic resources were introduced to certain parts of the world during the 1970s and 80s (e.g. Australasia: Stark, 1985; Europe: Chandler, 1970; Armitage *et al*., 1983; De Pauw and Vanhooren, 1983; Hellawell, 1986; Wright *et al*., 1989; North America: Karr, 1981; Hilsenhoff, 1987; Plafkin *et al.,* 1989; and South Africa: Chutter, 1972). Such methods were initially developed for rivers, where they have evolved rapidly and proliferated in accordance with the greater research and conservation attention given to these systems compared to wetlands. As a consequence, 'tried and trusted' approaches for the assessment and monitoring of rivers form the foundation of modern wetland assessment techniques (Danielson, 2002; Davis *et al*., 2006).

2.4 Modern wetland assessment: the hierarchical framework

The array of recent approaches to wetland assessment can effectively be synthesized into a 'three-tier framework' as proposed by Brooks *et al*. (2004). This approach divides assessment procedures into three hierarchical levels that vary in the degree of effort and scale. Broad landscape-level assessments using readily available remote sensing data constitute level one methods. Rapid on-site assessments form the level 2 category and intensive quantitative field methods are categorized as level 3. An assessment and monitoring programme has the option of concentrating efforts at one or more of these levels, depending on the availability of resources and the degree of confidence required from the results. Each level can be used to inform another; for example, quantitative field assessment results can be used to validate either level one or two results. Furthermore, assessment at level 1 can prioritize those catchments in need of more in-depth investigation of specific stressors degrading individual wetlands (Brooks *et al.,* 2004). Several authors have successfully applied this multi-level approach in assessment of catchments or other landscape units (Brooks *et al*., 2004; Fennessy *et al*., 2004; Wardrop *et al.,* 2007; Whigham *et al*., 2007). At each level within the 3-tier framework, two major types of assessment can be applied: functional and biological (Stevenson and Hauer, 2002; Butcher, 2003). Certain authors recognize habitat assessments as a third broad category of wetland assessment (e.g. DWAF, 2004), but in general, habitat availability is believed to be intimately linked with biological condition and should be incorporated as a sub-category of a biological assessment (Dickens and Graham, 2002; Helgen, 2002; Gernes and Helgen, 2002; Bowd, 2005; Dallas, 2007).

2.5 Functional assessment

Wetland functions can be described as the physical, chemical and biological processes which characterise a given wetland (Butcher, 2003). Methods that determine the level at which different types of wetlands perform these functions are known as functional assessments. Until the late 1990s, functional assessments were the most commonly used type of wetland assessment in the USA, where they were established principally to assess the impact of human developments on wetlands by evaluating change in wetland functioning over time (DWAF, 2004). The three major categories into which functions are generally assigned are hydrological (water storage), biogeochemical (element removal) and physical habitat (Findlay *et al*., 2002; Butcher, 2003).

At the broadest level, and requiring the least effort, level one functional assessment generally involves a desktop evaluation of wetland distribution in the context of different classes of land cover and land-use. The advantage of such techniques is that broad cumulative impacts on wetlands can be recognized (Preston and Bedford, 1988; Thiesing, 2001). This kind of evaluation is performed at a catchment scale and requires readily available GIS data, although landscape assessment of smaller clusters of wetlands is possible using aerial photographs, this technique being significantly more time-consuming than GIS (Brown and Vivas, 2005). Thiesing (2001) pointed out the problem of scale in wetland assessment and, in this regard, a general ignorance in the literature towards landscape level processes, which may be more important in determining wetland functional status than local processes. At the time of Thiesing’s comments, the only existing level 1 functional assessment approach was the Synoptic Approach to Wetland Designation (Leibowitz *et al*., 1992), which had been used in a few cases in the North-Western United States (Abbruzzese *et al*., 1990). This approach uses landscape-level data such as GIS to evaluate catchments for a variety of functions in terms of their capacity and sensitivity to wetland loss. Due to its limited application, shortfalls of the approach have not yet been described, however it could offer a useful approach for the evaluation of cumulative impacts. Since the review of Thiesing (2001), several studies have used land-use cover as a predictor of wetland condition (e.g. Brooks *et al.,* 2004; Brown and Vivas, 2005; Mack, 2006; Wardrop *et al*., 2007). Although land-use patterns do not completely describe disturbance levels, they are usually highly correlated with landscape and wetland condition (O'Connell *et al*., 1998; Wardrop and Brooks, 1998). Level one assessment of wetland functioning often aims to rank catchments in terms of the condition of wetlands contained therein and can act as a screening tool to establish catchments of concern, which would then warrant site-specific level 2 or 3 field investigations within catchments of concern in order to diagnose specific stressors (Brooks *et al.,* 2004). In summary, level 1 functional assessment techniques appear to have two kinds of roles to play in wetland assessment: firstly, they can be used as a coarse screening mechanism to identify priority catchments in which further level 2 and 3 assessments can be conducted (see e.g. Leibowitz *et al*., 1992; Brooks *et al*., 2004; Wardrop *et al*., 2007); secondly, a landscape approach can be used to assess cumulative impacts on the functioning of a specific wetland or grouping of wetlands, however examples of this type of assessment are rare (see e.g. Preston and Bedford, 1988; Mack, 2001; Ellery *et al*., 2010).

In terms of wetland-scale assessments, a proliferation of level 2 rapid functional assessment methods followed the USA's Clean Water Act of 1972 in response to its stipulation of "no overall net-loss" of the nation's remaining wetlands (for reviews see Bartoldus, 1999; Fennesy *et al.,* 2004; 2007). In South Africa, an urgent need for short to medium term assessment of wetland integrity and functioning led to the development of WET-Health (Macfarlane *et al*., 2008) and WET-Ecoservices (Kotze *et al.,* 2008), both level 2 rapid functional assessment approaches. WET-Health is designed for the rapid assessment of the integrity of wetlands in terms of a wetland's deviation from its natural state. WET-Ecoservices is designed for the rapid assessment of the delivery of ecosystem services by South African wetlands (Cox and Kotze, 2008). Perhaps the best known of all level two rapid functional assessment methods worldwide is the Wetland Evaluation Technique (WET) developed by the US Army Corps of Engineers (USACE), which considers broad groups of functions such as fish and wildlife habitat value, flood control and value of the wetland for recreation and education (Adamus *et al*., 2001). The advantages of rapid functional assessment techniques centre on efficiencies of time and expertise, and thus cost. Despite practical advantages, WET and other rapid functional assessments have at times been criticized for their lack of scientific basis, limited regional applicability and limited predictive power (Hruby, 1999; Thiesing, 2001; Cole, 2006).

The call for truly quantitative, data-driven assessment techniques (level 3) for assessing wetland function led to the development of the Hydrogeomorphic (HGM) approach under authority of USACE in the mid-90s (Brinson, 1993; Smith *et al*., 1995). The HGM classification approach was initially developed to address the inadequacies of the Cowardin *et al*. (1979) system for classifying wetland types in the USA and has since become the most widely used classification approach worldwide (Stevenson and Hauer, 2002; Ewart-Smith *et al*., 2006). In terms of wetland assessment, the HGM approach is generally regarded as setting the standard for level 3 functional approaches (Hauer and Smith, 1998; Butcher, 2003; DWAF, 2004) and is the only level 3 method, functional or biological, that has been designed specifically for wetlands (i.e. was not modified from a river assessment approach: Stevenson and Hauer, 2002).

The HGM approach initially groups the study wetlands into specific hydrogeomorphic classes, for which broadly based functions are characterized by comparing measured functions to those expected for least disturbed wetlands of the same class. Examples of broadly based functions include surface water storage, nutrient cycling and maintenance of aquatic food webs. Each function is characterized by a set of attributes. Attributes which respond to impact in *a priori* evaluation with a calibration dataset are incorporated as variables into logic models, with one model per function. Here the distinction of HGM classes is crucial in that different types of wetlands in different regions will possess varied natural ranges in terms of their functional abilities and thus quantitative comparisons can only be made between wetlands of the same class within the same eco-region. Wetlands within the calibration dataset are thus of the same HGM class and eco-region, ranging from least- to most-disturbed. Each logic model is written as an algorithm to produce a Functional Capacity Index (FCI) score for each function. The FCIs of test wetlands are compared with FCI ranges for the calibration dataset in order to score overall wetland functioning (Stevenson and Hauer, 2002).

The HGM approach uses objective, quantitative, level three assessments that include the use of reference wetlands as objective benchmarks for assessing functions (Smith *et al*., 1995; Hauer and Smith, 1998). Another key distinction is the iterative nature of the model, which allows for refinement and validation based on expert review at each step in the process (Thiesing, 2001). Perhaps the most criticized weakness of HGM-type models is the lack of empirical validation of structure-function links implicit within the models (see e.g. Hruby, 1999; Thiesing, 2001; Cole, 2006).

Models rely almost exclusively upon structural indicators that are assumed to directly relate to function. The actual rates or dynamics of environmental processes occurring in test wetlands are very difficult to measure because of high spatio-temporal variability and thus surrogates (structural indicators) for functional measurements are viewed as more practical for assessment purposes. Certain authors (e.g. Thiesing, 2001) suggest that HGM-type models may be under-scoring the functional importance of wetlands in terms of conservation and mitigation permit procedures, particularly highly impacted wetlands in urban landscapes, where they are performing valuable functions in the context of their degraded landscape but are under-scored relative to reference standards. Further criticisms of the HGM approach centre on the high cost of model development and the often complex and/or obscure interpretation of results (Hruby, 1999; Thiesing, 2001).

2 6 Biological assessment

Assessment techniques that aim to characterize various aspects of the environmental condition of wetlands using measures of the biota are known as biological assessments or bioassessments. Various definitions exist to help clarify exactly what bioassessment is. A commonly used definition is "…to evaluate a wetland's ability to support and maintain a balanced, adaptive community of organisms having a species composition, diversity and functional organization comparable with that of minimally disturbed wetlands within a region" (US EPA, 1998). More simply put, bioassessment can be defined as "…an evaluation of the condition of a water body using biological surveys and other direct measurements of the resident biota in surface waters" (Barbour *et al*., 1999).

For the purpose of this study, bioassessment is seen simply as "using bio-monitoring data of samples of living organisms to evaluate the condition or health of a wetland or river" (Helgen, 2002). In this context, the term bio-monitoring means "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 and Nedeau, 2000). Bio-monitoring thus relates to the actual collection of data and bioassessment relates to the analysis and interpretation of this bio-monitoring data. The term "bioassessment" will be used in this study as an umbrella term for both data collection and its subsequent analysis and interpretation.

A basic premise of bioassessment is that the community of plants and animals living in a wetland will reflect the biological integrity of the wetland (Danielson, 2002). As stated by Marchant *et al.* (2006), “Biological measurements provide direct information on the condition of groups of biota resident in the water resource, and therefore on the condition of the resource”. Importantly, biological communities integrate the effects of stressors over time, including short-term or intermittent stressors. In this regard, the ability of the biota to reflect past impacts on the aquatic system is seen as a key advantage of bioassessment over traditional 17hysic-chemical methods and modern functional approaches (Karr, 1991; Karr and Chu, 2000; Danielson, 2002; DWAF, 2004; Bonada *et al*., 2006). As discussed earlier (‘The early days of aquatic resource assessment’), the fundamental shortfalls of traditional physical and chemical monitoring approaches are now recognised as having contributed to the significant degradation of aquatic resources worldwide (see for reviews Karr, 1991; Karr and Chu, 2000). A modern paradigm for river resource management was set in motion by the likes of Karr and Dudley (1981) in the United States who pushed for the notion of conserving “biological integrity” rather than trying to conserve rivers merely for human use. They argued that conserving a river only in order to meet the needs of human water quality requirements was a short-sighted view and that it is only by considering the water quality and quantity requirements of all the river’s users (i.e. not just humans, but also plants and animals), that one can achieve the goal of long-term conservation of the river resource. This kind of holistic perspective is reflected in their now widely quoted definition for the biological integrity of a given resource: “the ability to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity and functional organisation comparable to that of natural habitat of the region”. Water resource management worldwide currently follows this holistic paradigm of managing water resources to conserve biological integrity (Ollis *et al*., 2006). In this regard, biological assessment approaches are now the most popular form of water resource assessment worldwide (e.g. Rader and Shiozawa, 2001; DWAF, 2004; Bonada *et al*., 2006). This popularity stems from the success of such approaches for monitoring river health (e.g. the SASS index in South Africa), whereas wetland bioassessment is a field in its relative infancy. However, several authors point out the good fortune for wetland ecologists and managers that they can draw on the vast resource of bioassessment knowledge gained through several decades of river research (e.g. Chessman *et al*., 2002; Danielson, 2002; Helgen, 2002; Davis *et al*., 2006).

2.7 Indicator taxa for aquatic bioassessment

In the context of wetland bioassessment, a given taxon can be considered an indicator species if it has a known narrow range of tolerance to one or several environmental variables and as such its presence/absence, abundance, behaviour or physical condition within a given wetland can be used to gauge the environmental conditions present in that wetland (Johnson *et al*., 1993). Here the word 'species' is used broadly to represent many levels of organization, ranging from sub-organismal (i.e., gene, cell, tissue) and organismal to population, assemblage, community, and even ecosystem levels (Bonada *et al*., 2006), This review focuses on indicator species within assemblages (i.e. taxa at the family-, genus- or species-level). A variety of faunal and floral assemblages have been tested for freshwater bioassessment purposes through the assessment of potential indicator taxa within assemblages. In this regard, macrophytes (e.g. Tremp and Kohler, 1995; Thiebaut and Muller, 1998; Ferreira *et al*., 2005; Brabec and Szoszkiewicz, 2006; Szoszkiewicz *et al*., 2006), periphyton (e.g. Van Dam *et al*., 1994; Barbour *et al*., 1999; de la Rey *et al*., 2004; 2008; Taylor *et al.,* 2007) and fish (e.g. Karr, 1981; Karr *et al*., 1986; Plafkin *et al*., 1989; Barbour *et al*., 1999) have all been used successfully in the bioassessment of rivers. The reader is referred to reviews by Barbour *et al*. (1999), Downes *et al*. (2002) and Harding *et al*. (2005) for more information on the advantages and disadvantages of using these taxa as bioassessment tools in lotic systems; more detail in this regard is beyond the scope of this review. It is the benthic macro-invertebrates, however, that are overwhelmingly the most successfully used assemblage of organisms for use in river bioassessment and their numerous advantages for this purpose have been well documented in the literature (for reviews see Hellawell, 1986; Metcalfe, 1989; Rosenberg and Resh, 1993; Dallas, 1995; Bonada *et al*., 2006; Ollis *et al*., 2006). Briefly, benthic macro-invertebrates are ubiquitous, largely non-mobile and generally abundant, occurring across a variety of habitats in rivers. Their taxonomic richness offers a spectrum of environmental responses and their sedentary nature allows spatial analysis of pollution effects. They are relatively easily sampled with inexpensive equipment. Taxonomy is well described for most genera and families and the sensitivities of many common taxa to pollution have been established. This is the case in most developed nations of the world, but it must be borne in mind that the taxonomy and pollution sensitivities of benthic macro-invertebrates is not necessarily well established in many developing nations of the world, including South Africa (Bonada *et al*., 2006; Ollis *et al*., 2006).

The use of suitable indicator assemblages for wetlands is not well researched compared to that for rivers and options are still currently being explored worldwide. Macrophytes (e.g. Mack, 2001; Wardrop and Brooks, 1998; Simon *et al*., 2001; Gernes and Helgen, 2002; De Keyser *et al*., 2003), invertebrates (e.g. Chessman *et al*., 2002; Gernes and Helgen, 2002; Helgen, 2002; Uzarski *et al*., 2004), algae (including diatoms, e.g. Lane and Brown, 2007), amphibians (e.g. Adamus *et al*., 2001; Sparling *et al*., 2002), birds (e.g. Adamus, 2002) and fish (e.g. Galastowitsch *et al*., 1998; Schulz *et al*., 1999) have all been considered to some degree for wetland bioassessment purposes. Of these, macrophytes and invertebrates have received the most research attention and have been incorporated into various biotic indices of wetland condition worldwide. Algae, amphibians, birds and fish have also been incorporated into biotic indices, though less often. Once again, the focus of this review is on aquatic invertebrates for bioassessment. For an overview of advantages and disadvantages of the other faunal and floral groups as indicators for wetland bioassessment, the reader is referred to Adamus and Brandt (1990), Adamus *et al*. (2001), Butcher (2003) and DWAF (2004).

Invertebrates show a variety of positive attributes for use as indicators in wetlands. Much less is known about the ecology and pollution sensitivities of wetland taxa than of their river counterparts, however. Several authors have reviewed the literature and reported possible advantages and disadvantages of wetland invertebrates as indicators, but this information is almost exclusively derived from North American studies and may or may not hold for the variety of wetland types and their associated invertebrates in other regions of the world.

Adamus and Brandt (1990) surveyed information in the technical literature on wetland bioassessment in the United States and compiled a list of the positive and negative attributes of invertebrates as indicators. Batzer *et al*. (1999) and Adamus *et al*. (2001) reviewed the attributes of wetland invertebrate indicators reported in the North American technical literature for the decade following Adamus and Brandt's (1990) report. Helgen (2002) compiled a summary of the generally recognised positive and negative attributes of invertebrates as potential indicators for wetlands (Appendix 1), incorporating information reported by Adamus and Brandt (1990), Batzer *et al*. (1999), Adamus *et al*. (2001) and from a variety of studies cited by these authors. Briefly, aquatic invertebrates are ubiquitous in many types of wetlands (Batzer *et al*., 1999). They have been shown to respond with differing sensitivities to a wide variety of stressors and have been used in eco-toxicology assessments of lakes and wetlands (Adamus *et al.,* 2001). However, toxicity tolerance and pollution sensitivity is considerably less understood for wetland invertebrates than for those in rivers, particularly in countries with limited research background (Chessman *et al*., 2002; Bonada *et al*., 2006). Many invertebrates complete their life cycles in wetlands and are thus exposed directly to environmental stressors (Merritt and Cummins, 1996; Fairchild *et al.,* 2000; Zimmer *et al.,* 2000). Winged insects, on the other hand, are able to escape by dispersal and may not be efficient indicators in this regard. Life cycles of weeks to months allow integrated responses to both short-term and chronic disturbance effects (Helgen, 2002). Studies aimed at assessment of longer-term disturbance effects or landscape-level effects might focus on other indicator taxa such as macrophytes (longer-term indicators), birds or amphibians (landscape-level indicators). Sampling methods are generally not difficult and are inexpensive (Helgen, 2002; DWAF, 2004). One of the major drawbacks of wetland invertebrates, however, is the laborious and often costly identification and enumeration process that follows the collection of samples (Batzer *et al*., 1999; Helgen, 2002). Taxonomic information for wetland invertebrates is remarkably scarce compared to that for rivers and samples may need to be contracted out to specialists for identification (Adamus and Brandt, 1990; Helgen, 2002).

Whilst the reviews discussed in the previous paragraph include aquatic and semi-aquatic insects, large crustaceans and molluscs, they do not include micro-crustaceans (e.g. cladocerans, copepods and ostracods), which certain authors (e.g. Lougheed and Chow-Fraser, 2002) have suggested are useful in wetland bioasessment. Throughout the literature, micro-crustaceans receive scant attention as potential wetland indicators. One of the major criticisms of wetland insects (e.g. odonates) as indicators is their ability to fly, thus escaping from adverse environmental factors. Micro-crustaceans do not have wings and are generally resident in wetlands for their entire life cycle, having adapted to a variety of period regimes mostly by an ability to survive desiccation. This makes them good candidates as indicators in that they are unable to escape environmental stressors. Micro-crustaceans are, however, difficult to identify due mostly to their small size and often-required dissection of soft parts to achieve meaningful identifications. They also may be extremely abundant and thus difficult to enumerate with reliability. Potential advantages of using micro-crustaceans in wetland bioassessment may well be overshadowed by difficulties encountered in making enumerations and identifications, especially in terms of developing rapid and user-friendly indices.

2.8 River bioassessment methods using aquatic macro-invertebrates

The use of macro-invertebrates as a bio-monitoring tool for flowing waters is well established nationally and worldwide (Plafkin *et al*., 1989; Rosenberg and Resh, 1993; Chessman and McEvoy, 1998; Chutter, 1998; Barbour *et al*., 1999; Dickens and Graham, 2002; Dallas, 2002; Ollis *et al.,* 2006). At a broad level, three major approaches exist for establishing a bioassessment study or programme:

- the multi-metric Index of Biological Integrity (IBI);
- multivariate indices; and
- numerical biotic indices.

An array of protocols and indices exist within each type of approach, depending on the assemblage being monitored and what region of the world implementation takes place. The usefulness of river macro-invertebrates as bioassessment tools is exemplified by their success in bioassessment programmes using all three approaches worldwide. All three approaches are discussed below.

2.8.1 The multi-metric Index of Biological Integrity (IBI)

The IBI approach was initially described by Karr (1981) as a means of assessing biological integrity of rivers using fish in the mid-western United States. The IBI has since become the most popular framework for freshwater bioassessment studies conducted in the United States and has been adapted for use with a multitude of biological assemblages in both rivers and wetlands (Plafkin *et al*., 1989; Barbour *et al*., 1999; Karr and Chu, 2000; Mack, 2001; Gernes and Helgen, 2002; Helgen, 2002). Essentially, the approach involves combining two or more biological metrics (e.g. number of families, % chironomidae, % predators) into a single index (i.e. it is known as a multi-metric index). Assessment of human health (using metrics such as blood pressure, urine analysis, white blood cell count, and temperature) and indices that summarise the economy are useful analogies (Karr, 1991). In the United States, the quantitative IBI approach has been modified into a wide variety (depending on the region) of rapid bioassessment protocols (RBPs) for wadeable rivers using aquatic macro-invertebrates. RBPs are semi-quantitative level 2 approaches generally aimed at on-site identification of invertebrate taxa and assessment of river conditions (Plafkin *et al*., 1989; Barbour *et al*., 1999). A later section of this review addresses the specifics of the IBI process for invertebrates, so a broader outline of a typical IBI process (applicable to any specified assemblage) is presented as follows (for reviews of this approach, see Karr, 1981, 1991; or Teels and Adamus, 2002):

Study sites are established *a priori* based on a gradient of human disturbance. Sites should be as similar as possible with regards to their natural state. Sites being compared should therefore be of the same classification type and should occur in the same eco-region. Standardised sampling protocols are applied at each site, the particular applied protocol depending on the assemblage being studied. Protocols should aim to collect a representative sample of the relevant biotic assemblage at each site. If resources permit, then two assemblage types (e.g. invertebrates and macrophytes) should be assessed concurrently in order to strengthen the validity of results. The biota should be sampled in the context of their ambient environmental conditions and thus basic physical and chemical measures describing habitats should be included in the sampling process. Various assemblage attributes (measures that summarise the assemblage e.g. number of intolerant species) are scored at each site and those found to correlate with the gradient of human disturbance are selected as metrics for inclusion in the multi-metric index. Metric scores at each site are added to produce an overall IBI score. The IBI score range is further divided into integrity classes, which offer a categorical description of the impairment range. Sites are classified into categories based on their IBI scores.

According to Karr (1991), analysis of the IBI scores allows one to: (1) evaluate current biological conditions at each site; (2) determine temporal trends at a site with repeated sampling; and (3) make comparisons between sites for which data are collected more or less simultaneously, presuming they follow the guidelines stipulated above.

The major scientific advantages of IBI extracted from the literature include: (1) it is quantitative; (2) it gauges a site against an expectation based on minimal disturbance in the region; (3) it reflects distinct attributes of biological systems, including spatio-temporal dynamics; and (4) it incorporates professional judgement in a systematic and ecological sound manner (Karr, 1981; Karr *et al*., 1986; Plafkin *et al*., 1989; Barbour *et al*., 1999; Danielson, 2002; Teels and Adamus, 2002). It should be stressed that multi-metric indices such as the IBI were designed to assess overall biological integrity or condition of a site and, in terms of investigating specific forms of impairment (e.g. trace metal toxicants), should not be claimed as a replacement method for more specific eco-toxicological, physico-chemical or functional assessments methods.

2.8.2 Multivariate indices

As mentioned previously, IBIs are reasonably simple and efficient to set up and implement and much of the popularity around these indices centres on this simplicity. More data-intensive and complex multivariate techniques are not as easy to implement and require a large research effort, although if properly established, these techniques have advantages over multi-metric methods in terms of their predictive power, ability to distinguish specific stressors, scientific rigour and regional applicability (Wright, 1995; Reynoldson *et al*., 1997; Boulton, 1999; Bonada *et al*., 2006).

The most advanced macro-invertebrate bioassessment indices worldwide are the predictive modelling approaches spearheaded by the British RIVPACS (River InVertebrate Prediction And Classification System) and Australian AusRivAS (Australian River Assessment System). AusRivAS (Simpson and Norris, 2000) was derived from RIVPACS (Wright *et al.,* 1993; Wright, 1995) and required certain fundamental modifications for use in Australian rivers. Both however have a common framework as multivariate models that seek to predict the aquatic macro-invertebrate fauna of a site in the absence of environmental stress (Simpson *et al.,* 1996). A comparison of the invertebrates predicted to occur at test sites with those actually collected provides a measure of biological impairment at the tested sites (Barbour *et al.,* 1999). Fundamental to these multivariate-type approaches is a need for extensive data collection from a set of reference sites, which forms the basis of such models.

2.8.3 Numerical biotic indices

Numerical biotic indices are calculated by assigning sensitivity weightings to individual taxa (generally at the family level) based on their known tolerance to pollution (usually organic pollution) and summing or averaging the values for all taxa or individuals in a sample. Numerical indices are rapid and effective and perhaps the most popular choice of rapid bioassessment method worldwide for detecting and monitoring water pollution and other forms of human impact using invertebrates (Chessman and McEvoy, 1998; Dallas, 2002). International examples of established biotic indices include the Biological Monitoring Working Party (BMWP) scoring system used in Great Britain (Armitage *et al*., 1983), the New Zealand Macro-invertebrate Community Index (MCI, Stark, 1985), the Spanish Biological Monitoring Water Quality (BMWQ) score system (Camargo, 1993) and the Australian SIGNAL index (Chessman, 1995).

South Africa has a simple, cost-effective numerical biotic index for the assessment and monitoring of river water quality called the South African Scoring System (SASS, Chutter, 1994). Through modification of an existing index used by the UK's Biological Monitoring Working Party (BMWP), SASS has become a very successful tool for detecting impairment of water quality through organic pollution in South African rivers and is currently in its fifth revised form (SASS5, Dickens and Graham, 2002). Although SASS5 is ineffective at distinguishing specific types of pollutants, it has proven very useful for detecting water quality impairment and river condition in general (Dallas, 1995, 1997, 2002; Dickens and Graham, 2002). SASS5 relies on the sampling of various biotopes within each site on a river's course and has proven to be more effective as the number of biotopes at a site increases (Dickens and Graham, 2002; Dallas, 2007). The macro-invertebrate taxa are identified to family level *in situ* and each taxon is given a pre-determined score from one to 15 based on its sensitivity to pollution (1=pollution tolerant, 15=pollution intolerant). Total score per site and average score per taxon are important values in determining the degree of impairment at a site. Thus, the system is carried out fairly quickly in the field and generally yields immediate meaningful results that cannot necessarily be gleaned by taking physico-chemical point measures in the river. SASS5 is currently considered the standard method for river bio-monitoring in South Africa and is an integral part of the South African National River Health Programme (Uys *et al.,* 1996). It has also been incorporated into the process of Ecological Reserve determination as required by the South African National Water Act No. 36 No. of 1998 (Dickens and Graham, 2002). SASS is also used extensively by various government departments (e.g. Department of Water Affairs, DWAF[1]), institutions (e.g. universities) and consultants (Graham *et al*., 2004).

2.9 Are macro-invertebrate river bioassessment methods applicable to wetlands?

Few studies have investigated the suitability of invertebrate river bioassessment methods for wetlands. In this regard, two schools of thought have arisen. The first is that rapid bioassessment protocols (RBPs) developed for rivers can be applied successfully to wetlands and many people believe that what works for rivers should work for wetlands, even though some modification may be required (Davis *et al*., 2006). A second smaller group of people hold the view that wetland invertebrate assemblages are too variable in their responses to environmental conditions, both within and between wetlands, and that rapid sampling and analysis will not produce meaningful bioassessment information at the wetland level, but rather only at the level of wetland category (Butcher, 2003). On the whole, the literature supports the use of region-specific invertebrate bioassessment protocols as effective tools to distinguish environmental change at a wetland level (Adamus *et al*., 2001; Butcher, 2003; Bowd, 2005), although the body of practical evidence for the effectiveness of such protocols is still reasonably thin. Two studies investigate applicability of river invertebrate protocols to wetlands, with contrasting results. Davis *et al*. (2006) tested a predictive model, derived from the Australian AUSRIVAS model for rivers, on wetlands of the Swan Coastal Plain region in Western Australia near Perth. Although observed-to-expected (OE) ratios were not significantly correlated with any qualitative indices of wetland condition, they found that the model could successfully detect nutrient enrichment through a correlation with pH levels. Greater discrimination among the test wetlands was provided by the model observed-to-expected ratios than either raw richness or a numerical biotic index (SWAMPS, Chessman *et al.,* 2002) developed specifically for that set of wetlands. The study of Davis *et al.* (2006) shows promise for transposing predictive models for river monitoring onto wetland systems, although the authors warn that such models can only be used for specific geographic regions and specific climatic conditions. In

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[1] Note that the Forestry division of DWAF has since been incorporated into the Department of Agriculture, Fisheries and Forests, and Water And Environmental Affairs have been linked into a single Department of Water and Environmental Affairs (DWEA).

addition to providing an objective method for assessing wetland condition, predictive modelling provides a list of taxa expected to occur under reference conditions.

Bowd *et al*. (2006a) tested the applicability of the SASS5 river bio-monitoring index on permanent depression wetlands (linked to river channels, see classification system of Ewart-Smith *et al.,* 2006) of the KwaZulu-Natal midlands region in South Africa. The SASS5 protocol (with slight modifications) was applied to three wetlands organically polluted by dairy effluent and four reference wetlands. SASS5 was ineffective at distinguishing impaired from reference sites and it was concluded that future 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 effluents. It was also recommended that a wetland biotope or habitat assessment index be developed for use in conjunction with macro-invertebrate assessment protocols. The field of wetland conservation in South Africa would benefit greatly from the establishment of a relatively easy-to-use SASS-like index for wetland bioassessment. The results of Bowd *et al.* (2006a) suggest however that significant further research and modification of the existing SASS5 index is required if the aim is to produce a user-friendly index for use in wetlands.

A derivation of the SIGNAL macro-invertebrate index used to assess river impairment in eastern Australia was applied to wetlands of the Swan Coastal Plain in western Australia (Chessman *et al.,* 2002). Although the index, known as SWAMPS, was specifically developed for wetlands, the framework of the index approach was essentially the same as the SIGNAL index. Study results indicated that the SIGNAL index, a numerical biotic index akin to SASS, could be applied to distinguish wetland impairment levels by modifying the invertebrate tolerance scores through empirical investigation. The findings of Chessman *et al*. (2002) provide preliminary justification that perhaps in South Africa, the SASS river index could be modified for use in wetlands given that important modifications to invertebrate tolerance scores are first made. The major problem in South Africa is the dearth of empirical information on responses of wetland invertebrates to human disturbances, including a complete lack of eco-toxicology data. The empirical component of this study makes an initial attempt to address this knowledge gap.

2.10 Wetland invertebrate bioassessment: existing programmes and scientific literature

Only two countries have really contributed significantly to the international literature on wetland bioassessment using invertebrates: the United States (e.g. Hicks and Nedeau, 2000; Gernes and Helgen, 2002; Lougheed and Chow-Fraser, 2002; Helgen, 2002; Uzarski *et al.,* 2004) and Australia (e.g. Davis *et al*., 1993; Chessman *et al*., 2002; Butcher, 2003; Davis *et al*., 2006). Work has concentrated almost exclusively on developing protocols for assessing wetlands using macro-invertebrates and much less attention has been given to micro taxa such as crustacean zooplankton, mostly because of the need for specialist taxonomic expertise and more complex laboratory processing (Lougheed and Chow-Fraser, 2002). Literature concerning the use of both macro- and micro-invertebrates as bioassessment tools for wetland monitoring is briefly covered in this review.

2.10.1 Macro-invertebrates

Several bioassessment indices have come out of protocols specifically tailored for wetland macro-invertebrate sampling. The leading agency in the development of such protocols is the United States Environmental Protection Agency (US EPA), which has a wetland bioassessment research programme, the Biological Assessment of Wetlands Working Group (BAWWG). Teels and Adamus (2002) formulated a multi-metric approach to assess the biological integrity of wetlands, which is a framework that can be applied to a chosen wetland category type within a specified eco-region. Briefly, the multi-metric approach aims to develop an index of biotic integrity (IBI) by sampling macro-invertebrates in wetlands across a gradient of disturbance and assessing which attributes of invertebrate community composition correlate with the gradient of disturbance. Invertebrate attributes which are good correlates with the degree of impact are incorporated into a set of metrics, which contribute to an overall index for the wetland type being studied. Examples of successful metrics (used for Minnesota depressional wetlands) include the number of chironomid genera, the proportion of corixids (as % of total sample abundance), the number of taxa known to be intolerant to water quality degradation and the number of odonata genera (Gernes and Helgen, 2002).

The multi-metric approach employs the sampling of ‘reference’ and ‘project’ wetlands. Reference sites are those minimally impacted by human disturbance that reflect the natural condition for the wetland type under study. Wetlands in a completely natural state may not exist for certain wetland types, depending on the region, and thus the term ‘least impaired’ is employed. Project wetlands are those that make up the remainder of the gradient of disturbance and may vary from a moderate impact state to severely impaired wetlands. The major strength of the multi-metric approach lies in its simplicity and the results are also relatively easy to interpret. However, difficulty may be encountered in finding a set of wetlands that meet the appropriate criteria for this approach (e.g. a gradient of disturbance or controlling for natural forcing factors). Another problem is that certain successfully used metrics in the USA (e.g. Minnesota: number of chironomid genera) do not lend to rapid assessment in that specialist expertise is required to make the given level of identification and these metrics would probably not be suitable in South Africa for this reason.

The multi-metric approach of the US EPA was applied to large depressional wetlands of the North Central Hardwood Forest eco-region of Minnesota by Gernes and Helgen (2002). It was found that macro-invertebrate IBI scores in large depressional wetlands were significantly correlated with Human Disturbance Scores (HDS), which score buffer and landscape use around the wetlands in terms of human land-use impacts. Further significant correlations were established between IBI scores and levels of turbidity, and concentrations of phosphorous and chloride. In summary, the US EPA has succeeded in producing a fairly transparent multi-metric approach for assessing the biological integrity of wetlands using macro-invertebrates and the usefulness of this approach needs to be tested for South African wetlands.

Hicks and Nedeau (2000) compiled a wetland macro-invertebrate bio-monitoring manual for volunteers. The manual is aimed to guide volunteers in collecting, identifying and analysing macro-invertebrate samples from permanent wetlands in New England, USA, and provides an example of the application of a multi-metric IBI framework. Hicks and Nedeau (2000) include a useful set of invertebrate identification diagrams (family level), suggest pollution tolerance values for each of the taxa (although only at the order level) and a set of invertebrate and habitat scoring datasheets ready to be filled out. Through a combination of Gernes and Helgen (2002) and Hicks and Nedeau (2000), a significant amount of information has been generated, allowing for the possible implementation of a multi-metric bioassessment approach in other parts of the world, although modifications (e.g. expected taxa) would be required.

Uzarski *et al.* (2004) validated an earlier macro-invertebrate index of biotic integrity (Burton *et al.*, 1999) during a period of lake level decline for a set of Lakes Huron and Michigan fringing wetlands (USA). With improvements, the index was able to place all sites in a comparable order of disturbance based *a priori* on adjacent land-use and various limnological parameters. The refined index was effective even during a period of significant lake level decline during 1998-2001.

The above studies suggest potential for aquatic macro-invertebrates as bioassessment tools in wetlands. Negative results, however, were also encountered in the literature and cannot be overlooked. Tangen *et al.* (2003) found weak correspondence between macro-invertebrate assemblages and human land-use practices in the Prairie Pothole Region wetlands of North Dakota, USA. These authors were unable to identify any effective IBI metrics indicative of human land-use disturbance and concluded that the influence of natural environmental fluctuations were more important in shaping invertebrate assemblages than were anthropogenic factors. Steinmann *et al*. (2003) investigated the influence of cattle grazing and pasture land use on macro-invertebrate assemblages in freshwater wetlands of south-central Florida, USA. They found little influence of cattle stocking densities on water column nutrient levels or invertebrate assemblages. However, they did find a correspondence between riparian vegetation types of different pasture land use practices and invertebrate assemblages and suggested that ostracods and culicid larvae might be useful as indicators of eutrophication in subtropical wetlands.

2.10.2 Micro-invertebrates

Davis *et al*. (1993) conducted a comprehensive examination of the physical, chemical and biological constituents of a large system of wetlands on the Swan Coastal Plain in Western Australia, near Perth. Multivariate classification and ordination of the wetlands on the basis of the aquatic macro- and micro-invertebrate fauna revealed that the main environmental factors affecting invertebrate assemblage composition were water colour, degree of eutrophication, salinity and seasonality. Invertebrate assemblage data were better able to distinguish levels of eutrophication among wetlands than were water chemistry measures, suggesting the potential of invertebrates as water quality indicators in wetlands. A wealth of more detailed information is included in the report of Davis *et al.* (1993), such as lists of useful indicator taxa, multivariate data analysis techniques for analysing invertebrate assemblages and methods for enumerating zooplankton sub-samples.

Lougheed and Chow-Fraser (2002) developed a wetland zooplankton index (WZI) to assess water quality in marshes of the Laurentian Great Lakes Basin, USA. Seventy coastal and inland marshes were sampled across a gradient of human disturbance during the period 1995-2000. The index was developed based on the results of partial canonical correspondence analysis (pCCA), which indicated that plant-associated micro-crustacean taxa such as chydorid and macro-thricid cladocerans were common in high quality wetlands, whereas more open water, pollution tolerant taxa such as *Brachionus* and *Moina* dominated degraded wetlands. The WZI was found to be more useful than indices of diversity and measures of community structure for detecting impairments in water quality. Furthermore, because the study covered wetlands across a broad geographic range, the index should be broadly applicable.

Despite the positive findings of Davis *et al*. (1993) and Lougheed and Chow-Fraser (2002) with regards the use of micro-invertebrates as a bioassessment tool for wetlands, any approaches developed worldwide are likely to be hindered by the difficulties involved in the identification and enumeration of micro-invertebrate taxa. Useful levels of identification of micro-crustaceans for bioassessment purposes may require expert taxonomists and simple distinction of different morpho-species, as required for bioassessment analyses, often requires tedious examination of body parts and may involve dissection of specimens.

2.11 Concluding remarks

The body of literature on wetland bioassessment protocols using aquatic invertebrates is small and centred almost entirely on research conducted in the USA and Australia in the last 10-15 years. Wetland invertebrates possess a number of advantageous attributes as biological indicators of disturbance. The majority of published findings in the literature suggest aquatic macro-invertebrates as a beneficial tool for the biological assessment of wetlands, but indices need to be modified, sometimes significantly so, in order to be used in different eco-regions and wetland types. With the latter point in mind, river macro-invertebrate indices appear to hold an advantage of often being applicable over broad spatial areas with little or no modification to indices. Certain studies (e.g. Tangen *et al*., 2003) indicate that aquatic macro-invertebrates are not a feasible tool for wetland bioassessment in areas where the influence of natural environmental disturbances outweighs anthropogenic-induced disturbances. Other potential pitfalls in developing macro-invertebrate indices for wetland bioassessment include the lack of empirical information on responses of invertebrates to human stressors and the dearth of taxonomic information for making correct identifications of wetland taxa. The use of micro-crustacean taxa could add an important complement of information to the more traditional macro-invertebrate assessment techniques, but is likely to be significantly hindered by the difficulties involved in identification and enumeration of such taxa.

3. INTRODUCTION TO THE FIELD STUDY

As stated in the opening section of this report, the objectives of the empirical component of this study are to:

- conduct an investigation into the response of aquatic invertebrates (including micro-crustaceans) to anthropogenic disturbances in isolated depression wetlands of the Western Cape, South Africa;

- identify candidate invertebrate taxa or metrics for assessing human impacts on isolated depression wetlands in the Western Cape; and if useful indicator taxa and/or metrics are established, to provide a protocol for developing an assessment method using aquatic invertebrates; and

- investigate the applicability of the SASS river index to wetlands; in this regard, both lentic (e.g. isolated depressions) and lotic (e.g. valley bottom) wetland types will be investigated.

The empirical research component of this study covers two of the most abundant wetland types in the Western Cape, namely isolated depressions and valley bottom wetlands. Investigation into the use of aquatic invertebrates as indicators of wetland condition was centred on isolated depressions, where relationships between invertebrates and human stressors were investigated. Applicability of the SASS index to this wetland type was also assessed. Valley bottom wetlands were assessed only in terms of the feasibility of using SASS to distinguish levels of water quality impairment among wetlands so as to address the issue of whether SASS can be used in wetlands with some degree of flow.

3.1 Isolated depression wetlands

Wetland invertebrate assemblages are affected by a multitude of natural and anthropogenic factors which act together to determine the structure of an invertebrate assemblage in a given wetland. Isolating specific factors that are responsible for the type of invertebrate assemblage found in a wetland is a difficult task hindered mainly by the difficulty in controlling for all the variety of factors that might be acting on an assemblage at any given time. Another complicating factor in the analysis of wetland condition in South Africa is the high diversity of natural wetland types that exist often even within a limited area. With large natural variations over small spatial scales, it is difficult to attribute the change in an invertebrate assemblage from one wetland to another to anthropogenic factors or merely to natural variation. With these complicating factors in mind, the aim of a wetland bioassessment study using aquatic invertebrates should be to control for major natural forcing factors whilst sampling across wetlands differentially affected by human activities. This is the basic premise of the US EPA multi-metric IBI approach successfully implemented in Minnesota and New England (see for example Danielson, 2002; Helgen, 2002; Gernes and Helgen, 2002; Hicks and Nedeau, 2002, section 2.10.1). In accordance with the IBI approach, this study concentrates on one type of wetland (isolated depressional wetlands, see section 1.5 for a description of this wetland type) in one region of South Africa (the winter rainfall region Western Cape Province) and incorporates the sampling of this wetland type across a gradient of human disturbance states.

The semi-arid climate of the Western Cape dictates that the majority of depressional wetlands in this area are naturally seasonal in their hydrological regime. However, many depressions have become dammed by farmers or receive increased runoff from hardened urban catchments and therefore have become perennially inundated due to human alterations. The aim of this study is to characterise invertebrate assemblages in natural depression wetlands of the Western Cape and to assess changes in these assemblages with increasing human impact. In order to avoid comparing seasonal and permanent wetlands, only seasonally inundated systems were sampled during this study. Least impaired, winter-inundated, isolated depression wetlands were thus compared with disturbed, winter-inundated, isolated depressions so as to control for the hydrological regime factor, which is often regarded as a primary determinant of wetland invertebrate assemblage composition (e.g. Batzer and Wissinger, 1996; Richard, 2003). Isolated depressions are by far the most abundant wetland type in the Western Cape (Silberbauer and King, 1991) and their abundance makes location of appropriate study sites more amenable. The aim of study site reconnaissance was to identify wetland sites that occur in close proximity (to control for variation of geographic factors), but that are differentially impacted by human activities. In this regard, isolated depressions are ideal as one area of low-lying coastal plain (e.g. Cape Flats) may contain a large number of depressions in close proximity, but human activities may also vary considerably among these wetlands. Finally, isolated systems were the preferred choice so as to control for aspects of import/export of invertebrates and environmental variables (e.g.

nutrient input) from upstream and downstream aquatic ecosystems that might otherwise determine invertebrate assemblage structure in more open systems.

3.2 Valley bottom wetlands

In order to test the SASS index in wetlands that are not truly lentic (i.e. have some flow), a set of valley bottom wetlands (refer to section 1.5 for a description of this wetland type) was investigated with a modified SASS sampling method. The ability of this method to differentiate sites based on the degree of human disturbance was investigated. It was decided that an analysis of SASS in wetlands with un-channelled flow would prove useful in determining at what flows SASS is an effective index. SASS has been demonstrated as an effective index for detecting impairment of South African rivers, but the ‘grey area’ in terms of its applicability is where river channels become less defined and flows become slow.

3.3 Proxy measures for human impacts on wetlands

3.3.1 Water quality impairment

Certain quantitative variables can be used as indirect proxies of human impacts in wetlands including macro-nutrients, trace metals and specific chemical compound pollutants (e.g. pesticides). Basic *in situ* physico-chemical variables (e.g. pH, electrical conductivity, temperature, dissolved oxygen etc.) can also be measured with relative ease, but although the level of these variables may well be altered by human impacts, they usually vary independent of human impacts and thus cannot necessarily be used as proxies for human disturbance. This is the case for studies covering broad geographical areas (such as the current study), however, these physico-chemical variables may be useful for inferring human impacts in more specific cases where the researcher has *a priori* knowledge of a study system and physico-chemical changes associated with a known impact gradient. Tests for specific trace metals and pollutant compounds such as pesticides were not a feasible option in a broad scale study such as this one, due to the large number of wetlands investigated and associated high costs. Furthermore, these tests are only useful if the researcher has some *a priori* knowledge of specific chemical pollutants associated with human impacts in the chosen study area, which is not the case for wetlands covered in this study. Macro-nutrient compounds (e.g. nitrates, phosphates and ammonium) offer a useful proxy for the organic pollution of surface waters and are a feasible option for broad scale studies with a large number of wetlands. Nutrient levels were thus used as a proxy measure for water quality impairment among wetlands in this study (see section 4.1.2.3 for further details).

3.3.2 Landscape impacts

Impacts to wetlands may be integrated across various sources and it is often the case that a wetland is not impacted by a single human stressor, but rather a combination of interacting stressors which cannot be directly measured in the wetland. These stressors may come in many different forms and can be assigned to general classes of disturbance such as hydrological, physical, habitat and water chemistry. Human impacts on a wetland should not be represented by a single score from a single source of disturbance, but rather should be assessed through a combination of scores from several prevailing disturbances from both the surrounding landscape and within the wetland (Teels and Adamus, 2002). The development and use of an index which scores multiple wetland stressors is of great benefit for developing a gradient of human disturbance among study wetlands (Danielson, 2002; Teels and Adamus, 2002).

No single standard protocol currently exists for the rapid assessment of human impacts on wetlands that meets the requirements of this project (i.e. establishing a gradient of human disturbance among Western Cape isolated depression wetlands), although several more comprehensive indices have been established (e.g. Mack, 2001; Macfarlane *et al*., 2008). Gernes and Helgen (2002) presented a rapid assessment index for scoring human disturbance on wetlands in Minnesota (USA), which they used together with measures of water and sediment chemistry to establish a gradient of human impairment among wetlands. Although their index was found to be useful in Minnesota, the types of landscape disturbance it scored are specific to the types of human activities in Minnesota and would not be particularly meaningful in the Western Cape. Furthermore, the index is over-simplified and does not appropriately score human impacts across different distances from each wetland (e.g. within wetland, within 100 m, within 500 m). Modifications were made to the indices of Mack (2001), Gernes and Helgen (2002) and Macfarlane *et al*. (2008) in order to produce an index that was suitable for scoring impacts in and around isolated depression wetlands in the Western Cape (see section 4.1.2.4 and Appendix

2 for details of index protocol). The final percentage scores produced by this index are referred to as the human disturbance scores (HDS), in line with the terminology used by Gernes and Helgen (2002). The rapid index scores were used to form the gradient of disturbance among wetlands in terms of general landscape-level impacts, whilst the nutrient measures were used to form the gradient of disturbance among wetlands in terms of more specific pollution impacts on wetland trophic state.

4. METHODS

4.1 Isolated depression wetlands

4.1.1 Study sites

The specific wetland type sampled in this study was isolated depression wetlands (see Ewart-Smith *et al*., 2006; SANBI, 2009). Sampling was conducted during the winter wet season of 2007 (July-September) when wetlands in the Western Cape were at maximum inundation. 125 wetlands were sampled, occurring on coastal plains of the Western Cape winter rainfall region. Wetlands were sampled in three broad areas (herein referred to as ‘clusters’) where they reach maximum abundance (Figure 4.1): west coast (least impaired and agriculturally-impacted wetlands, 51 sites); Cape Flats (least impaired and urban-impacted wetlands, 51 sites); and the Agulhus plain on the south coast (least impaired and agriculturally-impacted wetlands, 23 sites). The wetlands constituting these clusters varied in the degree of impact and a targeted sampling approach was followed whereby sites were chosen based on *a priori* judgement of their overall condition in accordance with the multi-metric wetland bioassessment approach (Helgen, 2002).

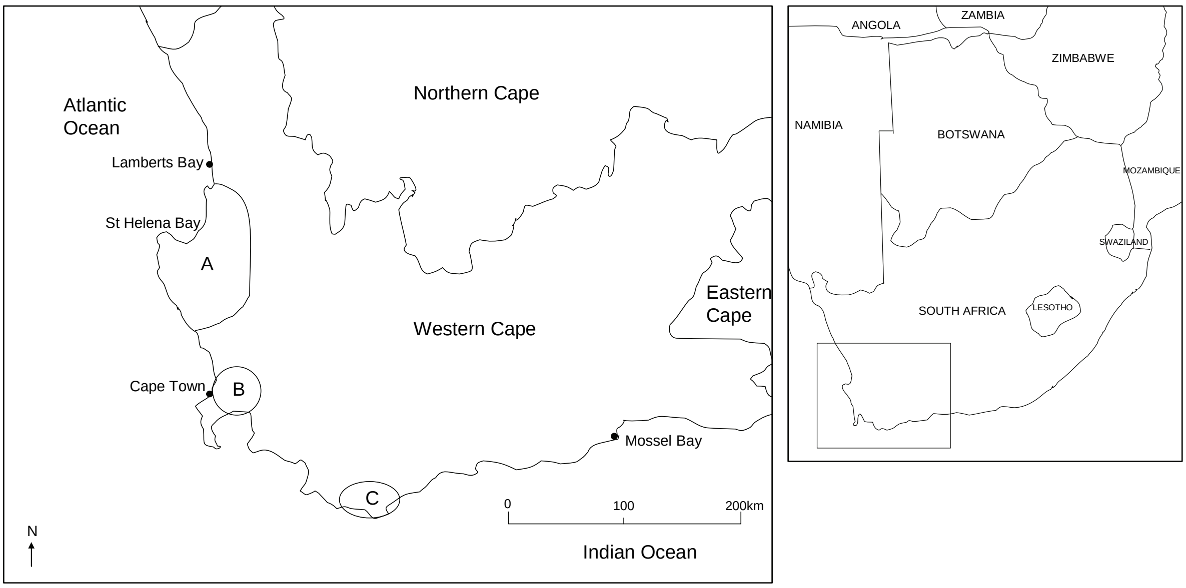

Figure 4.1: The three major clusters of wetlands sampled within the Western Cape Province, South Africa. A: West coast, B: Cape Flats, and C: Agulhas Plain.

4.1.2 Sampling

4.1.2.1 Aquatic invertebrates

All 125 sites were sampled for aquatic invertebrates and various environmental constituents. As a result of a preliminary investigation into the optimum sampling strategy (Bird, 2007), three biotopes (emergent vegetation, open-water and submerged vegetation) were sampled for invertebrates within each wetland using a square-framed, long-handled sweep net with a 23.5 cm mouth and 80μm mesh. The general literature suggests that sweep-net sampling of shallow wetlands is the most effective sampling method where the goal is comparing invertebrate assemblage composition between biotopes and between wetlands (e.g. Cheal *et al*., 1993; Turner and Trexler, 1997; Helgen, 2002; Bowd, 2005). In certain instances, a 'benthic unvegetated' habitat was sampled. This was appropriate for wetlands that had areas lacking in vegetated habitat that additionally were too shallow to collect true open water samples (defined in this study as water less than 30 cm deep).

The goal of the sweep net sampling was to collect a representative sample of the aquatic invertebrate biota from each wetland. The sampling of a variety of habitats from each wetland facilitates this goal and it was decided to choose this strategy over sampling of a single habitat per wetland. This approach avoids the problem of there not being a single standardized habitat which can consistently be sampled across all sites, since it was found in this study that a particular habitat was sometimes absent from certain wetlands. Where one of the three major habitats outlined above was absent (i.e. two out of three were present), a third 'mixed' sample was collected comprising sweeps from both of the first two habitats present in the wetland. For wetlands with homogenous habitat types (e.g. all submerged vegetation), three standardized samples were collected from three different areas of the wetland so as to maximise spatial representation. Samples were standardized and made as quantifiable as possible through a strict sampling method. Each sample comprised 3 × 1 m sweeps for three different areas of the wetland, so that one sample was a pooled combination of 9 × 1 m sweeps from three different areas where the habitat was found in the wetland. Three samples per wetland thus produced a total of 27 × 1 m sweeps evenly divided over the spatial area of the wetland and representing the major habitat types. Three samples from the different habitats of each wetland were preserved and stored separately.

All samples were fixed on site in a solution of 10% formaldehyde in water and replaced with a 70% ethanol solution after 24-48 hours for long-term preservation. In the laboratory, vegetation was removed from the samples and macro-invertebrates visible to the naked eye were picked for 30 minutes. To reduce bias in the macro-picking process (e.g. to not only pick the most abundant or largest individuals), emphasis was first placed on scanning the full area of the sampling tray and collecting the diversity of macro-invertebrate morpho-taxa (visual forms), followed by random selection of remaining individuals until all individuals had been picked or the 30 minute mark was reached (whichever came first). After conducting the macro-picking, the remaining sample contents were used to assess the micro-crustacean component. The micro-crustacean fauna (copepods, ostracods and cladocerans) was identified and enumerated using a 1/8th sub-sample of the remaining sample. The finest practicable level of taxonomic resolution for the various macro and micro taxa depends on information available for each taxon, but comprised mostly generic- and specific-level identifications. Macro-invertebrates were identified to family, genus or species, although for the majority of taxa it was possible to obtain generic- or specific-level identifications (Appendix 4). Micro-crustaceans were identified to generic- and specific-level, the only exception being familial-level identification of the Chydoridae (Appendix 5). Problematic taxa which required specific expertise were identified by a specialist taxonomist for the given taxon (e.g. Ostracoda). Because of the challenges faced in identifying and enumerating taxa, not all 125 wetlands could be analyzed for their micro faunal component. In this regard, a subset of 50 wetlands was chosen for micro-crustacean analysis from areas *a priori* assessed as containing the best gradients of human disturbance among wetlands. The final result of all the invertebrate laboratory work was a list of macro- and micro-invertebrate taxa identified per sample and a relative abundance estimate for each taxon per sample. Macro-invertebrate taxa were enumerated accurately with counts per 30 minute sample survey. Micro-crustacean taxa abundances per whole sample were estimated through extrapolation, i.e. by multiplying 1/8th sub-sample counts by a factor of eight.

4.1.2.2 Wetland hydromorphometry

Various hydromorphometrical constituents were measured at each wetland:

- maximum depth (cm) was measured with a calibrated depth stick (±0.5 cm accuracy);

- length (m) and breadth (m) measurements of wetland area inundated by surface water were made using a 100 m measuring tape, and for large wetlands, GPS points were taken to estimate length and breadth (± 3 m accuracy);

- total surface area (m^2) was estimated using the standard formula for an ellipse: Area = $\pi \times r_v \times r_h$, where r_v is the vertical radius and r_h is the horizontal radius. The equivalent here to r_v is half the width of the wetland and r_h is half the length; and

- habitat suitability for invertebrates at each wetland was ascertained by recording the proportion of surface water covered by the various habitat types.

4.1.2.3 Physico-chemical constituents

A number of *in situ* physico-chemical measures were taken at each habitat where an invertebrate sample was taken, producing three sets of *in situ* physico-chemical measures per wetland. Readings were taken at a standardized depth of 30 cm across all habitats. *In situ* physico-chemical measurements were averaged across the three habitats from where they were taken in each wetland and this mean value per wetland was used for further analyses. Measurements were taken as follows:

- pH was measured using a Crison pH25 meter;

- dissolved oxygen (mg/L) was recorded using a Crison OXI45 oxygen meter;

- electrical conductivity (mS/cm) was recorded using a Crison CM35 conductivity meter; and

- turbidity (NTU) was measured from the water column at two randomly selected points in the wetland using a Hach 2100P turbidimeter.

In order to compare wetlands in terms of their trophic status, water column nutrient concentrations were measured at each site. Five 1L surface water samples were collected from various parts of each site and pooled to form a bulk 5L sample, which was then thoroughly mixed and sub-sampled to obtain a 200 ml sample for analysis of nutrients levels in the laboratory. NO_3+NO_2 – N, PO_4 – P and NH_4 – N concentrations were estimated using a Lachat Flow Injection Analyser, as follows: NH_4 - N was measured using Lachat's QuikChem® Method 31-107-06-1, based on the Berthelot reaction in which indophenol blue is generated; NO_3 and/or NO_2 were estimated using Lachat's QuikChem® Method 31-107-04-1-E, in which NO_3 is converted to NO_2 and diazotized with sulfanilamide to form an azo dye; PO_4 was measured by forming an antimony-phospho-molybdate complex using QuikChem® Method 31-115-01-1. Approximate detection limits are: for PO_4 15μg/L P; for NO_3 and NO_2 2.5μg/L N; and for NH_4 5μg/L N. Details of the methods may be found at http://www.lachatinstruments.com.

4.1.2.4 Landscape disturbance index

A rapid assessment method for scoring land-use disturbances on depressional wetlands was formulated as part of this study in order to *a priori* classify wetlands in terms of their land-use impacts. The expected impact of various human land-use types on each wetland's water quality, hydrology and physical structure was scored semi-quantitatively (using rank scoring) as set out in Appendix 2. An additional category for plant community indicators was also included. Scores for each impact type and for the plant community indicators were summed and contributed to an overall 'human disturbance score' for each wetland. These 'human disturbance scores', herein referred to as HDS, were the output from the rapid assessment method and were used as a proxy measure for human disturbance. To ease interpretation, HDS values were produced as percentage scores through division of actual scores by the total possible score per site (a score of 70) and then multiplying by 100. The 'Extent' column in Appendix 2 was used as guide to aid the determination of impacts and was not in itself analysed quantitatively. Three distance bands were used to score local human impacts at each wetland (distance categories: within wetland; within 100 m radius of wetland edge; within 500 m radius of wetland edge). Within each distance band, the expected impacts of land-use activities were scored (semi-quantitative rank scoring from 0 = 'Best' to 5 = 'Poor', see Appendix 2 for details) in terms of the expected impacts of each land-use activity on the water quality, hydrology and physical structure of each wetland. For each column scored for human impacts (and in turn within each of the distance bands), the maximum score of impact across all land-use activities was used in the next step, which was to sum the maximum scores of impact across all impact categories (namely water quality, hydrology and physical structure) and distance bands. This score was added to the sum of the plant community indicator scores to produce the final HDS. This was divided by the maximum possible score (70) to obtain the HDS

(%) score for each wetland. Appendix 2 Table A2.1 presents the template score sheet and gives explanations for scoring criteria. Table A2.2 provides an example score sheet for calculating % HDS at KEN02 (a relatively impacted site) and Table A2.3 is an example score sheet for KEN12 (a least impaired site).

The other proxy used for human disturbance was water column nutrient levels (proxy for trophic state). HDS were used to form the gradient of land-use impairment among sites, whilst the nutrient measures were used to assess the gradient of trophic / water quality impairment among sites. The rapid assessment index to formulate human disturbance scores was the product of several sources of information: a workshop at the University of Cape Town collating local expertise on the topic of human land-use impacts on aquatic ecosystems; a modification of the rapid assessment index of Gernes and Helgen (2002) for Minnesota wetlands; a modification of the protocol stipulated by the Ohio Rapid Assessment Method (ORAM) for wetlands (Mack, 2001); and a modification of the WET-Health protocol of Macfarlane *et al*. (2008).

4.1.3 Data analysis

Both macro- and micro-invertebrate taxa counts per sample were converted to a standard density unit of number of individuals per cubic meter of water column (herein referred to as no/m^3). This was calculated by dividing the invertebrate count data per sample by the volume of water swept per habitat (i.e. per sample). Although the volume of water swept could not be exactly quantified, it was still useful to record abundances over a standardized unit volume among wetlands. Strict adherence to a standardized sampling protocol ensured that the data is as quantitative as possible with a sweep net technique. In this regard, analyses were focused on differences in relative, rather than absolute abundance data. Although habitats were sampled separately, it was decided to combine the abundances per taxon over the three habitats sampled to produce a density estimate per taxon for each wetland. The macro-invertebrate counts per taxon were summed over the three habitats to obtain a total abundance estimate at a wetland level (standardized to no/m^3). All macro-invertebrate count data in this study refers to relative abundance estimates and not absolute abundances in that the process of conducting 30 minute macro-picks per sample did not usually count all macro-invertebrate specimens in the sample, but rather provided a standardized process of enumeration.

Due to time constraints, only 20 of the wetlands analyzed for micro-crustaceans were processed over all three habitats. The remaining 30 sites were analyzed for either their submerged vegetation or emergent vegetation habitat only (depending on which was present) as the vegetated area of a wetland is where most of the taxa are present and in greatest abundance (Bird, 2007). To standardize the analysis over all 50 wetlands assessed for micro-crustaceans, those 20 sites with data from all three habitat types were averaged by dividing abundances through by 3 to obtain a comparable abundance estimate over all 50 sites. Because the focus of this study is on differences in invertebrate assemblage composition and abundance among wetlands differentially affected by human disturbance, the focus is on wetland-level data rather than habitats. However, it was important to sample habitats separately in a standardized manner so as to maximize the complement of taxa collected from each wetland. The output of laboratory processing of invertebrate samples is summarized in the form of a database of macro-invertebrate taxa in 125 wetlands and micro-crustacean taxa in 50 wetlands, both enumerated as counts per taxon (no/m^3) for each wetland (see “WHI Programme Sampling Database” attached to the summary report of the programme Day and Malan, 2010).

As described above (sections 4.1.2.2 and 4.1.2.3), a variety of physical and chemical measurements were recorded at each wetland, however, only the human disturbance-related variables were directly included in the analyses of this study due to the focus on invertebrate responses to human impacts. The remaining environmental variables provided valuable information for the WHI programme database and will greatly help to fill the ominous gap in baseline information on wetland environmental conditions in the Western Cape. It must be stressed that the approach throughout this study involves numerous univariate assessments of individual invertebrate taxa against individual human disturbance variables (in line with the multi-metric IBI analysis approach) and produces results which are purely exploratory correlations and cannot infer causality of the explanatory variables (HDS and nutrient variables). To achieve this purpose, more sophisticated multivariate exploratory approaches are required which take heed of an array of possible confounding factors, but produce complicated outputs which are difficult to relate to simple multi-metric or numerical biotic index development approaches (Chessman *et al.*, 2002). Of more primary interest in this study is an initial broad-scale assessment of potential linear relationships between invertebrates and human disturbance factors in order to provide suggestions of which taxa show potential as indicators and thus warrant further investigation in future studies.

Macro-invertebrate analyses for isolated depression wetlands were done using data from all 125 sites across the winter rainfall region of the Western Cape (i.e. for all three clusters of wetlands combined). The emphasis of these analyses was on detecting patterns or consistencies in the data in terms of macro-invertebrate taxa which showed response relationships with human impact variables. As previously mentioned (section 2.8.1), the multi-metric IBI approach for wetlands (Teels and Adamus, 2002; Helgen, 2002) recommends that an IBI should only be developed for a particular wetland type within a particular eco-region. No eco-region classification has at present been developed for South Africa with wetlands in mind, so one cannot develop an invertebrate IBI for a particular wetland eco-region. Kleynhans *et al*. (2005) have developed a level I eco-region classification for South African rivers, which suggests that the West coast and Cape Flats clusters of wetlands in this study occur within the South-Western Coastal Belt Eco-region, whilst the Agulhas Plain wetlands occur within the Southern Coastal Belt Eco-region. Cowan (1995) divided South Africa into wetland regions and using this system, all three clusters of wetlands in this study fall within the Western Coastal Slope Mediterranean wetland region. The bioregion approach of Brown *et al*. (1996) distinguishes geographical regions in South Africa based on differences in biotic composition. Their classification groups all three wetland clusters within one bioregion, in this case the Fynbos bioregion. With these discrepancies among classification schemes, it was decided to use all three clusters in a combined dataset so as to facilitate the goal of investigating index feasibility in a clearly and objectively distinguishable region, namely the Western Cape winter rainfall region. It was also decided that an invertebrate index will only really be useful if applicable over a relatively broad area, rather than just for a very specific cluster of wetlands.

Analyses conducted on isolated depressions in this study were divided into three broad categories:

1. firstly, an assessment of indicator taxa within the invertebrate community was conducted by relating individual taxa to human disturbance factors;

2. secondly, an assessment of potential invertebrate metrics for inclusion in a multi-metric index of biotic integrity was carried out; and

3. the third analytical approach was an application of the SASS scoring procedure to the macro-invertebrate dataset in order to test the feasibility of this approach for isolated depressions.

4.1.3.1 Indicator taxa

For both macro- and micro-invertebrate data, individual taxa were related to human disturbance factors across all wetlands analysed. The aim of this particular assessment was to search for taxa which were showing visible responses to human-imposed disturbances. The macro-invertebrate data were assessed at a low and a high level of taxonomic resolution. The low resolution analysis involved using family level data, whilst the high resolution analysis used a mix of species, genus and family level data, depending on how far it was practical to identify the various taxa. Micro-crustacean data was at the level of genus or species, the only exception being family level identification of the Chydoridae.

The factors used to represent human impairment were the HDS values produced from the rapid assessment index (proxy for landscape disturbances on each wetland) and nutrient levels measured in the water column at each site (proxy for water quality or trophic impairment at each site). Three variables were used separately as indicators of water column nutrient levels, namely NO_3+NO_2 –N, PO_4 –P and NH_4 –N. Correlations between individual taxa and the human impairment factors were assessed through linear regression analyses. This was carried out in conjunction with a categorical approach, where the range of HDS and nutrient scores were split into three categories (least, moderate, most) by trisection of the data ranges for each variable. Differences in the mean abundance of each taxon across the three categories of impairment were assessed using Kruskal-Wallis tests as the data were mostly non-parametric. Log-transformation of both biotic and environmental data was used to help linearise and normalize variables, which aids in linear correlation analysis and significance testing of mean differences (Quinn and Keough, 2003). The invertebrate abundance data (no/m^3) and nutrient data were $\log_{10}(x+1)$ transformed. HDS values were not transformed as they were in a percentage form. In cases where other environmental correlates were incorporated to assess confounding factors in human disturbance patterns, these data were $\log_{10}(x)$ transformed, with the exceptions of pH, latitude, longitude, temperature and habitat percentage cover, where transformation was unnecessary (latitude, longitude and temperature were approximately normal) or inappropriate (pH and habitat percentage cover).

4.1.3.2 Metric testing

In addition to exploring individual taxa as indicators of human disturbance, this study investigates applicability of the Index of Biotic Integrity (IBI) multi-metric approach (Helgen, 2002) on isolated depression wetlands in the Western Cape. For a review of the approach see sections 2.8.1 and 2.10.1 in the literature review component of this study. Briefly, a variety of attributes or summary measures of invertebrate assemblage composition are created using the basic invertebrate data per wetland. These attributes are regressed with human disturbance factors across a gradient of impairment among a selection of wetlands. Where predictable responses are found, the selected attribute is chosen as a metric for inclusion in a multi-metric index. The prescribed, and most simple way of testing for relationships between attributes and human disturbance factors, is through linear regression. Although more complicated non-linear techniques may be used for this purpose, it is difficult to incorporate non-linear relationships into the multi-metric scoring approach and linear correlations were the focus of this study. Feasibility of the multi-metric index approach hinges on how clear the linear response pattern of invertebrate attributes is to human impairment factors in wetlands. As with the indicator taxa testing, HDS percentages and nutrient levels (NO_3+NO_2 –N, PO_4 –P and NH_4 –N) were used as the human impairment factors that were correlated against invertebrate attributes and the same transformations of these variables applied. Abundance attributes were $\log_{10}(x+1)$ transformed as attributes often had very large ranges, making linear interpretation of raw abundances very difficult. Attributes expressed as percentages were not transformed.

This study empirically tested a wide variety of attributes extracted from the literature or developed specifically for this study. The focus was on testing the multi-metric IBI approach for macro-invertebrates in the Western Cape. There were no micro-crustacean attributes reported in the literature at the time of this study and thus any attributes tested would need to be created specifically for this study. Some crude attributes were tested for the micro-crustacean dataset, however, it was decided to rather concentrate on the responses of individual micro-crustacean taxa to human impairment factors when making an assessment of their feasibility for biotic index development, and in this regard, an IBI approach was not tested.

4.1.3.3 SASS testing

The macro-invertebrate data collected from the 125 depressional wetlands were used to test the SASS5 scoring approach in order to determine whether this index can be used to effectively classify wetland sites in terms of human impairment. SASS5 is the most up-to-date version of the SASS index approach and incorporates the most refined tolerance values of any of the versions of SASS. An important modification of SASS5 compared to SASS4 is that habitats are sampled and analyzed separately. However, the habitats found in the wetlands of this study did not correspond to those found in rivers, and hence the exact SASS5 sampling protocol was not possible. The general sampling approach used in this study, whereby information was pooled from three different habitats per wetland (see section 4.1.2.1), was used for the purpose of analyzing SASS scores.

A subset of the macro-invertebrate data, namely those taxa which are scored in the SASS5 index (i.e. occur in rivers), was used for SASS testing purposes. For details of the SASS5 scoring approach refer to Dickens and Graham (2002). Briefly, the SASS5 datasheet lists a set of invertebrate taxa (mostly families, but also some higher order taxa such as ‘Hydracarina’) with corresponding tolerance values, which estimate the sensitivity of the various taxa to pollution. The samples collected for each wetland were checked against the SASS5 datasheet and the relevant taxa were assigned SASS tolerance scores. The standard SASS protocol is to identify and enumerate invertebrate taxa in the field, whereas for this study identifications were done in the laboratory due to the practical constraints of time available in the field. This approach of identifying taxa in the laboratory under comfortable and controlled conditions is expected to be equally, if not more rigorous, than doing identifications in the field and does not compromise the reliability of SASS results. Although the SASS5 approach prescribes a log-scale count for each taxon, the assignment of a tolerance score to a taxon is based purely on its presence in a sample and quantitative information does not change the assigned score. The cumulative totals of tolerance values assigned to various taxa in each sample were calculated by summation of the tolerance scores, which produced the overall SASS score per site. Although this SASS score can be used for analyses of human impairment, a more reliable score is the ‘average score per taxon’ or ASPT. This ASPT score is simply the total SASS score divided by the number of taxa that were scored to produce this total score. ASPT values are considered more reliable than SASS total scores in that they account for the number of taxa constituting a sample, which aids in standardising scores across sites with different habitat diversity (total SASS scores can increase with increasing habitat diversity, Dickens and Graham, 2002). ASPT values were the focus of this analysis and were compared with HDS values and nutrient levels among wetlands to assess the degree of correspondence between ASPT and gradients of human impairment.

Tests of association were simple linear regressions and non-parametric tests of mean differences (Kruskal-Wallis ANOVA by ranks procedure) in ASPT across the three categories of HDS and nutrient impairment. These categories were produced in the same manner as for metric and indicator taxa testing whereby the constituent data were trisected to form categories of least, moderate and most impairment. The degree of correspondence between SASS5 ASPT values and gradients of human impairment were used to inform the feasibility of using SASS5 to infer wetland condition.

4.2 Valley bottom wetlands

Valley bottom wetlands are an abundant wetland type in the Western Cape and were chosen due to their suitability for testing the SASS index in wetlands with some degree of flow. Only the use of SASS in this wetland type was investigated and not the other index approaches that were tested on isolated depressions (IBI, numerical biotic index).

4.2.1 Study sites

A survey of valley bottom wetlands in the greater Cape Town and west coast areas revealed a lack of appropriate sites containing clear gradients of human impairment within individual wetlands (i.e. containing least impaired and disturbed sections within a single valley bottom system). It was therefore decided to sample a variety of sites over a broad spatial area and to compare reference sites (occurring in nature reserves) with impaired sites (occurring in agricultural or urban impacted areas) in terms of their ASPT (average score per taxon) values. Nine disturbed sites were compared with six reference sites in this manner. The null hypothesis being tested in this study is that sites occurring in disturbed areas have similar mean ASPT values to reference sites. The alternative hypothesis, as demonstrated in rivers and expected in valley bottom wetlands if the index works effectively, is that there is a significant and meaningful difference between the mean ASPT values of disturbed and reference sites. More specifically, one expects a significant increase in ASPT scores when one moves from disturbed to reference sites. Sites with decent accessibility allowed for replication of SASS sampling within sites, whereas some sites only had one entry point.

4.2.2 Sampling

To obtain each sample replicate, a standard SASS5 sampling protocol was carried out (Dickens and Graham, 2002) with one important modification in that sweeps from different habitats were pooled into one sample. This was deemed necessary because it was found that valley bottom wetlands lacked the habitat diversity usually present in rivers and thus distinction of biotopes for differentiated sampling was not possible. Ten sweeps were obtained across the variety of habitats found at each site using a typical SASS long-handled sweep net with mesh pores of 1 mm. Where movable rocks and pebbles were present, they were disturbed by kicking and the dislodged invertebrates were collected with the sweep net as per the SASS5 protocol. The pooled sample was assessed for macro-invertebrate taxa in the field using SASS5 criteria, except that once again ASPT values could only be compared at a site-level and not a habitat-level. Various *in situ* environmental parameters were quantified at each site: pH, dissolved oxygen (mg/L), conductivity (mS/cm) and temperature (^{o}C). Flow, turbidity, substrate, habitat description and riparian land use were assessed categorically. The trophic status of each site was ascertained through measurement of water column nutrient concentrations. The sampling and analysis protocol used for nutrients was as described for depressional wetland sites (see above). The nutrient data were used to create low, medium and high categories of relative nutrient impairment among sites (by simple trisection of the data range). The hypothesis of interest here is to test whether there is a significant difference in mean ASPT scores between low and high categories of nutrient enrichment and it is the extremes that are most likely to produce this result (i.e. there may well be a non-significant result when comparing with the ‘moderate’ category). If the SASS index is effective one expects a significant decrease in mean ASPT scores when moving from low to high nutrient categories.

4.2.3 Data analysis

Tests for mean differences in ASPT scores among impairment categories were non-parametric. Nutrient variables (NO_3+NO_2 –N, PO_4 –P and NH_4 –N) and HDS values displayed a non-normal distribution. Although the response variable (ASPT) was approximately normally distributed, its variance was uneven across the categories of HDS and nutrients and thus parametric tests would not be reliable. Mann-Whitney U tests were used to assess significant differences in ASPT between sites occurring in nature reserves and those occurring in disturbed areas. Kruskal-Wallis ANOVA by Ranks tests were used to investigate any significant differences amongst the three categories of impairment for the nutrient variables.

4.3 Seasonal pans of the Free State province

A fieldtrip to the Free State province in South Africa was undertaken during early March, 2008. The aim of the fieldwork was to collect data from wetlands of a similar type (seasonally inundated pans) to those sampled in the Western Cape in order to test index feasibility on a broader regional scale (i.e. for a similar wetland type, but in the summer rainfall region). Extensive reconnaissance of appropriate sites was carried out within a 200 km radius of Bloemfontein during the peak of the wet season. However, a sufficient number of suitable sites for testing of an invertebrate index could not be found. Free State pans appear to be too ephemeral in nature for establishment of an effective biological assessment index using aquatic invertebrates. Surface water inundation is highly unpredictable and the majority of pans contain surface water for only a few weeks of any given year. Furthermore, maximum depths seldom exceed 10 cm, making sweep net sampling unfeasible (Prof. Seaman, 2008, pers. comm., University of the Free State, Bloemfontein).

5. RESULTS

5.1 Isolated depression wetlands

5.1.1 Human disturbance variables

The magnitude of the human disturbance variables and additional environmental information collected for each wetland in this study are reported in Appendix 3. The most suitable gradients of human disturbance among wetlands were found in the Cape Flats and west coast areas. The Agulhas Plain on the south coast contained predominantly wetlands that were least impaired or moderately impaired by agricultural activities or upland alien vegetation invasion. In terms of human disturbance scores (HDS) generated by on-site assessments of human impacts at each wetland, the sampling distribution of sites on the Cape Flats and west coast areas was unavoidably centered on more disturbed sites and hence the overall distribution of this variable across the entire study area (Figure 5.1) was slightly skewed towards the left (i.e. low HDS scores formed a ‘tail’ on the frequency histogram). This is a consequence of the lack of remaining least impaired wetlands of this type in the Western Cape.

Nutrient variables were used as proxy indicators of water quality impairment throughout this study and were used to complement the HDS scoring of landscape impacts observed at each site. Nutrient results indicated that phosphate (PO_4) and ammonium (NH_4) gradients were best for analysis of invertebrate responses to water quality impairment, whilst results of ‘nitrates + nitrites’ (NO_3 + NO_2) produced a narrow range of concentrations not particularly suitable for the purposes of this study. The distribution of NO_3 + NO_2 concentrations (Figure 5.1) is heavily skewed towards unpolluted levels and only a few sites had high enough values to constitute a polluted comparison. Therefore it was decided to omit this variable from further analyses. Phosphate ranges were suitably broad in the west coast and Cape Flats areas, but were narrow for the Agulhas sites. The range in ammonium concentrations was best for the west coast, but rather narrow for Cape Flats and Agulhas. Both phosphate and ammonium distributions were suitable (closer to a normally-approximated distribution) when combined over the entire study area (Figure 5.1). Results of the distributions of the human disturbance variables further reinforce the decision to analyse all three wetland clusters as a single dataset, as this is the level at which the most suitable range of human disturbance was encountered, both in terms of the absolute ranges of the variables and their distribution shapes (more normally-approximated).

5.1.2 Indicator taxa – macro-invertebrates

A total of 40 aquatic macro-invertebrate families were sampled over the entire study area (Appendix 4). The decision was taken to include the Acarina (order Acarina: water mites), at the level of order, together with the rest of the macro-invertebrate data (represented at family level). Order is the practical level at which water mites can be identified in the field for bioassessment purposes. The families within this order are extremely difficult to identify and require a specialist taxonomist of this group, hence making family-level assessments unfeasible for user-friendly bioassessment.

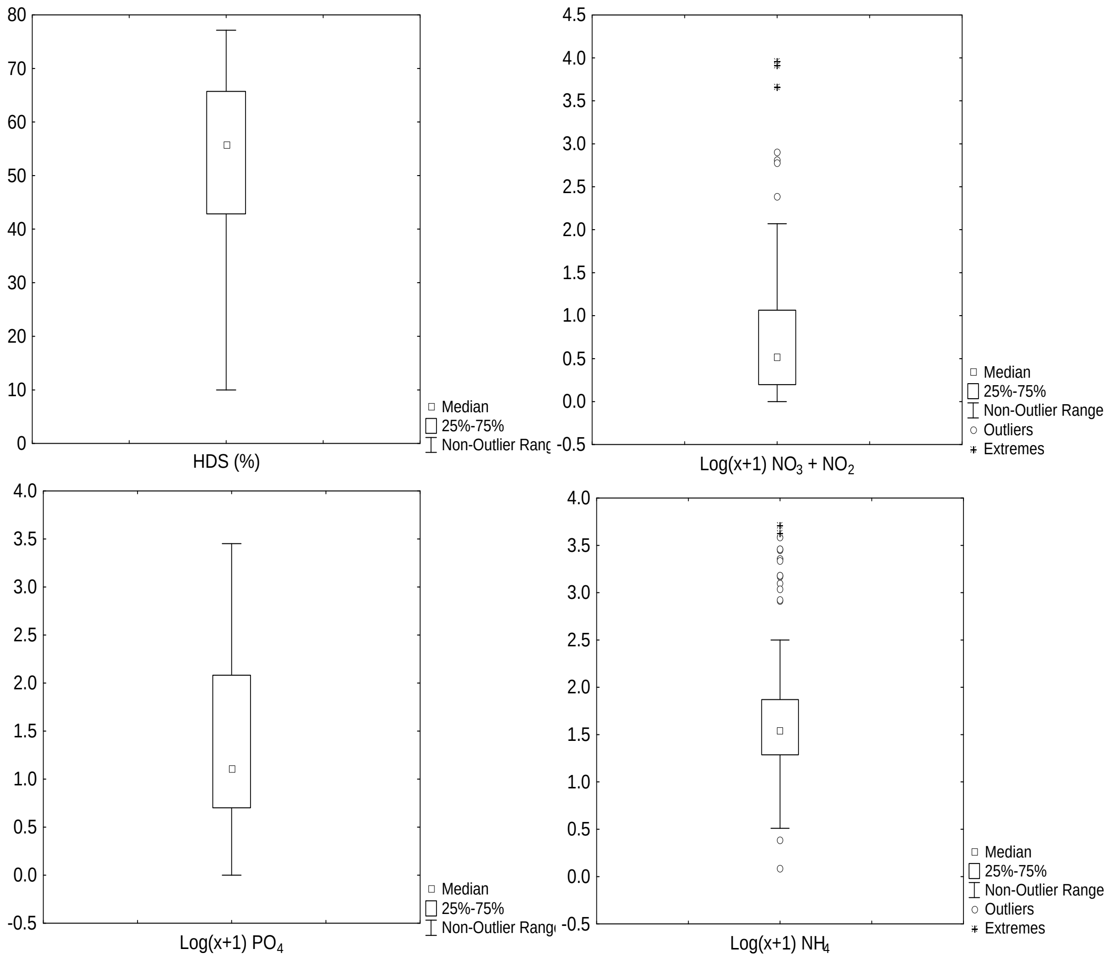

Figure 5.1: Box plots displaying the spread of each of the human disturbance variables recorded in this study. Nutrient concentrations are log(x+1) µg/L.

Eleven families showed observable patterns of response to HDS and/or nutrient variables. Fourteen families showed a generalist response to human disturbance and exhibited no clear response patterns. Fifteen families were too rare for inclusion in analyses (present in < 5% of samples). Although each family was related separately to HDS and nutrient variables, only those taxa that showed a pattern with these human disturbance factors are reported in further detail here. Figure 5.2 shows the abundance distribution of Culicidae in relation to human disturbance factors, displaying a typical non-response pattern as characteristic of the generalist taxa. Despite a significant p value for the relationship with HDS, the patterns are visibly too scattered for interpretation. The other generalist taxa are not depicted here, and rather Culicidae are given as an example of a typical generalist non-response pattern. Table 5.1 summarizes information on the invertebrate families which showed a discernable pattern with human disturbance variables, thus showing potential as biological indicators. Regression plots are presented for each family depicting their abundance distribution in relation to those human impact variables for which a significant pattern was evident. Appendix 8 presents the regression plots for all families which showed a discernable pattern with the human disturbance variables, whilst Figure 5.3 includes examples of only the strongest relationships.

Table 5.2 presents a summary of results of the non-parametric Kruskal-Wallis tests on categorized data, testing for a significant difference in the mean abundance of each invertebrate family across low, medium and high categories of HDS, PO_4, and NH_4. Categories were formed through simple trisection of the data range for each variable. These results are broadly similar to those obtained using the scatter plots (Figure 5.3); however there are some notable exceptions. For example, Hydrophilidae show good signs as an indicator of disturbance as there were statistically highly

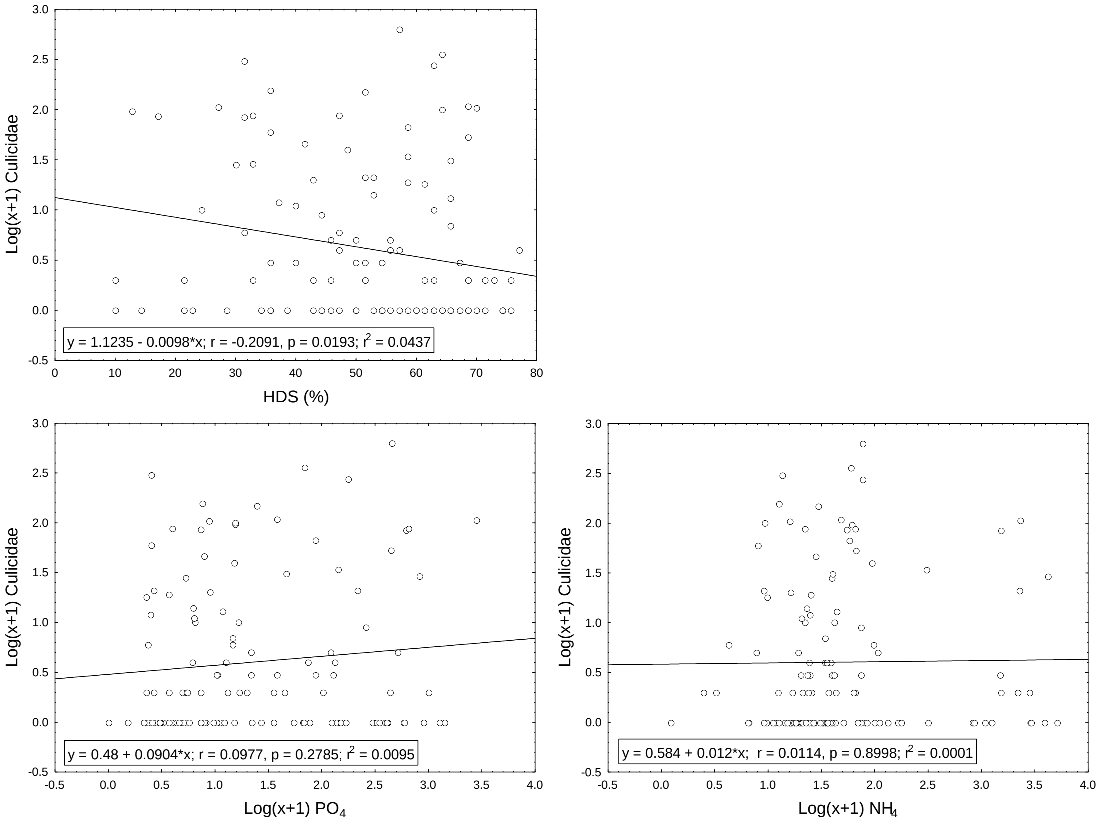

Figure 5.2: Abundance of Culicidae in relation to the human disturbance variables. Abundances are reported as log(x+1) no/m^3 and nutrient values as log(x+1) μg/L. Regression equations, Pearson correlation coefficients (r), coefficients of determination (r^2) and significance values (p) are provided.

Table 5.1: Invertebrate families which showed potential as indicators of human disturbance. Abundance values are in no/m^3. 'Human landscape disturbance' was proxied by HDS scores. 'Nutrients' refers to PO_4 and NH_4. 'n' refers to the number of wetlands at which taxa were present.

Taxon	Condition indicated	Confidence
Acarina	**Presence:** indicates low to moderate nutrient levels	High. Good sample size (n=49)
Belostomatidae	**Presence:** indicates low nutrients levels	Moderate (n=10)
Chironomidae	**Presence/absence:** inconclusive **Low abundance (<10):** associated with low nutrient levels **Moderate abundance (10-100):** inconclusive **High abundance (>100):** associated generally with more disturbed conditions both in terms of nutrients and human landscape disturbance	Low. Regression plots reasonably scattered (n=97)
Coenagrionidae	**Presence:** indicates low to moderate nutrient levels	Moderate (n=19)
Dytiscidae	Human landscape disturbance indicator **Presence/absence:** inconclusive **Low abundance (0-10):** impaired conditions **Moderate (10-100):** inconclusive **High (>100):** least impaired conditions	Low. Regression plots reasonably scattered (n=117)
Gyrinidae	**Presence:** indicates low nutrient levels	Moderate (n=12)
Hydraenidae	**Presence:** generally associated with higher nutrient levels and more disturbed landscapes, however, this inference is more reliable when abundance is >10.	Moderate (n=25)
Lymnaeidae	**Presence:** associated with low to moderate nutrient levels	Moderate (n=15)
Physidae	**Presence/absence:** inconclusive **Low abundance:** inconclusive **High abundance (>10):** associated with disturbed landscape conditions No nutrient relationship	Moderate (n=30)
Pomatiopsidae	**Presence:** associated with low to moderate nutrient levels	High. Good sample size and good regression patterns (n=44)
Scirtidae	**Presence:** associated with low to moderate nutrient levels	Moderate (n=15)

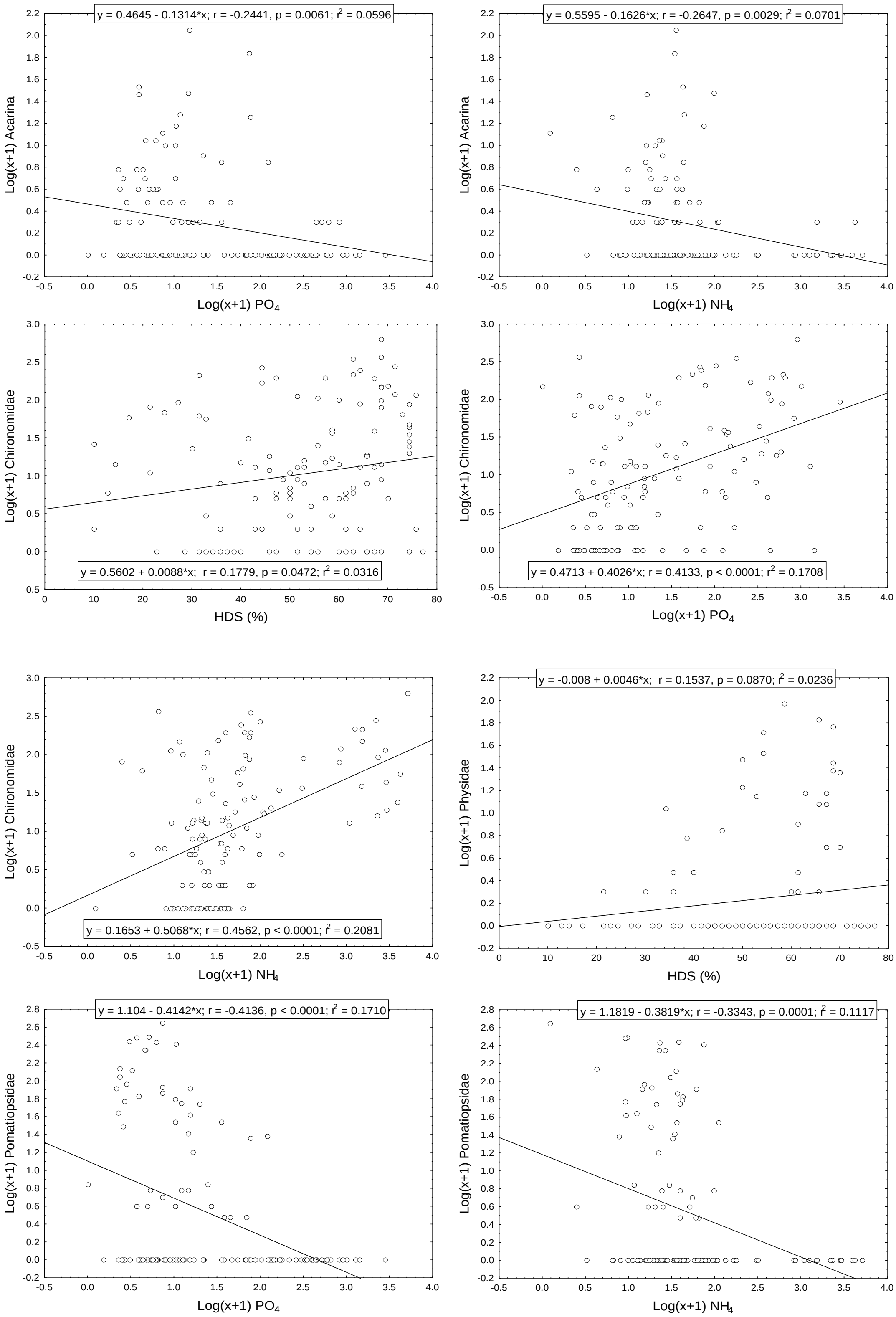

Figure 5.3: Abundance distributions of some potential macro-invertebrate indicator families in relation to human disturbance factors. Logged abundances are in no/m^3 and nutrient values in µg/L. Regression equations, Pearson correlation coefficients (r), coefficients (r), coefficients of determination (r^2) and significance values (p) are provided.

significant differences in the abundances of this taxon among the categories of impairment for all three human disturbance variables. This highlights the problem of making conclusions from statistical significance testing when one does not actually observe the distribution pattern of a taxon visually. The suggested approach is to examine a combination of significance testing results and scatter plot regression patterns to make an informed decision on whether a taxon is showing a response to human disturbance. For the case of Hydrophilidae, regression plots (Figure 5.4) indicate that there is no real pattern with HDS, and although abundances peak at low to moderate levels of the nutrient variables, the taxon is still present in wetlands with extremely high nutrient levels (in certain cases over a 1000µg/L PO_4 and NH_4). Thus, it is difficult to conclude any form of indicator status for this family.

Unfortunately time was not sufficient to include results of analyses of the macro-invertebrate data at a high resolution taxonomic level (mix of family, genus and species). The list of taxa identified to genus and species level is however included in Appendix 4. It is important to mention here that it is highly unlikely these results will be of use for bioassessment purposes as the level of difficulty in taking wetland macro-invertebrate identifications beyond family level is immense. Genus and species level identifications were made by specialists of each of the various taxa. In many cases, identification guides are not adequate for the average user of a biotic index to make reliable genus or species level identifications. The high resolution data for this project will contribute towards the author's PhD, where it will be used in more complex multivariate analysis techniques.

As stated in section 4.1.3, it was decided that all three wetland clusters should be analysed in combination for detecting macro-invertebrate indicator taxa. Analyses (regression scatterplots) of linear relationships between macro-invertebrate taxa and human disturbance variables within each of the wetland clusters were however undertaken as an exploratory exercise to compare results with those using data over the entire study area (i.e. all 125 sites). These exploratory regressions are not included as part of this report as they were voluminous and were not found to be particularly useful for determining trends, due to the effect of lower sample sizes and also the sometimes skewed distributions of human disturbance variables within each wetland cluster.

Table 5.2: p value results of the Kruskal-Wallis ANOVA by Ranks comparisons of mean family abundances between low, moderate and high categories of HDS, PO4 and NH4. N=125 (i.e. across all sites). Significant p values are presented in boldface. Taxa present in <5% of sites were excluded from analyses

Taxon	**HDS (%)**	**PO_4**	**NH_4**
Acarina	0.0507	**0.0021**	**0.0035**
Amphisopodidae	0.8534	0.5618	0.9127
Baetidae	0.1950	0.8644	0.6806
Belostomatidae	**0.0487**	**0.0172**	0.0717
Chironomidae	**0.0371**	**0.0001**	**0.0000**
Coenagrionidae	0.8640	**0.0457**	0.0551
Corixidae	0.4305	0.4382	**0.0243**
Culicidae	0.0696	0.2428	0.7425
Dytiscidae	**0.0085**	0.4544	0.6558
Gerridae	0.0611	0.2691	0.9540
Gyrinidae	0.0886	**0.0028**	**0.0209**
Haliplidae	0.2099	0.5392	0.7773
Hydraenidae	0.1561	0.9533	**0.0257**
Hydrophilidae	**0.0065**	**0.0001**	**0.0017**
Leptestheriidae	0.1594	0.4386	0.4083
Libellulidae	0.9212	0.5597	0.0580
Lymnaeidae	0.5018	0.2425	**0.0162**
Notonectidae	**0.0327**	0.2371	**0.0483**
Physidae	0.4735	0.2679	0.1105
Planorbidae	0.5624	**0.0033**	**0.0089**
Pleidae	**0.0292**	**0.0007**	**0.0079**
Pomatiopsidae	**0.0007**	**0.0000**	**0.0027**
Scirtidae	0.4770	0.0555	0.0731
Stratiomyidae	0.5511	**0.0251**	0.1664
Streptocephalidae	0.9651	0.9562	0.1501

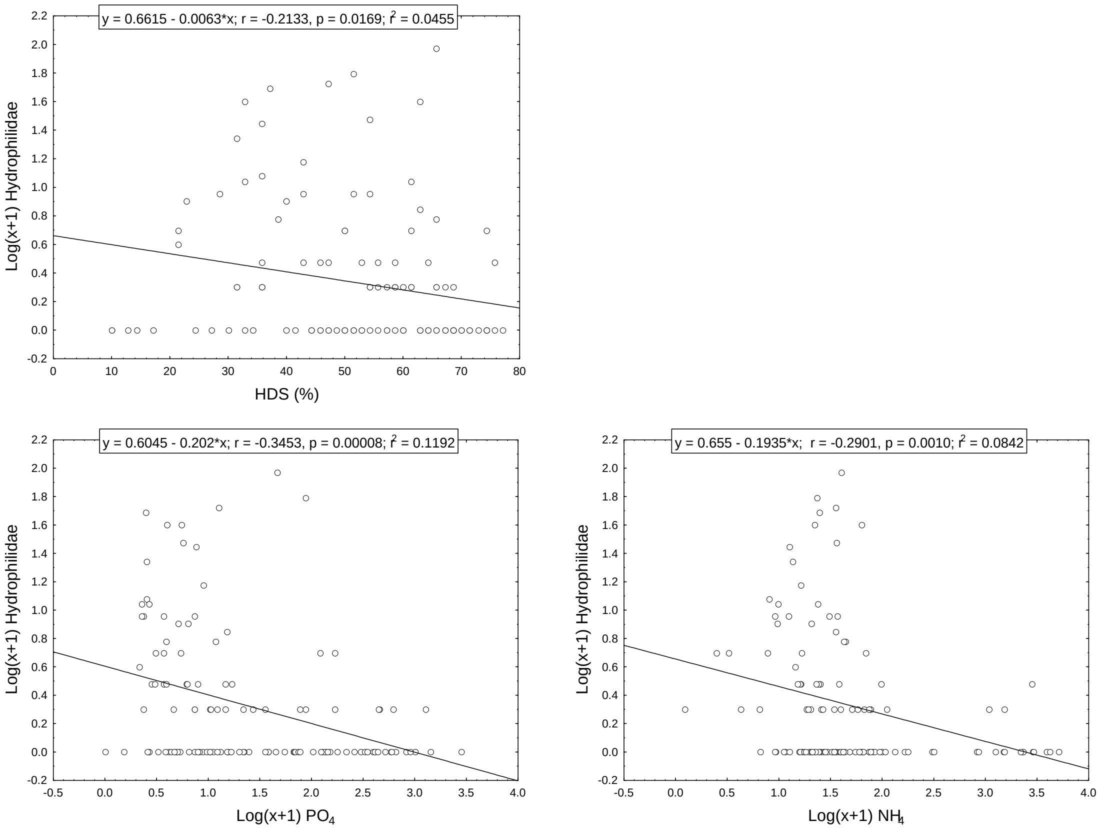

Figure 5.4: Abundance distribution of Hydrophilidae in relation to the human disturbance variables. Logged abundances are in no/m^3 and nutrient values in µg/L. Regression equations, Pearson correlation coefficients (r), coefficients of determination (r^2) and significance values (p) are provided.

5.1.3 Indicator taxa – micro-crustaceans

A total of 50 micro-crustacean taxa were sampled during this study (Appendix 5). Each taxon was regressed with the human disturbance variables in an analogous manner to the approach for macro-invertebrates in order to identify potential indicator taxa. Twenty-two taxa were present in fewer than 5 sites and thus were too rare to provide meaningful information. Of the remaining 28 taxa, 16 showed a generalist response to human disturbance variables and provide no evidence of potential as indicators. 5 taxa occurred in low abundance and displayed mixed results, thus preventing any interpretation of their overall likely response to disturbance. Seven taxa showed some potential as indicators of disturbance, but only 3 of these (*Metadiaptomus purcelli*, *Zonocypris cordata* and *Daphnia pulex/obtusa*) showed promising patterns drawn from good sample sizes (Figure 5.5). Table 5.3 summarizes results of the micro-crustacean indicator taxa testing and provides information on response patterns observed for each of the taxa to human disturbance.

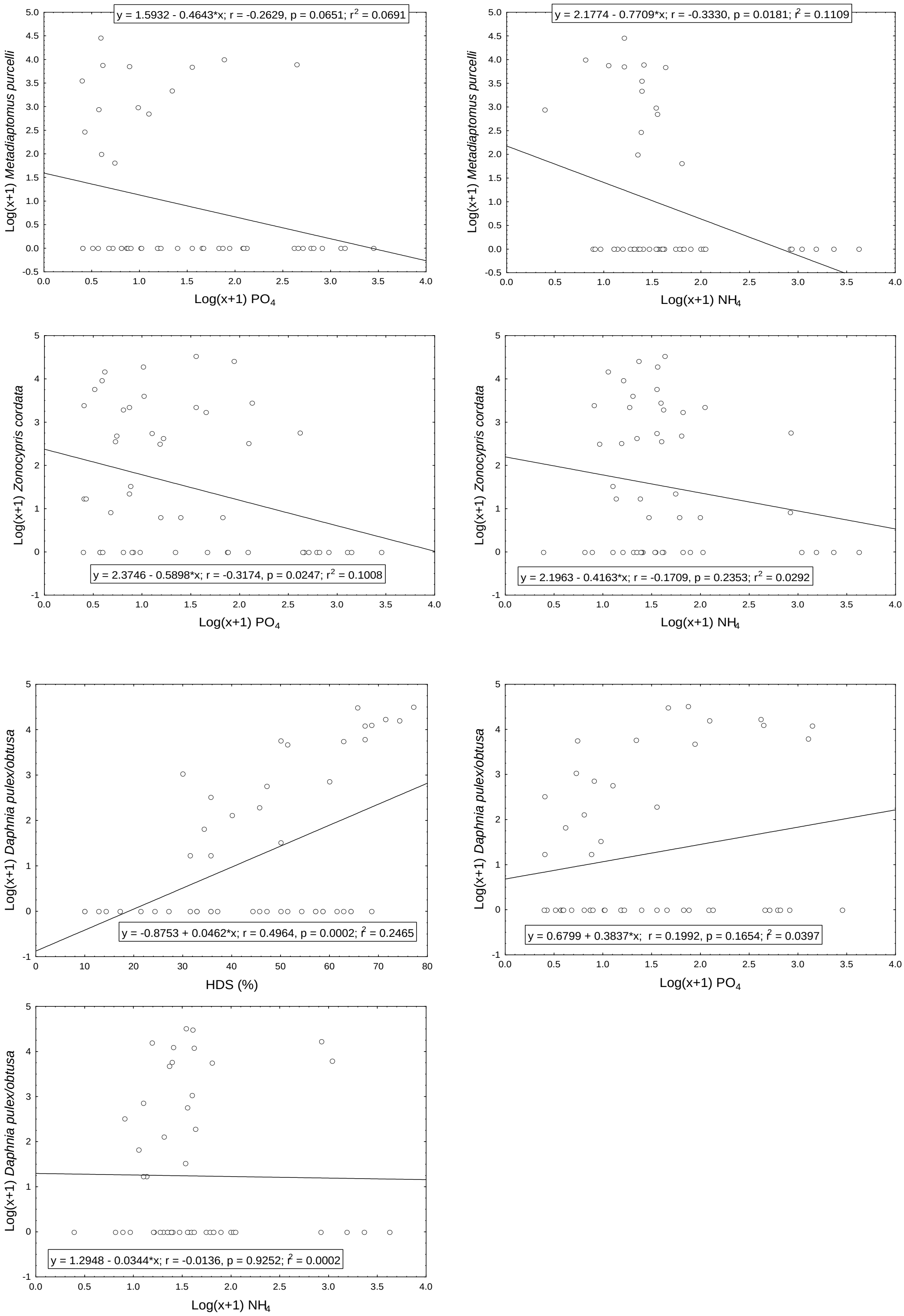

Figure 5.5: Regression plots depicting the abundance distributions of the three micro-crustacean species which showed potential as indicators of human disturbance. Logged abundances are in no/m^3 and nutrient values in µg/L. Regression equations, Pearson correlation coefficients (r), coefficients of determination (r^2) and significance values (p) are provided.

Table 5.3: Information on response patterns observed with human disturbance variables for the micro-crustacean taxa sampled in this study. N represents the number of wetlands at which the taxon was present

Taxon	**Relationship to human disturbance variables**	**Confidence (based on N)**
Acanthocyclops sp. A	N=1. Too rare for analysis	
Acanthocyclops vernalis	N=2. Too rare for analysis	
Bradycypris intumescens	N=1. Too rare for analysis	
Ceriodaphnia producta	HDS: Generalist Nutrients: Associated with low to moderate levels, but presence of a few notable outliers suggests more data required to decide on indicator status	Moderate
Chrissia sp. A	Generalist	Moderate
Chrissia sp. B	N=4. Too rare for analysis	
Chrissia sp. C	N=1. Too rare for analysis	
Chrissia sp. D	N=1. Too rare for analysis	
Chydoridae sp. A	N=1. Too rare for analysis	
Chydoridae sp. B	HDS: Mostly found at moderate levels Nutrients: Low levels only Potential low trophic state indicator, but need more data	Low. N=8
Chydoridae sp. C	N=1. Too rare for analysis	
Cypretta sp. A	N=2. Too rare for analysis	
Cypricercus episphaena	Generalist	High
Cypricercus maculatus	No response patterns	Low. N=6
Cypridopsis sp. A	N=1. Too rare for analysis	
Daphnia barbata	N=2. Too rare for analysis	
Daphnia dolichocephala	Generalist	Moderate
Daphnia pulex/obtusa	HDS: Positive correlation with HDS. An abundance-based pattern, so present in low abundance at low HDS sites and then increases linearly. Nutrients: Bulk of distribution in low nutrient sites, but some notable outliers.	Moderate
Daphnia sp. A (Sub-genus Ctenodaphnia)	Generalist	Low
Daphnia sp. B (Sub-genus Ctenodaphnia)	N=1. Too rare for analysis	
Daphnia sp. C (Sub-genus Ctenodaphnia)	N=1. Too rare for analysis	
Gomphocythere sp. A	N=1. Too rare for analysis	

(Table 5.3 continued)

Taxon	**Relationship to human disturbance variables**	**Confidence (based on N)**
Heterocypris sp. A	Generalist	Moderate
Lovenula simplex	Generalist	High
Macrothrix propinqua	Tends to prefer lower HDS and nutrient levels, but presence of a few notable outliers suggests more data required to decide on indicator status	Moderate
Megafenestra aurita	N=4. Too rare for analysis	
Mesocyclops major	N=4. Too rare for analysis	
Metadiaptomus capensis	Generalist	High
Metadiaptomus purcelli	HDS: Generalist response. Nutrients: Except for one outlier, only found at lower levels	Moderate
Microcyclops crassipes	Generalist	High
Moina brachiata	Generalist	Moderate
Moina sp. A	Generalist	Low
Nitocra dubia	N=2. Too rare for analysis	
Paracypretta acanthifera	Generalist	Moderate
Paracypretta sp. A	Only found at low to moderate HDS and nutrients	Low. N=5
Paradiaptomus lamellatus	Generalist	Moderate
Paradiaptomus sp. A	N=1. Too rare for analysis	
Physocpria capensis	HDS: Present at low HDS, but one high value Nutrients: Associated with low to moderate levels	Low. N=5
Pseudocypris acuta	No clear responses	Low. N=6
Ramotha capensis	N=3. Too rare for analysis	
Ramotha producta	Generalist	Low
Ramotha trichota	N=1. Too rare for analysis	
Sarscypridopsis sp. A	Generalist	Moderate
Sarscypridopsis sp. B	No response patterns	Low. N=6
Sarscypridopsis sp. C	No response patterns	Low. N=6
Sarscypridopsis sp. D	Generalist	Low
Scapholeberis kingi	N=2. Too rare for analysis	
Simocephalus spp.	Generalist	Moderate
Zonocypris cordata	HDS: Generalist Nutrients: Except for one or two outliers, associated with low to moderate nutrient levels	Moderate-to-high
Zonocypris tuberosa	N=1. Too rare for analysis	

5.1.4 Metric testing – macro-invertebrates

Linear relationships between macro-invertebrate attributes and human disturbance variables were weak and regression plots produced significantly scattered patterns in the data. A fundamental premise of the multi-metric IBI approach is that the strength of the index developed depends on the strength of linear relationships between metrics and human disturbance variables. Results of this study did not reveal strong candidate metrics for inclusion in a biotic index. Regression analyses conducted on the full list of macro-invertebrate attributes (Appendix 6) revealed that 12 attributes showed some sort of linear response pattern to the human disturbance variables. However, the predictive power of these regressions is low due to the amount of scatter observed in the plots. Inferences at one end of the scatter plots may be reasonably reliable, but at the other end were not. As a case example, Figure 5.6 presents the linear relationship between the attribute '% Chironomidae' and the human disturbance variable HDS (%). Although samples with higher percentages of chironomids tend to be associated with more disturbed sites (i.e. higher HDS values), one cannot reliably make any inferences about disturbance conditions when chironomid percentages are low because data points are scattered right across the range of HDS scores.

Twelve candidate metrics as extracted from attribute testing are reported in Table 5.4, including information on inferences that can be made for bioassessment purposes. These inferences were deduced from the scatter plot regressions and vary in their reliability, with even the best patterns still containing outliers to the general trends. Not all these attributes could be included in an index together by virtue of double scoring, for example, % Corixidae and Corixidae (as percentage of beetles and bugs) are both scoring the proportion of corixidae, but in two different ways. In this regard, a final list of the 9 best attributes was selected as candidate metrics for further analyses. These were the attributes as presented in Table 5.4, minus 'Corixidae (as percentage of beetles and bugs)', 'Number of Chironomidae' and 'Dominant taxon'. Although these were the best 9 attributes found in this study in terms of linear response patterns, they were still relatively weak candidates as metrics due to the degree of scatter around trends. The IBI approach is to assign a score of 1 to categories reflecting poor or impaired wetland conditions, a score of 3 represents moderate impairment and a score of 5 represents good or unimpaired conditions. In order to apply these candidate metrics in an IBI-type multi-metric index, it is required that these three levels of scoring are used for each metric and thus the scoring criteria as depicted in Table 5.4 would not produce sufficient information. Table 5.5 presents the 9 candidate metrics together with proposed scoring criteria. The US EPA suggests simple trisection of each metric's data range in order to delineate scoring categories. However, due to the weak trends found in this study and influence of outliers on data ranges, it was decided to divide each attribute's data range into three categories by examination of scatter plot results to produce more meaningful categories. Plots were depicted on a log scale for abundance attributes and thus interpretations had to be converted to raw abundances for these types of attributes.

Figure 5.7 presents the regression plots used to inform selection of the nine candidate metrics. Each candidate metric is presented in relation to those human disturbance variables with which a meaningful pattern was found. Seven of the candidate metrics were chosen based on their associations with the HDS variable representing human landscape disturbance. The remaining two candidate metrics were chosen based on their associations with the nutrient variables and could be considered as trophic state indicators. Table 5.6 reports the results of significance testing between categories of impairment for each of the nine candidate metrics. Mean values for each candidate metric were compared across the three categories of impairment for HDS, PO_4 and NH_4 as described in section 5.1.2 above. Once again, Kruskal-Wallis ANOVA by Ranks was employed as the appropriate statistical test due to the non-parametric nature of the data.Total IBI scores were produced for each wetland by scoring the 9 candidate metrics as 1, 3 or 5 and summing the scores. The total IBI scores, which are the final output of an IBI index, are used for delineating wetland impairment. In order to relate IBI results to the human disturbance variables measured in this study, IBI scores were regressed against HDS, PO_4 and NH_4 (Figure 5.8). Although slightly circular, in that IBI scores were produced using the same dataset as they are being tested on, the fit of these regression plots is useful in determining how well the IBI scores produced using metrics from this study were able to predict landscape disturbance (proxied by HDS) and/or trophic levels (proxied by nutrient variables). Results show that whilst the expected negative correlations are highly significant (p values < 0.05), visual analysis of the patterns reveals that there is considerable scatter in the data and predictive power is not particularly good. As an example, high IBI scores were associated with the full range of HDS scores instead of only with low HDS scores (as would be predicted with a good index). However, it must be noted that one must always expect outliers with exploratory analyses of this nature and the index scores will not predict wetland condition 100% correctly. In summary of the patterns produced by these IBI regressions, it appears that the overall expected trends have been met, but the data scatter or 'noise' around these trends is reasonably high.

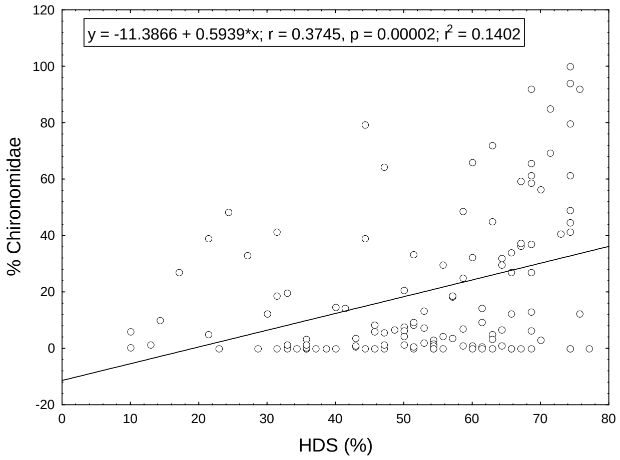

Figure 5.6: The '% Chironomidae' attribute versus HDS (%) scores. The regression equation, Pearson correlation coefficient (r), coefficient of determination (r^2) and significance value (p) are provided.

5.1.5 Metric testing – micro-crustaceans

Thirteen micro-crustacean attributes were developed for this study and assessed in relation to the human disturbance variables using linear regression plots as for macro-invertebrate metric testing. The complete absence of any micro-crustacean metrics in the literature meant that a list had to be created for testing in this study. Metric responses to human disturbance variables were poor and only 2 attributes (% Copepoda and % Ostracoda) showed potential for use in an index. Even these had rather low inferential power and would be expected to suffer from a reasonably high error rate. Table 5.7 provides a brief summary of the performance of micro-crustacean attributes tested in this study. Figure 5.9 depicts the response patterns observed for the two potential metrics, % Copepoda and % Ostracoda.

Table 5.4: The 12 candidate macro-invertebrate metrics extracted from attribute testing with regression plots, including information on inferences that can be made for bioassessment purposes. Abundances are no/m^3. Human landscape disturbance was proxied by HDS scores

Attributes	Condition indicated
Sum total organisms	Human landscape disturbance < 100: impaired site >100: inconclusive
Chironomidae abundance	Nutrient enrichment in agricultural areas West coast area: 0-5 = unimpaired site, >5 inconclusive Urban areas: No metric feasible, patterns unclear
% Chironomidae	Human landscape disturbance >50%: Impaired site <50%: Inconclusive Broad applicability
% Corixidae	Human landscape disturbance >40%: Impaired site <40%: Inconclusive Applicable on the west coast and Cape Flats, best pattern on West Coast
Physidae abundance	Human landscape disturbance >10: Impaired site <10: Inconclusive Pattern from Cape Flats and Agulhas Plain
Total number of Coleopterans	Human landscape disturbance <10: Impaired site 10-100: Inconclusive >100: Least impaired site
Abudance of the dominant taxon	Human landscape disturbance <50: Impaired site >50: Inconclusive
% Dominant 3 taxa	Nutrient enrichment <70%: Least impaired site >70%: Impaired site
Corixidae (as % of beetles and bugs)	Human landscape disturbance <50%: Inconclusive >50%: Impaired site
% Omnivores	Human landscape disturbance <60%: Inconclusive >60%: Impaired site
Number of families (incl. Acarina)	Nutrient enrichment >10: Least impaired site <10: Inconclusive
Dytiscidae abundance	Human landscape disturbance <10: Impaired site 10-100: Inconclusive >100: Least impaired site

Table 5.5: Summary of the nine most suitable candidate metrics which were included in IBI index testing. Abundances are no/m^3. See text for an explanation of IBI scores

Metric	Range *	Criteria *	Score
Dytiscidae abundance	~0-250	<10	1
		10-100	3
		>100	5
Sum Total Organisms	~10-1000	<100	1
		100-500	3
		>500	5
% Chironomidae	0-100	<33	5
		33-67	3
		>67	1
% Corixidae	0-94	<31	5
		31-62	3
		>62	1
Physidae abundance	~0-100	<33	5
		33-67	3
		>67	1
Total Coleoptera abundance	0-294	<10	1
		10-100	3
		>100	5
% Dominant 3 taxa	44-100	<44-63	5
		63-81	3
		>81	1
% Omnivores	0-100	<33	5
		33-67	3
		>67	1
No. of families (incl. Acarina)	1-19	<1-7	1
		7-13	3
		>13	5

* 'Range' refers to the full range of abundance values observed for each metric across the samples taken during this study. 'Criteria' are the 3 categories of abundance for each metric and are used to obtain the score for that metric. For example, if the number of Dytiscid beetles in a given sample is 50, then this number falls within the '10-100' criterion and the sample receives a score of 3 for the 'Dytiscidae abundance' metric.

Table 5.6: p value results of the Kruskal-Wallis ANOVA by Ranks comparisons of mean metric values between low, moderate and high categories of HDS, PO4 and NH4. N=125 (i.e. across all sites). Significant p values are presented in boldface.

Metric	**HDS**	**PO_4**	**NH_4**
Dytiscidae abundance	**0.0085**	0.4544	0.6558
Sum Total Organisms	**0.0015**	0.4577	0.2280
% Chironomidae	**0.0013**	**0.0000**	**0.0000**
% Corixidae	0.3402	0.5512	0.0998
Physidae abundance	0.5025	0.1536	**0.0481**
Total Coleoptera abundance	**0.0025**	0.2160	0.8648
% Dominant 3 taxa	0.1249	0.0707	0.0786
% Omnivores	**0.0015**	**0.0000**	**0.0000**
Number of families (incl. Acarina)	**0.0015**	**0.0001**	**0.0084**

Table 5.7: Micro-crustacean attributes assessed in this study with comments on their performance

Attribute	Comments
Total number of taxa	The sites with most taxa tended to be disturbed (in terms of HDS and nutrients) but otherwise the plot was scattered. There is not enough of a pattern to be useful for bioassessment.
Number of copepod taxa	No patterns with HDS or nutrient variables
Number of ostracod taxa	No patterns with HDS or nutrient variables
Number of cladoceran taxa	No patterns with HDS or nutrient variables
% copepod taxa (of total number taxa)	Tends to decrease slightly with increasing disturbance, in the form of HDS and PO_4, but not NH_4. The pattern is quite scattered however and will not have any predictive power for bioassessment.
% ostracod taxa (of total number taxa)	No patterns with HDS or nutrient variables
% cladoceran taxa (of total number taxa)	No patterns with HDS or nutrient variables
Total number of copepods	No patterns with HDS or nutrient variables
Total number of ostracods	Weak positive correlation with HDS and nutrients, but lots of scatter and no real predictive power. Difficult to enumerate due to extremely high abundances
Total number of cladocerans	Weak positive correlation with HDS and nutrients, but lots of scatter and no real predictive power. Difficult to enumerate due to extremely high abundances
% copepods (of total sample abundance)	The proportion of copepods in samples tends to decrease with increasing disturbance (in terms of HDS and nutrients). The pattern is clearest for PO_4. However, it's a one-sided metric in that one can only infer low disturbance at higher proportions, say >30% (reliable statement), but cannot infer anything at lower proportions.
% ostracods (of total sample abundance)	Weak positive correlation with HDS and nutrients, but very scattered. Can reasonably infer low nutrients from low proportions (<~30%) but there are a few outliers.
% cladocerans (of total sample abundance)	No patterns with HDS or nutrient variables

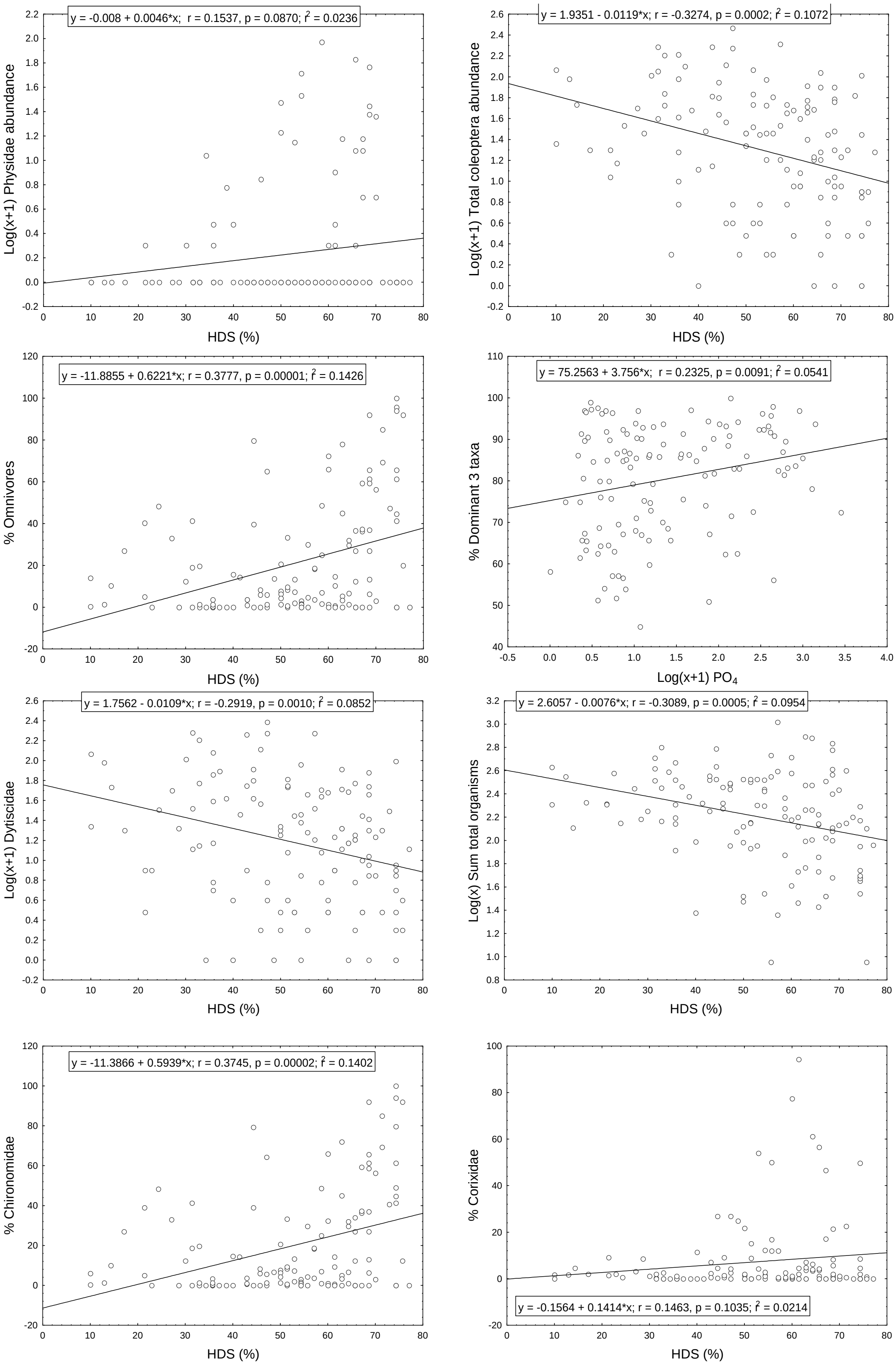

Figure 5.7:See below for explanation.

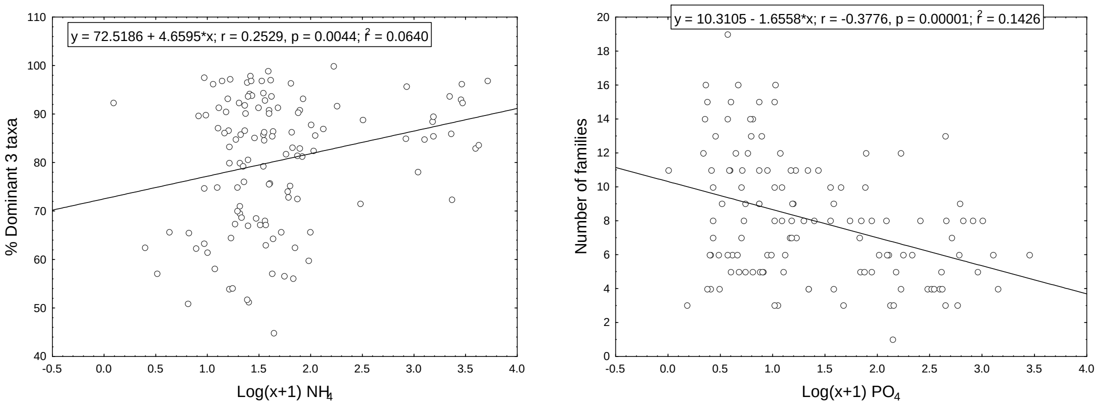

Figure 5.7: Regression plots used to inform decision making of the nine candidate metrics included in IBI testing. Logged abundances, where used, are no/m^3. Nutrient units are µg/L. Regression equations, Pearson correlation coefficients (r), coefficients of determination (r^2) and significance values (p) are provided.

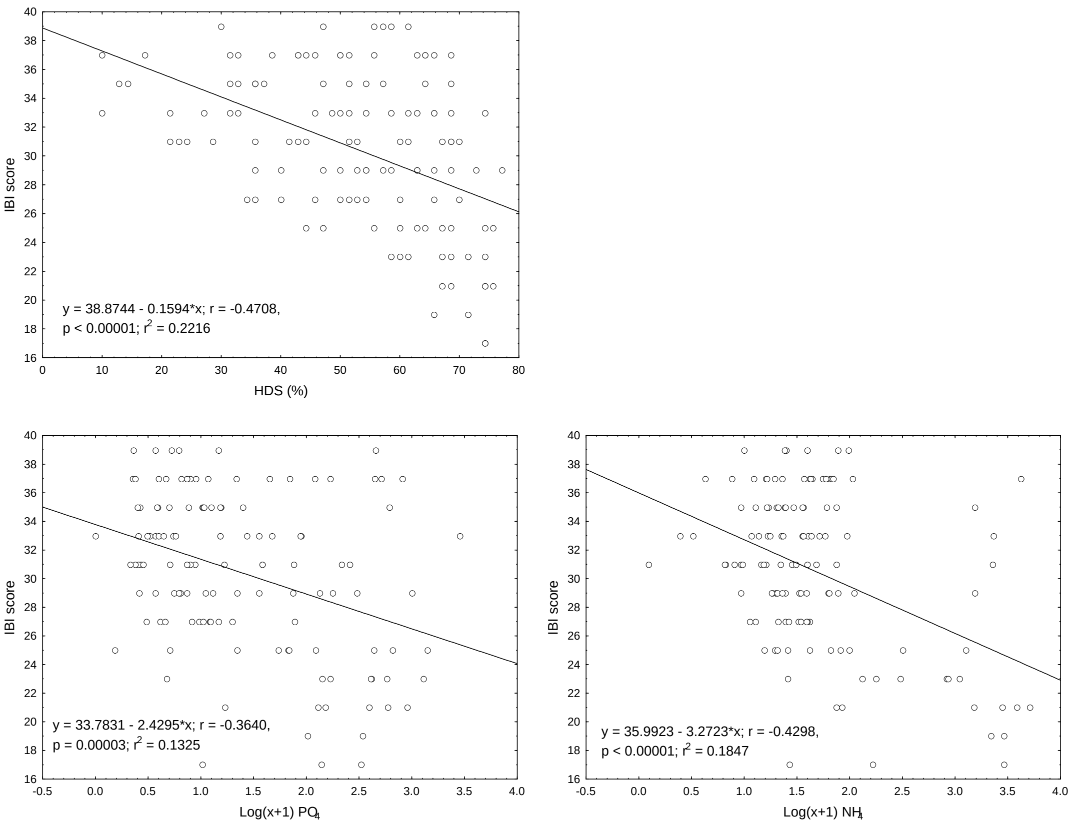

Figure 5.8: Regression plots of the IBI scores for each wetland against the human disturbance variables. Nutrient concentrations are log (x+1) µg/L. Regression equations, Pearson correlation coefficients (r), coefficients of determination (r^2) and significance values (p) are provided.

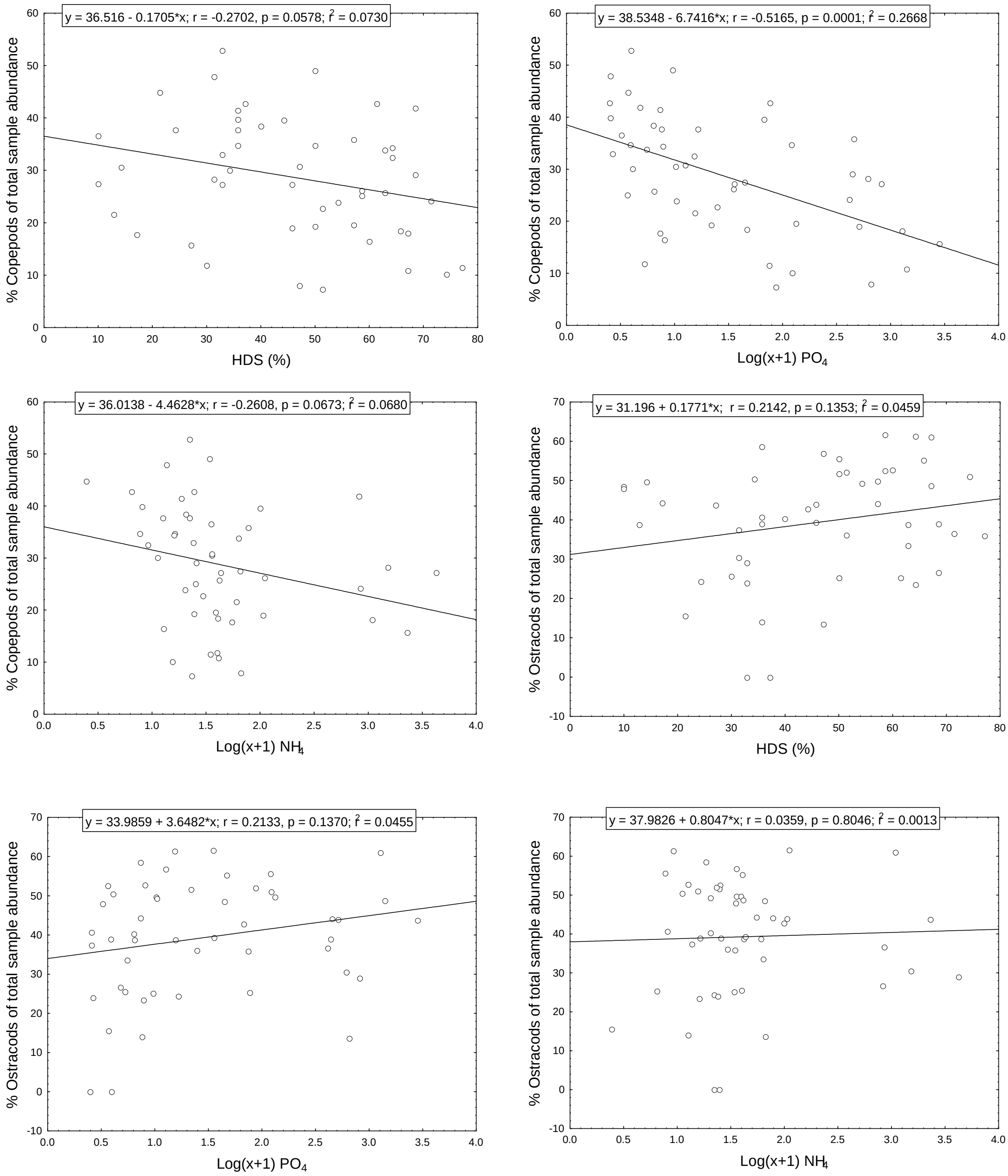

Figure 5.9: Regression plots for the two potential micro-crustacean metrics. Nutrient units are µg.L^{-1}. Regression equations, Pearson correlation coefficients (r), coefficients of determination (r^2) and significance values (p) are provided.

5.2 Testing a numerical biotic index approach using macro-invertebrates

As an alternative to the multi-metric IBI approach, the feasibility of a numerical biotic index for isolated depression wetlands in the Western Cape was assessed. Tolerance scores were assigned to each macro-invertebrate taxon (family level data) using the results of indicator taxa testing. Trends in the regression plots relating macro-invertebrate families to human disturbance variables were examined to inform the allocation of tolerance scores to each taxon. Once again, Acarina were included at the order level, as families within this taxon are too obscure to be useful for bioassessment purposes. Tolerance scores, reflecting sensitivity of the various taxa to human impairment, were allocated on a simple whole integer scale from 1-9. Although SASS5 incorporates a tolerance scale of 1-15, it was decided that the patterns of association between wetland invertebrate families and human disturbance observed in this study were not clear enough to warrant the use of a scale as broad as SASS5. Taxa showing a generalist response to human disturbance variables were allocated a median score of 5. A score of 9 is indicative of a taxon which was only found in least impaired conditions in this study. At the other end of the scale, a score of 1 is indicative of a taxon that was only found in highly disturbed sites. Scores were allocated between 5 and the extreme ends of the scale (i.e. 1 and 9) for taxa which showed varying degrees of response to human disturbance, but did show potential as indicators.

Table 5.8 presents the list of taxa sampled in this study together with suggested tolerance score allocations for use in numerical biotic index testing. Taxa present in <5% of sites have been omitted from this table. It should be noted however that if one is less conservative and only omits taxa present in <5 samples, this allows inclusion of taxa such as ‘Amphipoda’, which seem to indicate pristine wetland conditions, but were only found in 5 wetlands. Such a taxon would be designated a preliminary tolerance score of 9 until more data is collected. As with the SASS5 index for rivers, total index scores were produced per wetland by summing the tolerance scores of taxa present. However, an important difference to SASS5 is that certain taxa received tolerance scores weighted by their log-scale abundance in the wetland instead of a flat score based on presence/absence alone. ASPT values were produced in the same manner as for SASS5 by dividing total index scores at each site by the number of taxa scored to produce the total. These ASPT values were regressed against human disturbance variables to assess the effectiveness of this index approach for the wetlands in this study (Figure 5.10). This is a preliminary assessment and is partly flawed by the circular nature of testing results on the same set of wetlands used to develop the index. However, Figure 5.10 still provides a useful assessment of the effectiveness of this index approach relative to the multi-metric approach tested in this study. Examination of the regression plots reveals that the correlation fit is now better using a tolerance scoring approach than was observed using multi-metric IBI scores (Figure 5.8). Scatter in the data has been reduced and inferential power using this index approach appears to be stronger. However, there is still a reasonable amount of scatter and outlier points for all three regressions (HDS, PO_4 and NH_4), indicating that erroneous conclusions about a wetland's impairment state may still be reached using this index approach.

5.3 SASS index testing

The previous section tested what is essentially a modified version of the SASS index on isolated depression wetlands. This section presents results from applying the SASS index *per se* on isolated depression wetlands in order to assess how useful direct application of this index might be for classifying the impairment state of isolated depressions.

SASS 'average score per taxon' (ASPT) values were regressed against human disturbance variables in order to assess the inferential power of SASS for delineating wetland condition (Figure 5.11). Weak negative correlations were found between ASPT scores and each of the human disturbance variables. Due to the high amount of scatter, predictive power is weak and indicates a fairly poor relationship between SASS ASPT scores and human disturbance for the isolated depression wetlands in this study. The Kruskal-Wallis ANOVA by Ranks procedure was applied to test for significant differences in ASPT scores across the three categories of impairment (HDS, PO_4 and NH_4) as described in previous sections of this report. Results suggest a significant difference in the mean ASPT values among categories for both nutrient variables (PO_4: p=0.0002, NH_4: p=0.0032) and a nearly significant difference between the HDS categories (p=0.0860). Figure 5.12 presents box plots for this categorical data for each of the human disturbance variables. One can ascertain from the box plot trends that ASPT generally decreases from low (through moderate) to high categories, fairly uniformly for each of the human disturbance variables, but the overall decrease is small.

Table 5.8: Suggested tolerance scores (numerical biotic index) for macro-invertebrate taxa present in >5% of samples. For bioassessment purposes, certain orders have been included instead of families as this is the necessary level of identification for a biotic index. See text for explanation of tolerance scores

Taxa	**Suggested tolerance scores**
Acarina	6
Anostraca	4
Baetidae	5
Belostomatidae	8
Chironomidae	0-10 individuals: score 7 10-100: score 5 >100: score 2
Coenagrionidae	7
Conchostraca	5
Corixidae	5
Culicidae	5
Dytiscidae	5
Gerridae	5
Gyrinidae	8
Haliplidae	5
Hydraenidae	2
Hydrophilidae	5
Isopoda	5
Libellulidae	5
Lymnaeidae	5
Notonectidae	5
Physidae	<5 individuals: score 5 >5: score 2
Planorbidae	<10 individuals: score 7 >10: score 5
Pleidae	7
Pomatiopsidae	7
Scirtidae	6
Stratiomyidae	5

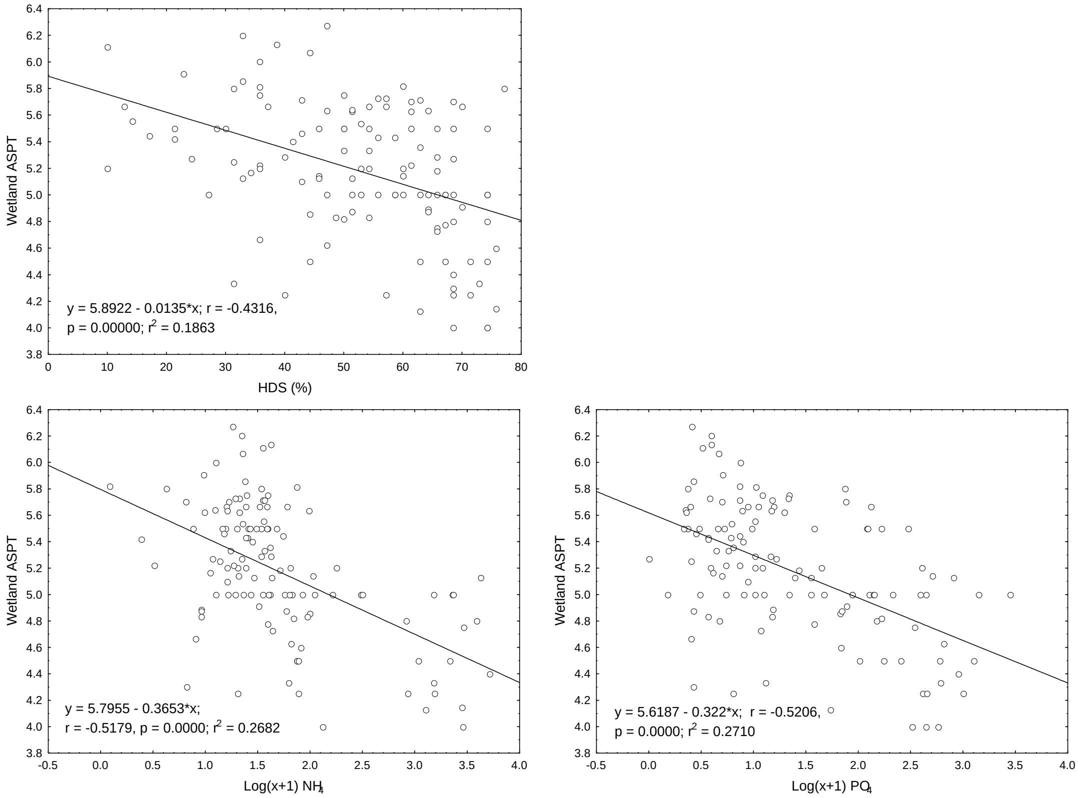

Figure 5.10: ASPT values using the tolerance scoring approach (numerical biotic index) regressed against human disturbance variables. Nutrient concentrations are log (x+1) µg/L. Regression equations, Pearson correlation coefficients (r), coefficients of determination (r^2) and significance values (p) are provided.

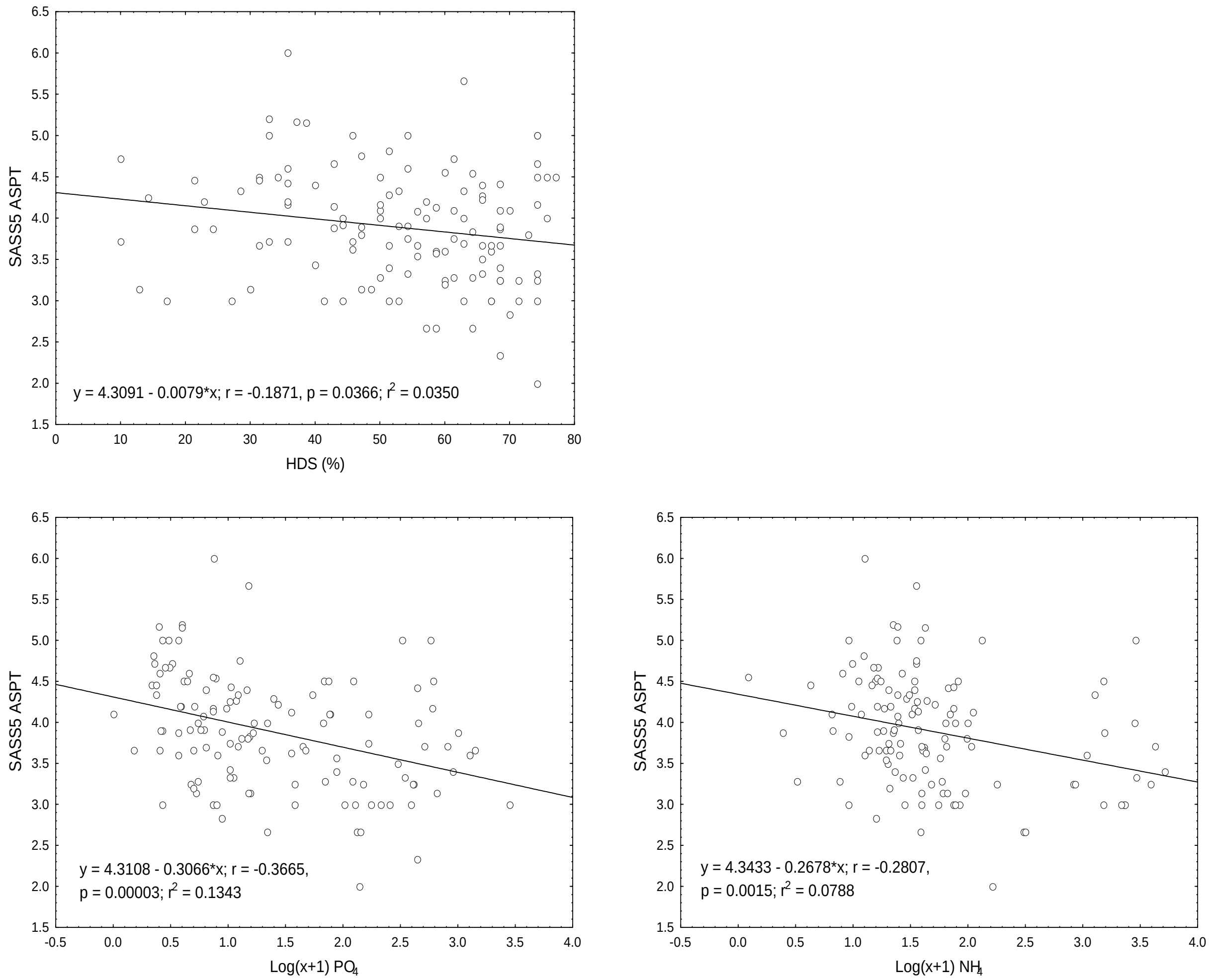

Figure 5.11: Linear regression plots of SASS5 ASPT scores against the human disturbance variables for isolated depression wetlands sampled during this study. Nutrient concentrations are log (x+1) µg/L. Regression equations, Pearson correlation coefficients (r), coefficients of determination (r^2) and significance values (p) are provided.

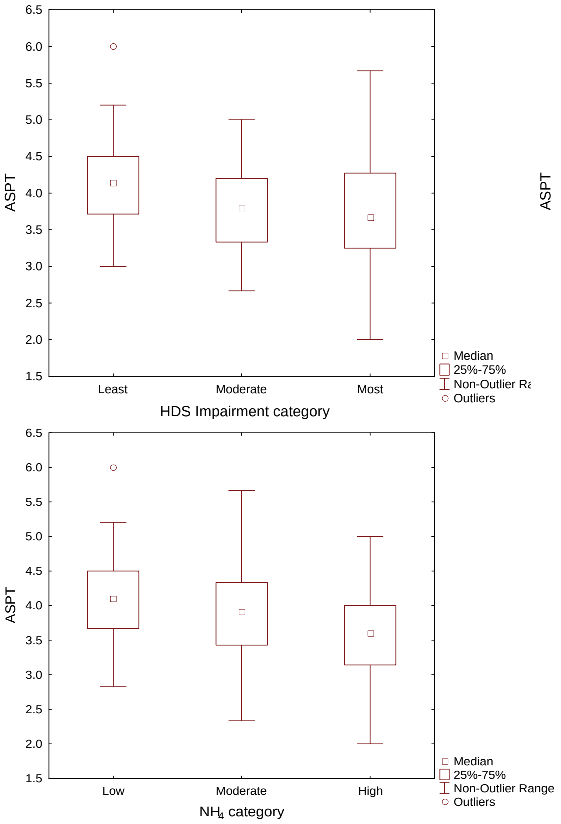

Figure 5.12: Box plots of SASS ASPT scores across the categories of impairment described for isolated depression wetlands in this study.

5.4 Valley bottom wetlands

A summary of study site information for the valley bottom wetlands sampled is included in Appendix 7. Mann-Whitney U tests revealed significantly higher mean SASS ASPT scores in valley bottom wetlands occurring in nature reserves compared to those situated within disturbed areas ($p=0.0451$, Figure 5.13). Kruskal-Wallis ANOVA by Ranks tests revealed no significant differences in mean ASPT scores among the three categories of nutrient impairment. Mann-Whitney U tests were used to test for differences in mean ASPT between the extreme nutrient categories (low and high) only, but no significant results were reported. Box plots indicate a pattern of decreasing mean ASPT values when moving from low to high nutrient categories (Figure 5.13), but the pattern is unclear and the 'moderate' category of nutrient enrichment fluctuates widely in terms of mean ASPT values. Regression plots did not reveal any relationships between ASPT values and nutrient variables for valley bottom sites (large amount of scatter in the data) and are thus not depicted here.

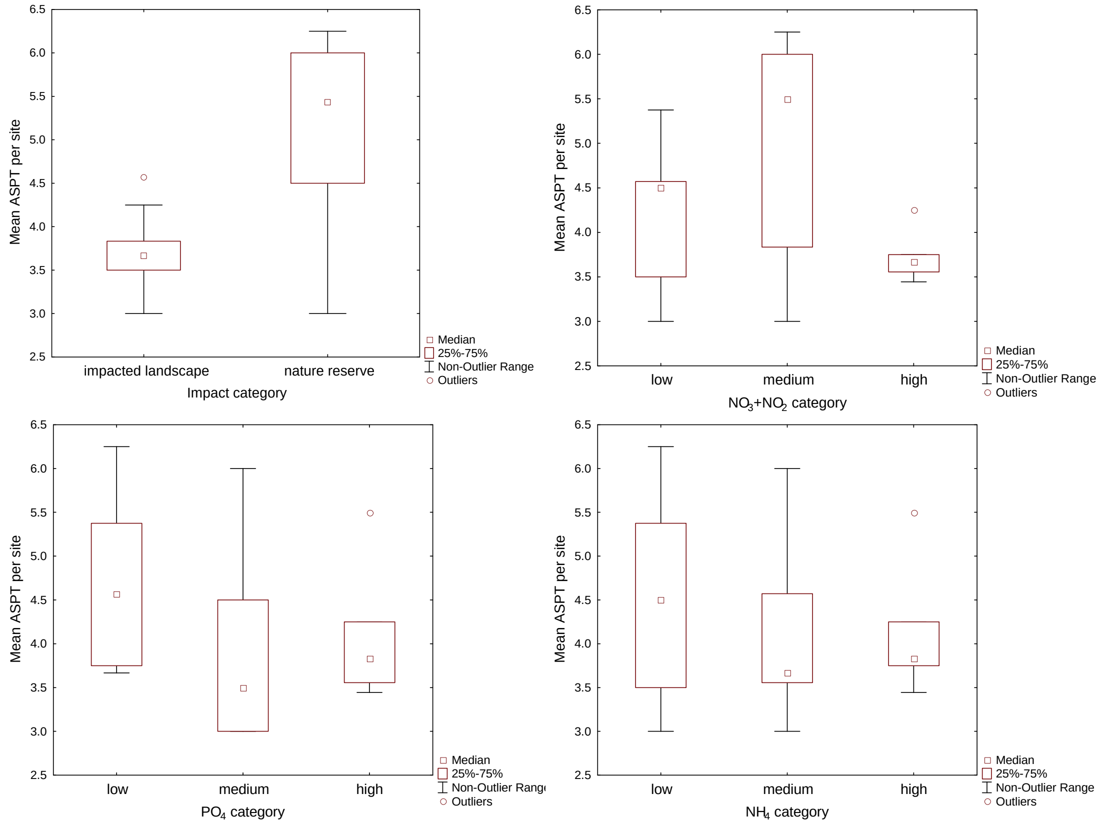

Figure 5.13: Box plots of SASS ASPT scores across the categories of impairment described for valley bottom wetlands in this study.

6. DISCUSSION

6.1 Isolated depression wetlands

6.1.1 Human disturbance variables

Due to a lack of literature information on what absolute concentrations constitute 'polluted' levels and those that can be considered 'least impaired', emphasis in this study was placed on the relative concentrations of PO_4 and NH_4 among sites. Due to some very high values in the nutrient data, log scales were applied when analyzing nutrients and thus levels were often inferred directly off plots using approximations (e.g. plot values of 1, 1.5, 2, 2.5 and 3 were approximated as 10, 30, 100, 315 and 1000 µg/L respectively). The gradients of PO_4 and NH_4 were suitable for the aims of this study in that there was a reasonable spread of data across a broad range of concentrations (Figure 5.1), whilst as mentioned it was decided to omit NO_3+NO_2 from analyses as almost all the values were at the low end of the range. Comparative information for these variables was sourced in the form of criteria for setting water quality standards according to DWAF (2002). The criteria were published for aquatic ecosystems in general and not specifically for wetlands. According to the criteria, water bodies with median SRP ('soluble reactive phosphorous', equivalent to PO_4) less than 25µg/L can be considered 'good', between 25 and 125µg/L can be considered 'fair' and greater than 125 can be considered 'poor'. These criteria thus fit reasonably well in line with the established gradient of PO_4 values recorded during this study (see Figure 5.1 and Appendix 3) and lend further support to PO_4 being the variable of preference for analyses over NO_3+NO_2 and NH_4.

Malan and Day (2005) reported a median SRP value of 20µg/L from a sample of 25 unimpacted endorheic wetlands (roughly equivalent to the wetland type in this study) in South Africa. This aligns with the grouping of wetlands in the 'low' category of PO_4 in this study. Although no guidelines have been published for NH_4 in the South African literature (guidelines are expressed in terms of the proportion of unionized ammonia to total ammonia or as a constituent of total inorganic nitrogen), Malan and Day (2005) reported a median NH_4 concentration of 90µg/L from a sample of 25 unimpacted endorheic wetlands in the region. This suggests that the majority of sites in this study could be considered unimpacted from an NH_4 perspective (see Figure

5.1 and Appendix 3), but once again emphasis was placed on relative values of NH_4 within this dataset rather than trying to ascertain pollution levels from a very scarce amount of published information. The use of NH_4 as a variable for analyzing biotic responses to nutrient enrichment may not be as suitable as PO_4 in that the majority of the spread was at the lower end of the spectrum, with only a few very high values offering good 'polluted' comparisons.

A major challenge in this study was finding appropriate reference wetlands in order to gauge what invertebrate assemblages can be expected in 'pristine' or un-impacted environments. The location of depression wetlands in low-lying coastal plain areas makes them especially vulnerable to human activities such as urban development and agriculture. As a consequence, very few least-impaired sites remain and thus the sampling distribution of wetlands in this study was unavoidably slightly skewed towards having more disturbed than un-impacted sites (see section 5.1.1). The graphical distribution of HDS (Figure 5.1) was not heavily skewed however, and still allowed for a sufficient gradient of human disturbance against which invertebrate taxa and metrics could be plotted.

6.1.1.1 Indicator taxa – macro-invertebrates

The majority of macro-invertebrate families sampled during this study showed a generalist pattern of response to the human disturbance variables. Although described as a generalist 'response' pattern, this essentially entails a 'lack of response' pattern in that these families seem to tolerate a wide range of human-imposed disturbance conditions. Similar findings were reported by Tangen *et al*. (2003), who investigated the feasibility of developing a macro-invertebrate IBI for the Prairie Pothole Region (USA) using a dataset of 24 seasonal wetlands. They ascribed the lack of correspondence between land use and macro-invertebrates to the high degree of natural disturbance associated with temporal changes in seasonal wetlands, which probably override human-induced disturbance. The view that seasonal wetland invertebrates have developed a natural resilience to disturbance is thus supported by the high proportion of generalist taxa found in the present study.

Results obtained from this study indicate that 14 families could be described as 'generalists', whereas 11 families showed some observable response to human impairment. A considerable number of families appear to be very localized in their distributions (15 families were present in <5% of sites) and were too rare for the purpose of deducing patterns. Although these rare taxa may indeed respond to human disturbances, it is unlikely that they would be particularly useful in an index in the Western Cape as they wouldn't be encountered enough for scoring purposes. A recommendation is that further sampling at different times of year be conducted to establish if these rare taxa become more abundant in samples and can be incorporated into an index. Sampling for this study was mostly conducted during the 'index period' (Helgen, 2002) when wetlands were at maximum inundation and invertebrate assemblages were reasonably well developed (mature successional phase). Early- to mid-winter sampling was avoided as far as possible due to the generally lower diversity of taxa present during the early successional phases.

Regression plots (for example Figure 5.3) were found to be a useful tool in this study for the purpose of depicting the distributions of taxa in relation to human disturbance variables. Results from this study suggest that quantitative relationships between invertebrate taxa and human disturbance variables appear to be important for wetland bioassessment purposes rather than simple presence-absence relationships as often used for river bioassessments (e.g. Dickens and Graham, 2002). However, it was decided to include the absence of taxa from sites (i.e. zero values) on regression plots as the value of presence-absence data could not be disregarded in this study. Unfortunately, with the information collected in this study it could not be ascertained whether the absence of a given taxon from sites was due to an intolerance of environmental conditions at those sites or whether it was due to factors not related to bioassessment, such as geographic distribution of the taxon. This makes it difficult to interpret correlation values (e.g. r, r^2 and p values) for plots containing many zero values (i.e. a taxon is absent from many sites) as it was not clear whether taxa were absent from sites due to intolerance of those sites or simply because of geographic or stochastic factors. There is no substitute for visual assessment of scatterplot distributions in these cases.

Certain bioassessment studies have concentrated on correlation coefficients (r), coefficients of determination (r^2) and statistical significance (p) of correlations (e.g. Chessman *et al.,* 2002; Gernes and Helgen, 2002). Results from the current study, however, suggest that these values do not provide the full story for bioassessment purposes and emphasis should rather be placed on a visual analysis of plots. Furthermore, the non-normal distribution of the majority of invertebrate taxa in this study makes r, r^2 and p correlation statistics unreliable and the allocation of taxa as indicators based purely on these values is not advocated. As an example, Appendix 8 indicates the potential of Belostomatidae as an indicator of low nutrient levels by virtue of the distribution of this taxon being only towards the left of the plot (x axis). The pattern is not particularly linear however, and thus the r values are weak (PO_4: r = -0.21, NH_4: r = -0.18). Visual analysis seems a better option here than only looking at r values. The number of non-zero (i.e. presence) points, amount of scatter, linearity and lateral distribution (left or right on the x axis) of invertebrate abundance data were all useful attributes assessed when determining whether a genuine response pattern existed for each taxon. The optimal indicator taxon would have a large sample size (i.e. a widespread taxon), minimal scatter, a clear linear correlation and furthermore should portray these characteristics against more than one type of disturbance. None of the taxa in this study came close to this ideal indicator pattern. Response patterns closer to this ideal have been reported elsewhere in the literature (e.g. Chessman *et al*., 2002; Gernes and Helgen, 2002).

Indicator taxa identified from Western Cape isolated depression wetlands tend to give inferential type information from one side of the regression plot only (e.g. Figure 5.6), whereas from the results of Gernes and Helgen (2002), for example, one can infer wetland condition at both ends of regression plots. To illustrate this point, hypothetical scenarios are produced in the figure below (Figure 6.1). Scenario A depicts a useful metric with inferential power at both ends of the spectrum of a given human disturbance variable. The results reported by Gernes and Helgen (2002) are more aligned with the scenario A model, than scenario B, which depicts the kind of results reported for the best metrics/indicator taxa established during this study (e.g. Figure 5.6). Figure 6.1B represents a positive correlation scenario and reciprocal patterns for negative correlations were also observed in the results of this study (i.e. inferential power at the low end of the disturbance spectrum only). For example, let's say a given taxon's numerical abundance gives information on wetland pollution state in terms of nutrient levels in that a pattern has been found that the taxon tends to be abundant in eutrophic sites, whereas it is absent or rare in oligotrophic wetlands. After gathering a sweep net sample from a wetland with an unknown pollution history, it is established that the taxon is abundant in the sample. Given that this taxon has shown a scenario A type of pattern when tested in other wetlands of the same type (and region), we can infer quite reliably that the wetland in question is likely to be in a eutrophic state. However, even the best indicator taxa in the current study conformed to a scenario B type of model and in this case only if the given taxon is rare in a sample does it suggest one can infer wetland trophic status (oligotrophic), whilst an abundance of the taxon would present ambiguous information.

Categorical presentation of data in this study, by means of testing for significant differences in the abundance of taxa between impairment categories, was not found to be as useful for choosing invertebrate indicators as was investigating scatter patterns on regression plots using continuous data. Problems arise, in this author's opinion, when one does not actually observe the distribution pattern of a taxon in relation to a human stressor variable. The case of Hydrophilidae (Figure 5.4) was described in section 5.1.2 to highlight the discrepancies that may arise between significance testing results and those obtained by visual assessment of regression plots. The suggested approach is to examine a combination of significance testing results and scatter plot regression patterns to make an informed decision on whether a taxon is showing a response to human disturbance or not.

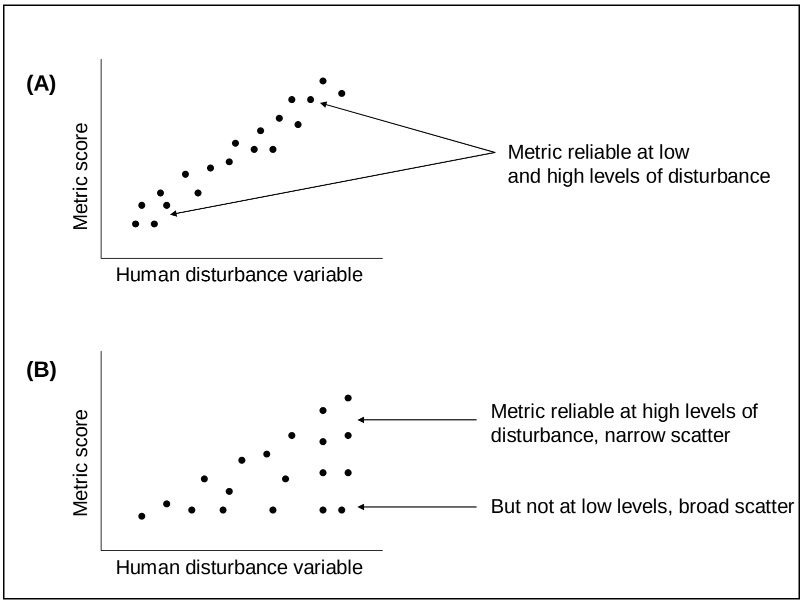

Figure 6.1: Hypothetical scenarios illustrating: A) regression scatterplot with inferential power at both ends of the disturbance spectrum; B) regression scatterplot with inferential power only at one end of the disturbance spectrum only (in this case the high end).

Identification of wetland invertebrate taxa beyond family level is not recommended for future index development in South Africa. Although genus and species level data were available for analysis in this study, the task of gathering such data was enormous and required specialist expertise for most major groups. It is of the author's opinion that this would defy the point of having a simple and relatively easy-to-use bioassessment index for wetlands in this country, in that taxa should be identifiable on site using field guides or at least be reasonably quickly identified in the laboratory. Family level identifications would suit this purpose, however certain taxa should be identified at the order level, such as Acarina, which contains families that are too difficult to identify in the field. Furthermore, preliminary exploration of the genus and species level data using correlation analyses revealed that many of the taxa were too rare (in terms of distribution among wetlands) at this taxonomic level to provide useful indicator patterns. Although certain taxa showed potential as indicators of disturbance, these patterns were upheld when scaling the data up to family-level. These preliminary analyses did not reveal any significant increase in the resolution of bioassessment information provided by genus and species level data compared to family data. Even if this increase was significant, the use of fine resolution taxonomic data for bioassessment would be limited to longer-term comprehensive studies and would have to involve the contracting of specialist expertise. In the shorter term, more user-friendly wetland bioassessment methods are required in South Africa.

6.1.1.2 Metric testing – macro-invertebrates

As observed with macro-invertebrate families, relationships between metrics and human disturbance variables (Figure 5.7) were characteristically 'one-sided' in that metrics could only predict wetland condition at one end of the spectrum. A supposed strength of the multi-metric index is that even a collection of mediocre metrics, used in combination, may produce a reliable score (Teels and Adamus, 2002). It was hoped the reasonably weak metrics found in this study could produce meaningful overall IBI scores (i.e. when used in combination). This was tested through regression of total IBI scores against human disturbance variables (Figure 5.8) and produced poor results. Once again, although r and p values implied significant correlations, emphasis was rather placed on visual analysis of the plots, which indicate a large amount of scatter and low predictive power. Due to the circularity of regressing total IBI scores against the data from which they were derived, one expects a bias towards good patterns. The reasonably weak patterns observed in Figure 5.8 thus become even more unreliable.

Metric relationships with human disturbance in this study were not as clear-cut as those reported by Gernes and Helgen (2002) in their development of a multi-metric index for depressional wetlands in Minnesota. They found metrics were able to predict wetland conditions at both ends of the spectrum (i.e. for low and high values of any specific metric), as opposed to the 'one-ended' results of this study, where inferences from metrics were generally only applicable at one end (either high or low) of the range of metric values. Another potential weakness of the metrics developed in this study is that 7 out of 9 were based solely on their relationship with the HDS variable, a proxy for landscape impairment at each wetland. Only 2 of the metrics were related to nutrient levels, suggesting the index would not be particularly useful for detecting nutrient enrichment in wetlands, which is often a primary concern in terms of wetland conservation and rehabilitation. Although generic and species level information has been incorporated into invertebrate metrics developed by agencies within the US EPA, it is not a suggested strategy in South Africa, where resources (taxonomic expertise and financial) are not comparable to the USA.

6.1.1.3 Testing a numerical biotic index approach using macro-invertebrates

The suggested tolerance scoring criteria for wetland macro-invertebrates sampled in this study covers a fairly narrow range of scores (1-9, with a median score of 5) compared to the SASS index (1-15, with a median score of 8). This is an unavoidable consequence of the lack of observed clear-cut responses from macro-invertebrates in wetlands to disturbances and thus it was considered that allocation of a larger range of scores would be superficial. Furthermore, sensitivities of river macro-invertebrates in South Africa have received more research attention than wetlands and this has allowed some refinement of the SASS tolerance scores. The range suggested here for wetlands is not based on extensive research or eco-toxicology testing results, but simply the correlational results from this study.

The proposed preliminary tolerance scoring approach for isolated depression wetlands (Table 5.8) is essentially a modification of the SASS index approach, with three important differences:

- as noted above, the preliminary tolerance scoring range needs to be narrower than the 1-15 range for SASS;

- abundance data appears to be important in wetlands rather than just presence-absence data (SASS scores taxa based on presence alone) and the suggested approach is to weight the scoring of certain taxa according to a simple count scale that can be performed in the field (e.g. rank abundance using log scale counts); and

- lastly, a different sampling technique to SASS will be required for wetlands as habitats are significantly different to rivers and are often less distinct within wetlands.

Otherwise the approach is the same as SASS in that a total score is produced per wetland by summing the individual tolerance scores. An ASPT score can then be produced by dividing the total score by the number of taxa. The use of these total scores and ASPT scores as a means of inferring wetland condition will be tentative until further research clarifies indicator taxa. The regressions of wetland ASPT scores (derived from wetland tolerance scoring) against human disturbance variables (Figure 5.10) produced considerably better patterns than both IBI (Figure 5.8) and SASS ASPT (Figure 5.11) regressions. Although the regressions are partly flawed by a circular approach, they still allow useful relative comparisons of the effectiveness of the different indices applied to this dataset. There is still some degree of scatter in the plots of Figure 5.10 and erroneous conclusions in terms of bioassessment are unavoidable at this early point in the development of a wetland index. A slightly less conservative method would incorporate rarer taxa, say those in five or more samples as opposed to 5% or more samples (as used in this study) and would allow for inclusion of taxa such as Amphipoda, which seem to indicate pristine wetland conditions, but were only found in five wetlands. Such a taxon would be allocated a preliminary tolerance score of nine until more data is collected.

6.1.1.4 Indicator taxa – micro-crustaceans

Only 7 of the 50 micro-crustacean taxa identified from this study showed potential as indicators of human disturbance (Table 5.3) and only 3 of these taxa produced good patterns with reliable sample sizes (*Metadiaptomus purcelli*, *Zonocypris cordata* and *Daphnia pulex/obtusa,* Figure 5.5). The majority of taxa analyzed against human disturbance variables showed a typically generalist-type response and would not be of any particular use for bioassessment purposes. Almost half the taxa (22) were too rare for analysis (present in less than 5% of sites), indicating that their distributions are most likely too localized for use in a bioassessment index. The most important point to stress when it comes to micro-crustaceans is the difficulty involved in obtaining identifications. Ostracods often require complete dissection for family and genus level identification, which is a tedious task best attempted by a specialist. Morpho-species distinction of ostracods based on external characters is also difficult as they are a very specious group and differences between individuals are subtle. There are no existing keys which adequately cover the cladocerans in South Africa (Prof. J Day, 2009, pers. comm., University of Cape Town, South Africa). Copepods are more easily identified, but the use of a compound microscope is required for most identifications. One option is to make coarse identifications to the level of family or even sub-order (e.g. calanoids, cyclopoids, harpacticoids), but even this will require that samples are examined in the laboratory under dissection microscope and precludes on-site assessment. The lack of response patterns seen in this study using high resolution taxonomic data (genus and species level) indicates that the use of lower resolution data (order and family level) is also not likely to provide useful information for bioassessment.

6.1.1.5 Testing metrics – micro-crustaceans

Thirteen metrics were assessed using micro-crustaceans and provided little information for bioassessment purposes. Relationships were weak between metrics and human disturbance variables and produced only two feasible metrics (% Copepoda and % Ostracoda, Figure 5.9), both of which had low inferential power and would be expected to suffer from a reasonably high error rate. Although metrics only required identification to the level of order, the difficulties in enumerating the extremely abundant micro-fauna in order to calculate metrics does not appear to be worth the effort, in terms of the usefulness of the resultant metrics for inferring human impairment levels among wetlands. Although more research in other wetland types and regions would offer clarification of this issue, the findings of this study provide a lack of preliminary evidence from metrics or indicator species to suggest micro-crustaceans as useful for inclusion in wetland bioassessment indices in South Africa. This conclusion is reached partly based on the laborious identification and enumeration procedures involved and partly because of the lack of good indicator patterns observed in this study.

6.1.1.6 SASS index testing

Analysis of the significance testing results for SASS using the categorical approach in this study, together with box plots depicting these categories (Figure 5.12), indicate a significant decrease in the ASPT scores of isolated depression wetlands from low (through moderate) to high categories of PO_4 (p=0.0002) and NH_4 (0.0032), whilst a nearly significant difference was found between the HDS categories (p=0.0860). The greater distinction between nutrient categories compared to HDS is expected when considering that the SASS index best responds to organic pollution in rivers (Dickens and Graham, 2002). The overall decrease in ASPT between low and high categories of impairment may be statistically significant, but is considerably less discernable than would be observed for a similar comparison in rivers (e.g. Vos *et al*., 2002). The invertebrate sampling approach described for depressional wetlands in this study (see section 4.1.2.1) is expected to produce a representative sample of the invertebrate biota in each wetland and was thus considered adequate for the purpose of testing SASS among wetlands. This study is likely to present a conservative test of SASS5 in that the sampling of wetlands was more rigorous than the rapid protocol prescribed for SASS5 sampling (cf. Dickens and Graham, 2002).

Although ASPT scores do show a certain degree of correspondence to impairment in this study, the pattern as depicted in Figure 5.11 is blurred. The relationship between ASPT scores and human disturbance variables was weak and there was a high amount of scatter in the trend. Once again, more emphasis should be placed on visual analysis of the regression trends rather than just looking for statistically significant differences between categories of impairment. The predictive power is very low and many erroneous conclusions would be made with regards to wetland condition. The regression of ASPT scores on human disturbance variables produced the least suitable degree of correspondence out of the three macro-invertebrate index approaches tested in this study (c.f. Figures 5.8 and 5.10). A modification of the SASS index as described in a previous section (6.1.1.3) produced considerably more accurate results (relatively speaking) and is recommended as a more feasible approach than applying SASS *per se* to wetlands.

Bowd *et al*. (2006a) found the SASS index to be unsuitable for determining organic pollution levels in permanent palustrine wetlands of the KwaZulu-Natal midlands and also recommended modification of the index for future use in wetlands. Given evidence of the unsuitability of SASS for assessing the condition of large permanent wetlands in KwaZulu-Natal (Bowd *et al*., 2006a) and isolated depression wetlands in this study, it appears highly unlikely that SASS constitutes a feasible option for determining the condition of lentic wetlands in South Africa. However, as shown in this study, modifications to this index may produce feasible indices for wetlands.

6.1.2 Valley bottom wetlands

Whilst the majority of evidence from this study and the literature (discussed in the previous section 6.1.1.6) indicates that SASS is unsuitable for use in truly lentic (non-flowing) wetlands, a remaining ‘grey area’ is whether SASS may be useful in wetlands with some degree of flow. Valley bottom wetlands are ideal for addressing this ‘grey area’ in that, unlike isolated depressions, they are situated within low gradient landscapes and thus tend to have weak flows (although flows may be strong during flood events).

Results for the valley bottom wetlands sampled in this study indicate a lack of correspondence between nutrient levels and ASPT scores. Closer examination of box plots in Figure 5.13 shows that high nutrient sites produced a narrow range of ASPT scores, which were consistently lower than those observed at the low nutrient sites (except one outlier). This suggests that SASS was able to detect nutrient enrichment among wetlands to some degree, but making inferences about nutrient enrichment from ASPT scores would be unreliable. This pattern was evident once again when looking at median ASPT values of wetlands within nature reserves versus those within disturbed areas (Figure 5.13), where one observed a narrow spread for the impacted sites and broad spread for those situated in nature reserves. The Mann-Whitney U test indicated a significant difference in ASPT scores among the two land-use categories, but the significance of this difference may not be particularly meaningful bearing in mind the large spread in ASPT values among wetlands in nature reserves.

SASS is very effective at detecting impairment among sites (particularly in terms of nutrient enrichment) in rivers and thus, although the number of wetlands sampled in this study is not large (n=15), one is able to conclude that a certain degree of inferential power is lost when transferring SASS from rivers to valley bottom wetlands. Essentially, this preliminary data suggests that the distinction between ‘good’ and ‘poor’ condition sites becomes more blurry for valley bottoms compared to rivers and the reliability of SASS decreases. This could be partly attributed to the inadequacy of the SASS sampling protocol for the habitats found in valley bottoms, which were found to be considerably different to rivers in that they lacked distinctive SASS biotopes. Another significant problem encountered during this study was that valley bottom wetlands only contained sufficient surface water (for SASS sampling purposes) for a small fraction of the wet season and thus for an overwhelming majority of the year, suitable SASS sampling cannot be undertaken. These factors, combined with the less than definitive results, suggest that other bioassessment methods may be required for this type of wetland. It is suggested that methods less reliant on surface water are pursued. More research into the applicability of SASS for valley bottom wetlands would help clarify the situation, but based on these preliminary results, it is concluded that the use of SASS as a bioassessment index for this type of wetland cannot be advocated.

Another study using SASS in a similar type of wetland was by Vlok *et al*. (2006) who used a modified SASS approach on a floodplain wetland (Nylsvley) in the Mpumalanga province. They found that SASS was inconclusive in terms of its ability to distinguish sites within the greater Nylsvley system, but did concede that pollution ranges within the system were perhaps too narrow to offer a proper test of the index. It was suggested that a modified version of SASS would be more suitable for use on floodplain wetlands, and that future indices should incorporate land-use and habitat quality ratings. Results for the present study on valley bottom wetlands in the Western Cape fit broadly in line with those of Vlok *et al*. (2006) in terms of SASS index applicability.

Dallas (2009) very recently reported preliminary bioassessment results for a macro-invertebrate study in the Okavango Delta, a floodplain wetland system in Botswana. She developed and tested a macro-invertebrate IBI and a numerical biotic index (OKASS), which was a direct modification of SASS, against gradients of human disturbance (% HDS) for three focal areas (two within Moremi Game Reserve and one near the town of Maun) within the greater wetland system. Her results indicated comparable performances of the two index approaches and both indices were effective at detecting the most disturbed sites, but were not effective at detecting slight-to-moderate impairment in the wetland system. The findings of Dallas (2009) are generally in line with those of Vlok *et al*. (2006) for Nylsvley in suggesting that macro-invertebrate indices are only able to detect large gradients in human impairment within floodplain wetlands and do not tend to detect more moderate impacts.

7. A POSTERIORI INDEX TESTING WITH AN INDEPENDENT DATASET

7.1 Introduction and study sites

The IBI and numerical biotic indices developed for isolated depression wetlands during the current study (from herein referred to either as the ‘training dataset’ or ‘training study’) were tested on an independent dataset in order to assess index performance without elements of circularity or ‘double-testing’ of the data (cf. sections 5.1.4 and 5.1.6). The test dataset was made available from the study of De Roeck (2008). As part of her PhD study, Els De Roeck sampled a number of isolated depression wetlands for macro-invertebrates and environmental variables between July-September 2004. She sampled 58 wetlands, covering an area very similar to that of the training study (winter rainfall region of the Western Cape) and sampled comparable areas: west coast (28 sites), Cape Flats (12 sites), Agulhus Plain (12 sites) and Cape Peninsula (six sites). The only area covered by De Roeck, but not covered during the training study, is the Cape Peninsula (her sites occurred in the Cape Point area specifically). The latter sites are valid for index testing purposes in that they are coastal low-lying isolated depression wetlands. Although a comparison of GPS points among the two datasets was not undertaken, it can be reasonably expected that several sites (most probably less than 5) are the same among both studies. For the purposes of index testing, however, her data can be considered largely independent of the training dataset.

7.2 Assessing gradients of human disturbance

The sampling regime of De Roeck (2008) was not undertaken with the objective of sampling wetlands across a range of human disturbances and thus no specific assessments aimed at measuring human disturbances were made. However, sites were classified according to the predominant land use in their immediate landscapes and these land use classes were useful in testing for mean differences in index scores among land use categories. The classification of her sites in this regard was: ‘agriculture’ (29 sites); ‘nature reserve’ (21 sites); ‘adjacent to road’ (6 sites); and ‘residential area’ (two sites). The main impacts being evaluated are therefore related to agricultural activities, as few sites had urban impacts. Her nutrient data (NO_3+NO_2–N: nitrates + nitrites; and SRP: Soluble Reactive Phosphorous) were used to proxy for water quality impairment among sites. NH_4–N was not measured, so NO_3+NO_2–N and SRP (equivalent to PO_4–P, Malan and Day, 2005) were the data used for index testing and for comparison with the training dataset. The spread for NO_3+NO_2–N using De Roeck's data (Figure 7.1), although lacking heavily disturbed sites (in terms of water quality), is more evenly spread (normally distributed) than is seen for the training dataset (cf. Figure 5.1) and should be more useful in this regard for index testing. The spread for SRP (Figure 7.1) using De Roeck's data is almost identical to that reported in Figure 5.1 for PO_4-P and thus presents a suitable gradient of trophic disturbance for the purposes of index testing.

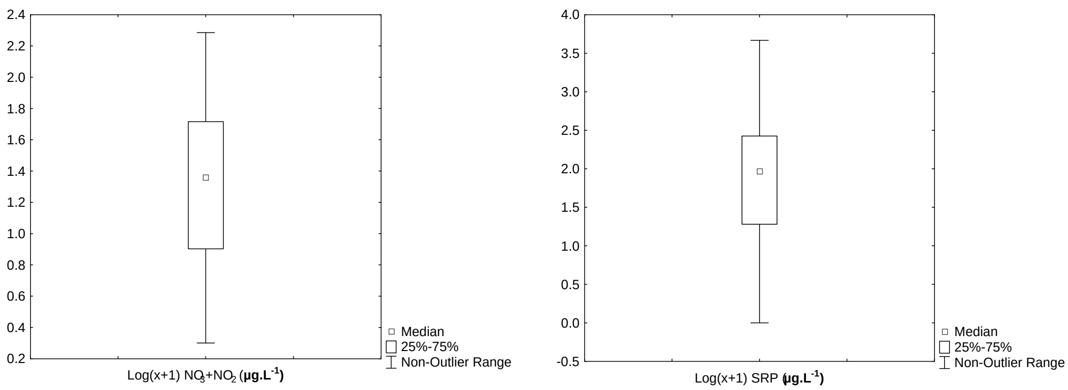

Figure 7.1: Box plots displaying the spread of each of the nutrient variables recorded in the study of De Roeck (2008). Nutrient concentrations are log(x+1) µg/L.

7.3 Comparison of sampling protocols

The sampling protocol of De Roeck (2008) was slightly different from that of the training study. De Roeck swept all habitats within each wetland (effort per habitat proportional to its cover in the wetland) for a cumulative total of five minutes per wetland (cf. 27 × 1 m sweeps per wetland in training study). She used a sweep net with a catch-surface of 500 cm^2 (cf. 529 cm^2 in training study) and a mesh size of 250µm (cf. 80µm in training study). The time-based sampling approach of De Roeck cannot be converted to a no/m^3 density estimate and so cannot be directly compared with the macro-invertebrate abundances used to formulate and test indices in the training study. An approximation of the total volume of water swept per wetland using the De Roeck sampling protocol was, however, undertaken to assess comparability among datasets:

- first, the average time spent sampling each habitat in the training study was two minutes, and thus per wetland approximately six minutes were spent sampling;

- given that an estimated ~1.49 m^3 of water column was sampled in these six minutes [0.0529 m^2 (catch-surface of net) × 27 m (27 sweeps)], it can be estimated that De Roeck sampled a volume of between 1 and 1.5 m^3 of water during her five minutes of sampling (her net catch-surface of 0.05 m^2 being directly comparable); and

- therefore, her macro-invertebrate abundances are likely to be comparable on the whole with those used to develop indices in this study, although she probably swept slightly more on average than 1 m^3 in five minutes.

Despite certain differences in sampling protocols, the dataset of De Roeck (2008) still provides a useful test of indices developed during the training study. Her invertebrate abundance data is standardised in terms of sampling effort among her study wetlands and thus one expects relative differences in abundances among wetlands to be reflected in the index scores, if indeed invertebrates respond predictably to human disturbances.

7.4 Assessment of index performance

Index scores were assigned to each of De Roeck's study wetlands by applying the IBI and numerical biotic index scoring procedures (developed in this study, see section 5.1.4) to her macro-invertebrate data (family level). In accordance with the simple index testing procedure used during the training study, linear regressions (continuous/quantitative variables) and box plots (categorical variables) were used to assess the general performance of indices. Kruskal-Wallis non-parametric tests (ANOVA by Ranks) were used to test for significant differences among the categorical variables. Figure 7.2 presents the linear regression plots used to assess correlations between wetland ASPT scores (using the numerical biotic index approach), IBI scores (using the nine metrics developed earlier in this study) and the nutrient variables (nitrate + nitrite and SRP). Figure 7.3 presents box plots depicting the median and spread of index scores among the different categories of land use adjacent to wetlands.

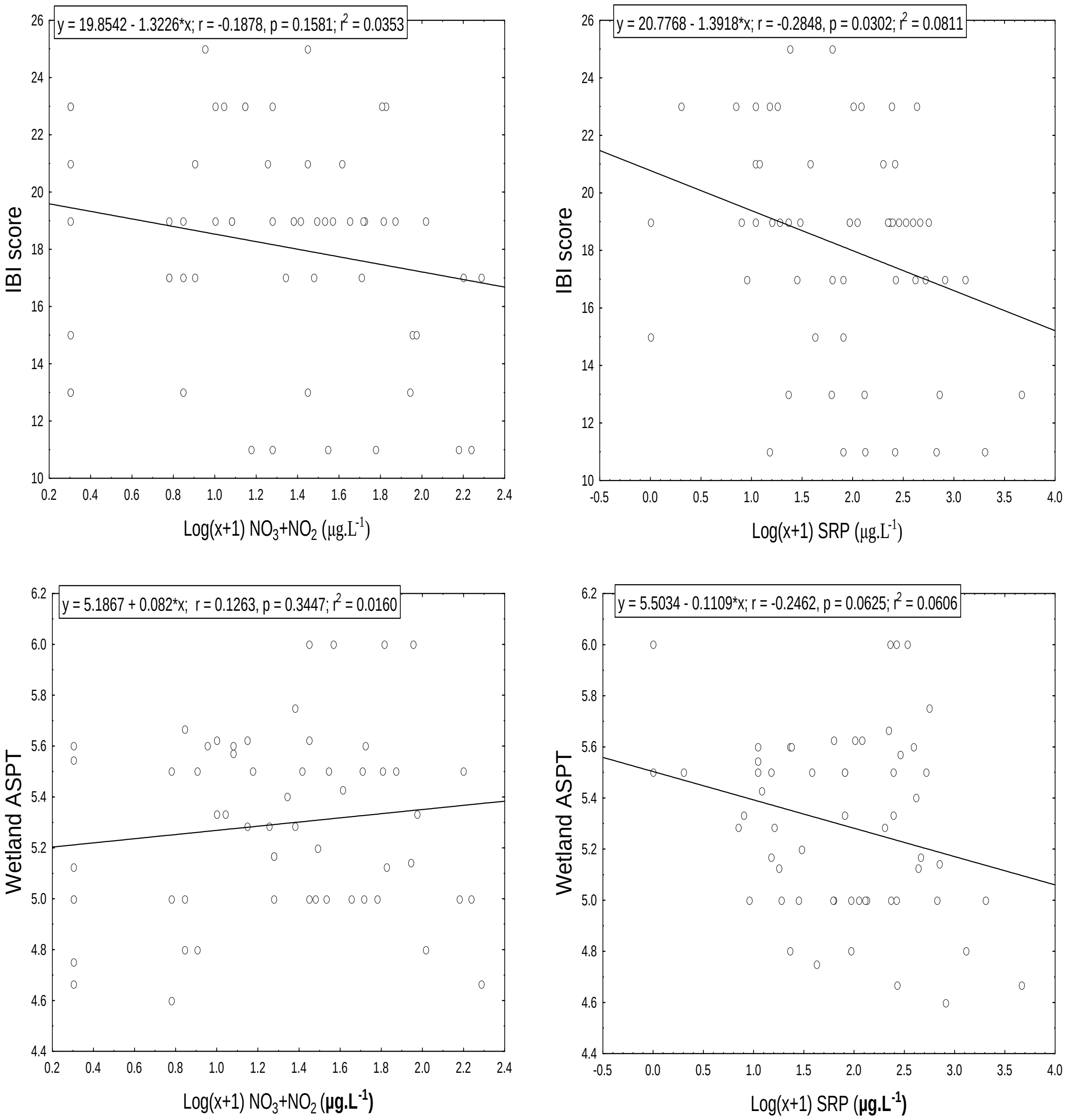

Figure 7.2: Linear regression plots of IBI scores (multi-metric IBI approach) and wetland ASPT scores (numerical biotic index approach) versus nutrient variables, using data from De Roeck (2008). Nutrient concentrations are log (x+1) µg/L. Regression equations, Pearson correlation coefficients (r), coefficients of determination (r^2) and significance values (p) are provided.

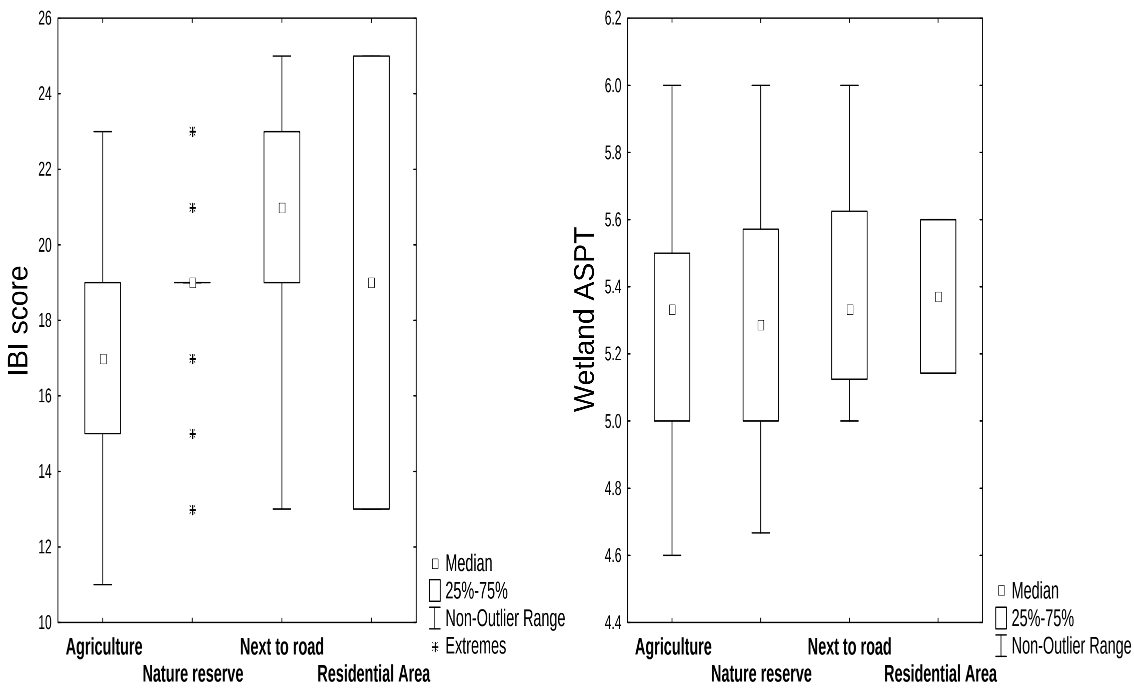

Figure 7.3: Box plot representations of the spread of IBI scores (multi-metric IBI approach) and wetland ASPT scores (numerical biotic index approach) amongst the different land uses adjacent to wetlands, as recorded by De Roeck (2008).

7.5 Discussion of trends

The IBI and numerical biotic index performed poorly in the test dataset both in terms of relationships with nutrient values and land use categories. The only significant correlation (for $\alpha = 0.05$) was between IBI scores and SRP ($r = -0.28$, $p = 0.03$, Figure 7.2), however, the amount of scatter was high in all four regression plots and inferences made from these index scores would not be useful in terms of determining nutrient/trophic status of wetlands. Nitrate + nitrite values greater than ~100µg/L (~2 on x axis of Figure 7.2) and SRP values greater than 1000µg/L (1 mg/L, ~3 on x axis of Figure 7.2) tended to be associated with lower index scores, but the lowest nutrient values were associated with a full range of index scores and there was no consistency in the index scoring of intermediate nutrient values. Kruskal-Wallis ANOVA by Ranks tests revealed no significant differences among the four categories of land use (adjacent to wetlands) in terms of IBI scores or numerical biotic index ASPT scores ($p = 0.18$ and $p = 0.78$ respectively). The most useful categorical comparison (in terms of sample size) is between index scores in agricultural wetlands versus those in nature reserves. Figure 7.3 shows that the spread within each grouping was very high for both IBI and numerical biotic index ASPT scores and that the medians were very similar. One concludes that the indices do not appear to be affected by land use impacts.

The poor results described above could be at least partly attributable to differences in sampling protocol among the training and test datasets. The study of De Roeck (2008) was aimed at assessing the general ecology of seasonal wetland invertebrates in the Western Cape and thus was not designed to incorporate the full range of human impacts nor a range of types of human impacts (hence the lack of urban-impacted wetlands). The only quantitative proxy for human impacts came in the form of the two nutrient variables, which suggest that water quality ranged from least impaired to moderately impaired, but lacked highly impaired sites (cf. the training dataset and Malan and Day, 2005). However, the indices did not associate in any consistent manner with low nutrient sites and this inability to classify wetlands with good water quality (oligotrophic sites) indicates a serious weakness in the inferential power of the indices.

The numerical biotic index performed better using the training dataset than when tested using the independent data of De Roeck (2008), but testing in the former dataset did suffer from an element of circularity in that the index was tested using the same data from which it was derived. It must be reiterated that the test dataset was not ideal in terms of factors discussed in section 7. Ideally, one would need to conduct another study specifically designed for testing the indices, with exactly comparable sampling methods and covering a full range of human impacts.

8. THE WAY FORWARD: WETLAND INVERTEBRATE INDICES IN SOUTH AFRICA

The inconsistent results of this study do not provide encouragement for the use of aquatic invertebrate indices in isolated depression wetlands of the Western Cape. The majority of evidence in this report (from both training and test datasets) points towards a generalist-type response of aquatic invertebrates to human disturbances for this wetland type. This conclusion is likely to apply to seasonal wetlands in general across the country, given a similar generalist response of the invertebrate biota as a reflection of adaptations to naturally high levels of disturbance induced by constantly fluctuating water levels in these systems (Tangen *et al*., 2003). This suggests that instead of pursuing index development for this wetland type, a more useful avenue for future empirical research may be to develop and test indices for other wetland types and regions in South Africa. To achieve this purpose, results from this study indicate that numerical biotic indices should be the approach of choice and a basic prototype has been presented for application in other wetland types and regions, given suitable modifications for taxonomic differences are incorporated (see paragraph below). Empirical results drawn from this study and those of Bowd *et al*. (2006a), Vlok *et al*. (2006) and Dallas (2009) indicate that the SASS river index *per se* should not be pursued for further use in wetlands without modifications.

Unlike the broad applicability of SASS, it is expected that a wetland numerical biotic index will require modification for different wetland types and regions and that one wetland index will not simply be applicable throughout the country. In this regard, the development of indices for the major wetland types in South Africa will be an evolutionary process and will require refinement at various steps within the process, as was observed for each of the 5 versions of SASS. Importantly, further research should increase the sensitivity of indices by broadening the range of tolerance scores beyond 1-9. The prescribed identification of wetland invertebrates at the family level is relatively user-friendly and due to the simplicity of this index approach, it is suggested that testing on further wetland types and regions can be achieved without too much difficulty. Although eco-toxicology testing for the various wetland taxa would be optimal, resources and expertise may limit this approach in South Africa and instead it is recommended that similar correlational methods are used to ascertain tolerance scores as conducted in this study. The reader is referred to similar methods employed by Chessman *et al*. (1997, for rivers) and a derivation of this approach applied to wetlands (Chessman *et al*., 2002). According to the latter study, a numerical biotic index framework should be applicable broadly and over different wetland types, but will require modification based on the different suites of invertebrate taxa among different areas and wetland types.

Another important consideration for establishment of this index approach in different regions and wetland types of South Africa is that the protocol will only be suitable for wetlands with sufficient surface water for the sweep netting protocol described in this study. The inapplicability of this protocol for ephemerally inundated pans of the Free State province (as described in section 4.3) illustrates this point. It is of the author’s experience that sweep-net sampling of aquatic invertebrates generally requires wetlands with surface water depths not shallower than 10 cm. Wetlands occurring in the drier parts of South Africa (both winter and summer rainfall areas) are usually only ephemerally inundated and are not likely to be suitable for establishment of an aquatic invertebrate index in that only a few brief times of each year will one be able to collect invertebrates to produce index scores. In this regard, wetland types and regions that are conducive to providing surface water for reasonable lengths of time and with increased annual predictability will be more appropriate for establishment and application of aquatic invertebrate indices.

As already stated, a suggested outcome from this study is to test the numerical biotic index approach on other wetland types and regions of South Africa. In this regard, effort should first be concentrated on wetland types that meet the criteria described above. Permanent wetlands, though not characteristic in this generally arid country, would be suitable for the purposes of testing and establishment of invertebrate bioasessment indices. The baseline scoring criteria provided in this report can be used in other regions/wetland types, but it is recommended that the correlational testing approach used in this study is applied to invertebrate taxa that are characteristic in these other regions/wetland types in order to properly calibrate the numerical biotic index. The suggested index framework can, however, remain the same.

Perennial endorheic depressions (known locally as ‘pans’) are a regular feature in the landscape of the Mpumalanga province in South Africa and are found across differentially impacted landscapes, thus presenting an ideal opportunity to investigate the use of aquatic invertebrates as indicators of human disturbance. A PhD study is currently being undertaken by Martin Ferreira (University of Johannesburg) which examines the biotic and abiotic components of various pans occurring in the minimally disturbed area of Lake Chrissie and areas impacted by coal mining activities. Although the results have not yet been published, preliminary findings suggest that there is a large degree of variation in the biotic and abiotic components of the various pans. The large amount of variation observed in water quality and invertebrate communities makes separation of natural changes and changes induced by human activities quite difficult. A shift in focus from invertebrate diversity to the functional roles of the different taxa in the community may help to elucidate patterns in terms of human disturbance effects on the invertebrate fauna of these wetlands (Martin Ferreira, 2009, pers. comm. University of Johannesburg, South Africa). These findings do not offer early encouragement in terms of index potential for this wetland type and suggest that perhaps not only seasonal wetlands, but also more perennial wetlands in South Africa, are characterized by high amounts of natural variation and thus may not be entirely suitable for index development purposes. A key difference between permanent depression wetlands found in South Africa and those, for example, reported on by Gernes and Helgen (2002) in Minnesota, USA, may well lie in the amount of natural variation inherent to the areas. Although not discussed by Gernes and Helgen (2002), natural variation among Minnesota wetlands may be quite low, thus enabling patterns in the invertebrate fauna to be more directly attributable to variation in human disturbance than is possible in South Africa, for example, among the heterogenous Mpumalanga pans. Clarification on the issue of index potential in perennial wetlands of South Africa can be achieved through further index testing in other perennial wetland types and regions of the country using the framework approach presented during this study.

Comparison of multi-metric IBI and numerical biotic index approaches tested in this study suggest significant advantages of the latter over the former as a way forward for South African wetland bioassessment using aquatic macro-invertebrates, both in terms of user-efficiency and strength of results. Although combining taxa as summary metrics may provide additional bioassessment information compared to scoring taxa individually, the gains from such an approach appear to be considerably outweighed by the effort required (in terms of time, money and expertise) to create and test such metrics in South Africa. A significant drawback of the multi-metric IBI approach is the need for quantitative data to calculate metrics, which precludes rapid on-site assessments, and rather a combination of presence-absence and rank abundance data (*sensu* the numerical biotic index approach) is prescribed for use in more rapid wetland assessments.

9. CONCLUSIONS AND RECOMMENDATIONS

9.1 Isolated depression wetlands

- The macro-invertebrate families sampled in this study did not show clear relationships with human disturbance variables as proxied by landscape use (HDS) and nutrient levels (PO_4 and NH_4) among wetlands. The majority of families showed a generalist response to human disturbances and results do not provide encouragement for establishment of an invertebrate index for this wetland type.

- Despite relatively poor bioassessment results for isolated depression wetlands in the Western Cape, a prototype framework for a numerical biotic index has been developed during this study (essentially a modification of the SASS river index), which shows potential for testing in other wetland types and regions of South Africa. In this regard, the prescribed approach is to first use a training dataset in order to modify tolerance scoring criteria according to the prevalent taxa for a given wetland type/region; followed by testing of the index with an independent set of data to clarify its inferential power.

- The lack of clear indicator taxa for seasonally inundated wetlands investigated in this study is likely to be a common pattern in seasonal wetlands throughout South Africa due to the ‘generalist-type’ adaptations of taxa to these transient environments. Only more research on seasonal wetlands found in other areas of the country can confirm this prediction. Evidence presented in this study, however, suggests that research effort towards the development of aquatic invertebrate indices in South Africa should rather be concentrated on perennial wetlands, where more specialist invertebrate taxa are likely to be found and are thus more likely to show responses to human disturbance. This recommendation is also relevant in the context of developing wetland indices using other biotic assemblages (e.g. diatoms) in that more specialist taxa are likely to inhabit perennial wetlands and thus bioassessment research for other biotic assemblages is expected to be more fruitful in perennial environments.

- The identification of wetland macro-invertebrate taxa to family level is appropriate for future index testing and development in South Africa.

- The multi-metric IBI approach, although shown to be useful in certain parts of the United States, is not recommended as a way forward for rapid wetland bioassessment in South Africa. This conclusion is reached due to a combination of factors: the need for quantitative data; the often laborious process of calculating metrics; the sometimes required identification of taxa beyond family level; and the relatively poor performance of this approach compared to the numerical biotic index as observed during the empirical component of this study.

- Based on results from this study and those of Bowd *et al*. (2006a), the use of SASS for determining the impairment state of truly lentic wetlands appears unfeasible, however a modified version of this index shows some potential.

- Preliminary evidence from metrics and indicator species testing suggests that micro-crustaceans are not useful for inclusion in wetland bioassessment indices in South Africa. This conclusion is reached partly because of the laborious enumeration and identification procedures involved and partly because of the lack of good indicator patterns observed in this study. More research in other wetland types and regions would offer clarification of this issue.

9.2 Valley bottom wetlands

- Although the number of valley bottom wetlands investigated in this study was comparatively low (n=15), SASS appeared unable to reliably distinguish impairment levels among sites in comparison to the precision witnessed when using this index in rivers. It is concluded that a certain degree of inferential power is lost when transferring SASS from rivers to valley bottom wetlands. Bioassessment methods less reliant on surface water (e.g. soil indices, macrophyte indices) may prove more feasible for this wetland type as the SASS sampling protocol requires the presence of a suitable amount of surface water for sweep netting.

- Empirical evidence collected from this study and the literature (Bowd *et al*., 2006a; Vlok *et al*., 2006; Dallas, 2009) reaches a firm conclusion that the SASS river index should not be directly applied in the bioassessment of wetlands (including those with flow) without some degree of modification for the different suite of macro-invertebrate taxa and habitats characterizing wetlands.

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11. GLOSSARY

Abiotic: not pertaining to living organisms; describes features such as temperature, rainfall, etc.

ASPT (Average Score Per Taxon) values: these values are the key output of the SASS (South African Scoring System) rapid assessment index and are calculated by dividing the total SASS score for a site by the number of taxa scored.

Bioassessment: the use of living organisms to assess conditions (usually with reference to some aspect of conservation).

Biotope: an area of uniform environmental conditions.

BMWP (Biological Monitoring Working Party): a rapid macro-invertebrate bioassessment method developed for scoring the degree of impairment of streams in Great Britain.

BMWQ: Spanish Biological Monitoring Water Quality score system. Developed for the rapid bioassessment of Spanish streams using macro-invertebrates.

Branchiopoda: primitive crustaceans (*q.v.*) belonging to the Anostraca (fairy and brine shrimps), Conchostraca (clam shrimps) and Notostraca (shield or tadpole shrimps)

CCA: canonical correspondence analysis, a type of multivariate statistical analysis

Chironomidae: non-biting midges

Cladocera: water fleas such as *Daphnia*

Copepoda: minute shrimp-like and mostly planktonic crustaceans (*q.v.*)

Crustacea: a large group of usually aquatic invertebrate animals characterized by two pairs of antennae and usually having many pairs of appendages

Eco-region: A region defined by similarity of climate, landform, soil, potential natural vegetation, hydrology and other ecologically relevant variables.

Ecosystem condition: the quality of an ecosystem relative to that of an undisturbed or fully functional state

Eco-toxicology: the study of the effects of toxic chemicals on the biotic constituents of ecosystems.

Eutrophication: the process whereby high levels of nutrients result in the excessive growth of plants.

FCI (Functional Capacity Index): used to indicate the degree (capacity) to which a wetland performs a given function under the HGM functional assessment method.

Functional unit: A level 3 discriminator in the South African National Wetland Classification System hierarchy (Ewart-Smith *et al.,* 2006). Functional units are distinguished on the basis of several different discriminators, which vary between the different 'Systems' (level 1 discriminator). The reader is referred to Ewart-Smith *et al.* (2006) for examples.

Fynbos: the low-growing vegetation found in much of the part of the Western Cape province which experiences a Mediterranean climate

Generalist: An organism that is able to thrive in a broad spectrum of environmental conditions.

GIS data: 'Geographical Information System' is a computer-based system that stores, manages and analyzes data linked to locations of physical features on earth.

Halophyte: a salt tolerant plant

HDS (Human Disturbance Scores): Summary output of the rapid assessment index used in this report to quantify the integrated effects of various landscape stressors on wetlands.

HGM (Hydrogeomorphic) classification: a classification system based on the shape of the land (landform setting) and the patterns of surface and subsurface flow.

IBI (Index of Biological Integrity): An integrative expression of the biological condition of a site that is composed of multiple metrics.

Index period: A defined interval of the season that serves as the sampling period for biological assessments.

Indicator species: a species whose presence in an ecosystem is indicative of particular conditions (such as saline soils or acidic waters)

Invertebrate: an animal without a backbone

Isolated depression wetland: ‘A basin-shaped area with a closed elevation contour that allows for the accumulation of water and is not connected via a surface inlet or outlet to the drainage network i.e. it receives water by direct precipitation, groundwater or as limited runoff from the surrounding catchment but no channelled surface inflows or outflows are evident’ (Ewart-Smith *et al*., 2006).

Least impaired: pertaining to wetlands which have incurred a minimal degree of human impairment, relative to other wetlands in a region.

Lentic: of standing waters (ponds, lakes etc.).

Lotic: of running waters (streams and rivers).

Macro-invertebrate: Animals without backbones that are retained by a 500-1000 micron mesh (mesh size depending on definition used).

MCI (Macro-invertebrate Community Index): rapid bioassessment index used to score the impairment of New Zealand streams using macro-invertebrates.

Metrics: A summary measure of assemblage composition which shows empirical change along a gradient of human disturbance.

Micro-crustacean: Crustaceans of length greater than 63-153 microns (mesh size depending on definition used), dominated by the taxa Cladocera, Ostracoda and Copepoda in freshwater environments.

Multivariate index: in a bioassessment context, models that seek to predict biotic assemblage composition of a site in the absence of environmental stress. A comparison of the assemblages predicted to occur at test sites with those actually collected provides a measure of biological impairment at the tested sites.

Numerical biotic index: in a bioassessment context**,** a simple index format involving the assignment of sensitivity scores to individual taxa, which are then summarized as a total score or average score per taxon from a representative sample of a site.

OKASS (Okavango Assessment System): a modified version of the SASS index used for bioassessment in the Okavango Delta.

ORAM (Ohio Rapid Assessment Method): Rapid technique for assessing human impacts on wetlands in the state of Ohio, USA.

pCCA: partial canonical correspondence analysis, a direct gradient analysis technique which allows one to partial out the effects of covariables.

Reference sites: those sites that are minimally impacted by human disturbance and that reflect the natural condition of a wetland type in a given region.

SASS (South African Scoring System): a system for the rapid bioassessment of water quality of streams in South Africa using macroinvertebrates, currently in its 5th version (SASS5).

Seasonally inundated wetlands: those wetlands which are inundated with surface water during a particular season of the year only.

SIGNAL index: rapid macro-invertebrate bioassessment index developed for use in Australian streams.

Valley bottom wetland: 'a functional unit at the bottom of a valley that receives water from an upstream channel and/or from adjacent hill slopes. The area is not subject to periodic over-bank flooding by a river channel' (Ewart-Smith *et al*., 2006).

Vlei: a South African term for a wetland; in the Cape, any wetland; in the rest of the country, a reedbed in a river course

WET (Wetland Evaluation Technique): rapid assessment technique developed through the US Army Corps of Engineers, which uses the presence or absence of a large set of wetland characteristics as qualitative predictors of wetland functions.

WET-Ecoservices: a technique for rapidly assessing ecosystem services supplied by wetlands in South Africa.

WET-Health: a technique for rapidly assessing wetland health in South Africa.

WZI (Wetland Zooplankton Index): index developed for the assessment of wetland condition based on multivariate pattern analysis of water quality and zooplankton associations with aquatic vegetation in the Laurentian Great Lakes Basin.

Zooplankton: animal plankton (*q.v.*)

APPENDIX 1: SUMMARY OF ATTRIBUTES OF AQUATIC INVERTEBRATES AS BIOASSESSMENT INDICATORS FOR WETLANDS

Table A1.1: Summary of attributes, beneficial and otherwise, of aquatic invertebrates as bioassessment indicators for wetlands (from Helgen, 2002)

Advantages	**Disadvantages**
Invertebrates can be expected to respond to a wide array of stresses to wetlands, such as pollutants in water and bottom sediments, nutrient enrichment, increased turbidity, loss or simplification of vegetation, siltation, rearing of bait or game fish, input of storm water or wastewater runoff, introductions of exotic species, or alterations of the landscape around the wetland.	Because it is likely that multiple stressors are present, it may not be possible to pinpoint the precise cause of a negative change in the composition of invertebrates. However, data from major sources of human disturbance, e.g., water and sediment chemistry, the nearby wetland landscape features, sources of hydrologic alteration, and other disturbance factors can be assessed in relation to the invertebrate data to see which factors have the greatest effects.
Life cycles of weeks to months allow integrated responses to both chronic and episodic pollution, whereas algae recover rapidly from acute sources, and vertebrates and macrophytes may take longer to respond to chronic pollution	Information on short-term, pulse impairments (using algae, zooplankton) or more long-term impairments (using macrophytes, vertebrates) or more landscape-level (using birds, amphibians) impairment may be desired.
Toxicological/laboratory based information is extensive. Invertebrates are used for a large variety of experimental approaches.	Toxicological response data may not be available for all invertebrates; data for some wetlands' species are less extensive than for stream species.
There is an extensive history of analysis of aquatic invertebrates in biological monitoring approaches for streams.	Using invertebrates to assess the condition of wetlands is now under development in several States and organizations.
Invertebrates are used for testing bioaccumulation of contaminants to analyze effects of pollutants in food webs.	Tissue contaminant analyses are always costly. This is true for tissue analysis of any group of organisms: vertebrate, invertebrate, or plant.
Invertebrates are important in food webs of fish, salamanders, birds, waterfowl, and predatory invertebrates.	Aquatic invertebrates tend not to be valued by the public as much as fish, amphibians, turtles, or birds. However, citizens do respond to invertebrates
Many invertebrates are ubiquitous in standing water habitats.	Invertebrate composition will differ in different wetland classes, as will other groups of organisms (plants, birds) that might be used to assess wetlands.
Many invertebrates are tightly linked to wetland conditions, completing their life cycles within the wetlands. They are exposed to site-specific conditions.	Some invertebrates migrate in from other water bodies; these taxa are not as tightly linked to the conditions in the specific wetland.

Table A1.1: Continued

Many invertebrates depend on diverse wetland vegetation; some depend on particular types of vegetation for reproduction.	Loss of invertebrates may be a secondary effect from the loss of wetland vegetation, e.g., from herbicide treatments. Vegetation loss is an impairment.
Invertebrates have short and long life cycles and they integrate stresses to wetlands often within a 1-year time frame.	Many complete their life cycle within a year; they are not as "long-lived" as birds, amphibians or perennial vegetation.
Invertebrates can be easily sampled with standardized methods.	Picking invertebrate samples is labour-intensive.
Invertebrates can be sampled once during the year, if the best index period is selected for optimal development of invertebrates.	Invertebrate composition of wetlands often varies within the seasons of the yearly cycle. Invertebrates mature at different times. This necessitates selecting an "index period" for sampling once, or alternatively, sampling more than once in the season.
Invertebrates can be identified using available taxonomic keys within labs of the entities doing the monitoring. Staff help develop bio-monitoring programs.	Expertise is required to perform identifications of invertebrates. Some may choose to contract out some or all the identifications. There is a cost involved.
High numbers of taxa and individual counts permits the use of statistical ordination techniques that might be more difficult with just a few species, e.g. with amphibians.	Large numbers of taxa and individual counts make the sample processing more labour intensive than other groups. Adequate training and staff time are required. More lab time is needed than for some other groups of organisms.
Citizens can be trained to identify wetlands invertebrates and become interested and involved in wetlands assessment. Citizens are excited to see the richness of wetland invertebrates.	Citizen monitoring requires training to learn many invertebrates in a short time, a structured program, and a commitment by volunteers and local governments; citizens may tend to underrate high quality wetlands.

APPENDIX 2: SCORE SHEETS FOR CALCULATING HUMAN DISTURBANCE SCORES AT EACH SITE

Template score sheet

The score sheet consists of two main components, firstly, the land-use characterization table used for scoring the expected effects of immediate and surrounding land-use on wetland water quality, hydrology and physical structure. ‘WQ’ refers to ‘water quality’, ‘Hydrol’ refers to ‘Hydrology’ and ‘Phys struc’ refers to ‘Physical Structure’. Secondly, the table is used for scoring plant community indicators.

Table A2.1: Template score sheet for calculating HDS at each site

Rate areal extent: 0 = none, 1 = (< 25%), 2 = (25-50%), 3 = (50- 90%), 4 = (>90%). If present, score as per below SCORE table												
Present Land-use / Activity	**In wetland**				**Within 100 m**				**Within 500 m**			
	Extent	**Score Impact on:**			**Extent**	**Score Impact on:**			**Extent**	**Score Impact on:**		
		WQ	Hydrol	Phys struc		WQ	Hydrol	Phys struc		WQ	Hydrol	Phys struc
Commercial afforestation												
Agriculture – crops												
Agriculture – livestock												
Abandoned lands												
Rural development												
Urban development												
Suburban gardens												
Deep flooding (too deep for emergent vegetation)												
Shallow flooding												
Dead brush piles of alien vegetation												
Dead/dying plants												
Drowned vegetation												
Stranded aquatic vegetation												
Old high water marks												
Industrial												
Informal settlement												
Mining / excavation												
Recreational (sports field, golf estate etc.) specify												
Infilling												
Stormwater outlets												

Rate areal extent: 0 = none, 1 = (< 25%), 2 = (25-50%), 3 = (50- 90%), 4 = (>90%). If present, score as per below SCORE table												
Present Land-use / Activity	**In wetland**				**Within 100 m**				**Within 500 m**			
	Extent	**Score Impact on:**			**Extent**	**Score Impact on:**			**Extent**	**Score Impact on:**		
		WQ	Hydrol	Phys struc		WQ	Hydrol	Phys struc		WQ	Hydrol	Phys struc
Sewage disposal												
WWTW outlets												
Solid waste disposal (including dumping and litter)												
Weirs												
Berms												
Dams												
Water abstraction												
Drainage channels												
Roads / Railway												
Culverts												
Dredging												
Pedestrian paths												
Off road vehicle use												
Habitat modifiers fish stocking												
Dense woody alien vegetation patches												
Dense aquatic alien vegetation patches												
Erosion e.g. gullies / headcuts												
Deposition / sediment												
Other												

SCORE TABLE: total impact on scale of 0 to 5:
5 = Poor: currently active and major disturbance to natural hydrology
4 = Fair: less intense than “poor”, but current or active alteration
3 = Immoderate: active alterations that have changed the hydrological potential of the wetland
2 = Moderate: low intensity alteration that has minor impact on natural hydrology
1 = Good: low intensity alteration or past alteration that is not currently affecting wetland
0 = Best : as expected for reference, no evidence of disturbance

Plant community indicators						
Approximate width of upland vegetation buffer	Unlimited: (0) surrounding land use not transformed from natural state	Wide: (1) buffer averages > 50 m around wetland perimeter	Medium: (2) buffer averages 25-50 around perimeter	Narrow: (3) 10- 25 meters on average	Very Narrow: (4) less than 10 meters on average	None: (5)
Indigenous monospecific plant stands (opportunistic species)	Absent (0)	Nearly Absent (1) < 5% cover	Sparse (2) 5-25 % cover	Moderate (3) 25-75% cover	Extensive (4) >75% cover	Complete cover (5)
Alien vegetation coverage	Absent (0)	Nearly Absent (1) < 5% cover	Sparse (2) 5-25 % cover	Moderate (3) 25-75% cover	Extensive (4) >75% cover	Complete cover (5)
Dryland or upland plant invasions	Absent (0)	Nearly Absent (1) < 5% cover	Sparse (2) 5-25 % cover	Moderate (3) 25-75% cover	Extensive (4) >75% cover	Complete cover (5)
Horizontal plan view – heterogeneity *	High hetero-geneity (0)	Moderately High (1)	Moderate (2)	Moderately Low (3)	Low (4)	None (5) No veg / monospecific veg

* Degree of interspersion of distinct plant communities and thus habitats within the wetland

Example score sheet 1

Shown below is the score sheet used for calculating % HDS at KEN02, a relatively impacted site (see Appendix 3 for further site details). Only the land-use categories which received a score at this site are presented. Table A2.1 provides details of scoring criteria. 'WQ' refers to 'water quality'; 'Hydrol' refers to 'Hydrology' and 'Phys struc' refers to 'Physical Structure'. For each column scored for human impacts (and in turn within each of the distance bands), the maximum score of impact across all land-use activities (see 'max. impact scores') was used in the next step, which was to sum the maximum scores of impact across all impact categories (namely WQ, Hydrol, Phys struc) and distance bands (see 'sum of max. impact scores'). The plant community indicator scores were summed and this score was added to the 'sum of max. impact scores' to produce a final impact score for the site. This was divided by the maximum possible score (70) to obtain the final HDS (%) for each wetland.

Table A2.2: Example score sheet for calculating % HDS at KEN02, a relatively impacted site (see Appendix 3 for further site details)

Present land-use	**In wetland**				**Within 100 m**				**Within 500 m**			
		Score Impact on:				Score Impact on:				Score Impact on:		
	Extent	WQ	Hydrol	Phys struc	Extent	WQ	Hydrol	Phys struc	Extent	WQ	Hydrol	Phys struc
Recreational (sports field, golf estate etc) specify					1	4	3	3	2	3	3	4
Stormwater outlets					1	3	3	2				
Roads / Railway					1	2	0	2				
Pedestrian paths	1	0	0	2	1	0	0	2				
Dense woody alien vegetation patches	1	1	2	3	1	1	2	4	2	1	3	4
Other (grassy pioneer invasion)	2	1	1	4	3	1	1	5	2	1	1	4
Max. impact scores (per column):		**1**	**2**	**4**		**4**	**3**	**5**		**3**	**3**	**4**
Sum of max. impact scores:	**29**											

Plant community indicators	
Buffer Width Score	2
Indigenous monospecific extent score	4
Alien veg extent score	3
Upland plant invasion score	4
Horizontal plan view or Heterogeneity score	4
Plant community indicators: sum of scores	**17**
Total impact score = 29 + 17	**46**
Maximum possible impact score	**70**
% Impact score (%HDS) = 46/70 * 100	**66**

Example score sheet 2

Shown below is the score sheet for calculating % HDS at KEN12, a least impaired site (see Appendix 3 for further site details). Only the land-use categories which received a score at this site are presented. Table A2.1 provides details of scoring criteria. 'WQ' refers to 'water quality'; 'Hydrol' refers to 'Hydrology' and 'Phys struc' refers to 'Physical Structure'. For each column scored for human impacts (and in turn within each of the distance bands), the maximum score of impact across all land-use activities (see 'max. impact scores') was used in the next step, which was to sum the maximum scores of impact across all impact categories (namely WQ, Hydrol, Phys struc) and distance bands (see 'sum of max. impact scores'). The plant community indicator scores were summed and this score was added to the 'sum of max. impact scores' to produce a final impact score for the site. This was divided by the maximum possible score (70) to obtain the final HDS (%) for each wetland.

Table A2.3: Example score sheet for calculating % HDS at KEN12, a least impaired site (see Appendix 3 for further site details)

Present land-use	**In wetland**				**Within 100 m**				**Within 500 m**			
		Score Impact on:				Score Impact on:				Score Impact on:		
	Extent	WQ	Hydrol	Phys struc	Extent	WQ	Hydrol	Phys struc	Extent	WQ	Hydrol	Phys struc
Recreational (sports field, golf estate etc) specify					1	3	3	3				
Urban development									1	3	3	3
Dams									1	1	2	2
Roads/Railway					1	1	2	2				
Off road vehicle use					1	0	0	2				
Dense woody alien vegetation patches									2	1	3	3
Max. impact scores (per column):		**0**	**0**	**0**		**3**	**3**	**3**		**3**	**3**	**3**
Sum of max. impact scores:	**18**											

Plant community indicators	
Buffer Width Score	1
Indigenous monospecific extent score	0
Alien veg extent score	0
Upland plant invasion score	0
Horizontal plan view or Heterogeneity score	4
Plant community indicators: sum of scores	**5**
Total impact score = 18 + 5	**23**
Maximum possible impact score	**70**
% Impact score (%HDS) = 23/70 * 100	**33**

APPENDIX 3: MEASUREMENTS OF HUMAN DISTURBANCE VARIABLES AND ENVIRONMENTAL INFORMATION COLLECTED AT EACH STUDY SITE

Table A3.1: Measurements of human disturbance variables and environmental information collected at each study site

WC = Western Cape, CF = Cape Flats, AGU = Agulhas Plain, Ref = reference wetland, Urb = urban impacted wetland, Agri = agricultural impacted wetland, Other = Other impacts. **HDS** – human disturbance scores; **NTU** – nephelometric turbidity units; **TSA** = total surface area.

Site code	Latitude (Decimal degrees)	Longitude (Decimal degrees)	Region	Impact category	HDS (%)	$NO_3 + NO_2$ (µg/L)	PO_4 (µg/L)	NH_4 (µg/L)	TSA (m^2)	Depth (cm)	Altitude (m)	pH	Conductivity (mS/cm)	Temperature (°C)	Oxygen (mg/L)	Turbidity (NTU)
KOE01	33.687167	18.435583	WC	Ref	14	1.56	9.29	34.97	3309.7	55	6	8.29	8.193	18.5	9.47	1.9
KOE02	33.685278	18.434889	WC	Ref	10	0.37	43.87	64.42	3793.5	35	5	8.20	6.453	19.5	7.60	2.7
KOE03	33.684167	18.436778	WC	Ref	17	1.26	6.33	54.11	1885.0	35	8	8.23	9.253	22.4	5.63	1.7
KOE04	33.685472	18.437444	WC	Ref	13	1.47	14.51	59.75	706.9	35	10	8.18	10.640	22.6	4.97	1.2
KOE05	33.686889	18.436944	WC	Ref	10	0.99	2.25	34.44	4712.4	45	8	8.64	7.723	22.6	13.33	0.9
KOE06	33.692194	18.438639	WC	Ref	30	0.91	4.28	38.75	2199.1	45	7	8.12	3.473	23.7	3.33	8.8
SOU01	33.709611	18.454417	WC	Urb	50	0.01	119.92	6.70	716.3	38	6	9.16	15.617	18.7	14.33	2.3
SOU02	33.690194	18.454722	WC	Urb	59	0.06	2.67	24.14	1394.9	27	13	8.41	12.467	22.8	11.23	15.7
SOU03	33.703306	18.468722	WC	Urb	64	0.02	14.31	8.22	1374.4	150	16	8.22	20.833	22.2	16.70	2.5
SOU04	33.700111	18.468333	WC	Urb	51	1.36	23.90	28.38	471.2	21	20	7.25	5.233	23.1	7.13	17.5
DIE01	33.514639	18.654583	WC	Other	53	11.62	213.88	2283.93	450.8	80	120	6.90	0.306	18.2	1.73	7.4
DIE02	33.544944	18.635611	WC	Agri	59	9.66	87.39	56.77	2827.4	44	97	7.38	0.585	22.5	1.93	1.8
DIE03	33.591222	18.605861	WC	Other	69	11.24	37.11	47.04	1570.8	70	90	7.25	0.261	20.5	4.60	4.5
DIE04	33.647861	18.569000	WC	Agri	66	1.04	301.25	19.00	4021.2	60	117	7.39	0.549	19.3	4.07	4.7
PIK01	32.772278	18.818333	WC	Agri	44	14.58	66.27	98.39	11781.0	90	126	7.27	0.488	17.0	5.87	9.8
PIK02	32.702389	18.836139	WC	Agri	76	2.56	15.86	2803.87	15708.0	40	125	6.95	0.353	17.7	3.33	157.5
PIK03	32.700167	18.841056	WC	Agri	63	66.01	53.61	1257.23	490.9	30	125	7.12	0.268	18.0	4.33	397.0
PIK04	32.694472	18.855111	WC	Agri	76	4655.60	67.31	81.02	3141.6	30	124	7.55	0.250	16.9	8.53	1000.0
PIK05	32.683694	18.884000	WC	Urb	67	13.84	127.37	1506.27	1178.1	50	95	7.51	1.279	15.8	3.00	39.7
PIK06	32.688556	18.932667	WC	Agri	74	5.63	137.35	164.10	11781.0	50	159	7.21	0.354	16.9	3.93	53.9
PIK07	32.686528	18.935278	WC	Agri	74	22.12	150.09	3881.72	392.7	36	163	7.34	0.305	16.6	6.57	494.3
PIK08	32.686778	18.934722	WC	Agri	74	10.18	326.43	2879.63	235.6	30	158	7.15	0.411	17.6	5.07	850.0
PIK09	32.677611	18.934500	WC	Agri	69	5.92	999.41	1534.37	2513.3	38	151	7.35	0.436	19.7	4.17	38.1
PIK10	32.627472	18.928500	WC	Agri	74	9196.39	390.78	83.12	414.7	50	232	9.11	5.910	19.1	24.20	256.0
PIK11	32.640889	18.890917	WC	Agri	74	47.65	579.56	130.81	3711.0	40	139	7.72	0.260	18.3	6.57	713.0
PIK12	32.901583	18.798944	WC	Agri	73	0.06	12.03	61.72	1570.8	40	120	7.31	0.265	12.3	3.83	439.5

Site code	Latitude (Decimal degrees)	Longitude (Decimal degrees)	Region	Impact category	HDS (%)	$NO_3 + NO_2$ (µg/L)	PO_4 (µg/L)	NH_4 (µg/L)	TSA (m^2)	Depth (cm)	Altitude (m)	pH	Conductivity (mS/cm)	Temperature (°C)	Oxygen (mg/L)	Turbidity (NTU)
PIK13	33.370833	18.706222	WC	Agri	54	13.19	10.12	32.04	1570.8	30	236	7.47	1.047	14.9	10.67	17.0
DAR01	33.373000	18.382222	WC	Urb	57	654.63	131.97	38.04	23561.9	54	105	7.18	0.738	14.9	3.07	247.5
DAR02	33.358917	18.395861	WC	Other	69	99.34	3.78	825.86	8246.7	75	95	7.27	0.529	16.7	5.87	30.6
DAR03	33.287556	18.445111	WC	Other	71	1.06	413.10	850.18	7539.8	46	50	7.29	3.110	17.7	3.63	37.0
DAR04	33.008333	18.351528	WC	Ref	27	28.95	2827.36	2314.41	1665.0	68	30	7.04	0.570	17.3	1.20	4.4
DAR05	33.084222	18.397056	WC	Ref	46	0.87	510.89	105.21	78539.8	50	50	8.21	3.697	18.7	8.50	14.7
DAR06	33.145722	18.461250	WC	Agri	57	0.98	453.14	76.85	314.2	30	40	7.66	11.027	14.4	3.10	14.0
DAR07	33.086972	18.397667	WC	Ref	31	10.55	615.00	1524.48	1885.0	25	44	7.27	2.733	17.6	1.38	4.0
DAR08	33.085278	18.398083	WC	Ref	33	17.95	816.31	4231.53	1413.7	29	50	7.57	3.313	24.3	3.13	18.5
DAR09	33.072361	18.371889	WC	Agri	47	10.26	655.65	65.20	15708.0	39	62	8.08	0.583	26.3	6.27	7.0
VEL01	32.807333	18.359778	WC	Agri	59	5.91	140.79	303.03	706.9	30	38	8.27	12.983	22.3	2.80	10.5
VEL02	32.768167	18.239111	WC	Agri	69	0.79	444.40	66.16	1413.7	78	23	8.71	7.653	18.5	5.50	4.0
VEL03	32.770417	18.230833	WC	Agri	74	4.39	594.89	73.38	587.5	20	15	8.68	2.697	21.7	9.10	9.0
VEL04	32.825556	18.101500	WC	Agri	49	13.44	14.14	93.84	94247.8	45	14	8.46	22.133	22.6	11.43	1.5
VEL05	32.942583	17.927444	WC	Agri	66	245.14	343.48	2908.83	17671.5	130	70	7.71	0.467	19.7	5.20	546.0
VEL06	32.945611	17.927778	WC	Agri	64	116.37	20.97	315.25	11781.0	70	72	8.53	2.723	21.8	9.50	35.5
VEL07	32.953861	17.925389	WC	Agri	71	0.03	101.60	2178.22	4712.4	78	67	7.71	8.433	18.6	2.67	41.5
VEL08	32.953639	17.932194	WC	Agri	61	0.06	167.13	24.63	2513.3	120	71	9.42	45.333	19.4	10.33	12.0
VEL09	32.976722	17.960639	WC	Agri	69	13.92	903.47	5146.28	2356.2	40	12	7.63	0.758	21.4	1.63	21.5
VEL10	32.970611	17.952917	WC	Agri	60	37.89	405.86	176.46	471.2	57	37	8.14	0.743	20.0	6.00	73.5
YZE01	33.341111	18.184833	WC	Other	51	0.12	1.67	8.21	3180.9	150	5	9.69	10.773	22.1	15.47	4.0
YZE02	33.340417	18.183722	WC	Ref	44	0.18	256.12	73.27	518.4	30	4	8.42	9.190	23.3	6.50	34.0
YZE03	33.336667	18.242389	WC	Other	56	0.21	0.53	18.37	5026.5	120	75	4.69	4.207	21.6	8.53	16.0
YZE04	33.403750	18.279250	WC	Other	63	4.39	175.36	76.22	1256.6	34	63	7.93	8.100	21.5	4.97	5.0
YZE05	33.403472	18.279639	WC	Other	64	1.71	68.31	58.76	1963.5	28	63	8.27	7.847	20.9	6.77	6.7
MFU01	34.012417	18.681306	CF	Urb	66	0.04	10.75	42.98	3141.6	55	29	7.51	1.146	20.0	3.07	1.0
MFU02	34.009028	18.680556	CF	Urb	69	0.04	1.69	5.61	1256.6	45	28	8.19	0.869	23.0	7.33	2.0
MFU03	34.009639	18.678528	CF	Urb	69	0.06	0.00	10.64	17867.8	64	18	8.23	1.437	22.0	7.33	2.0
BAD01	34.037917	18.725000	CF	Urb	66	2.57	13.67	33.44	1256.6	45	11	7.86	0.429	15.1	5.07	18.6
BAD02	34.036278	18.725333	CF	Urb	74	0.88	9.38	26.00	471.2	25	11	8.57	0.258	19.4	10.03	2.1
BAD03	34.041389	18.724111	CF	Urb	66	57.00	26.06	50.33	3141.6	35	9	7.94	2.730	17.5	8.27	1.1
BAD04	34.037389	18.722583	CF	Urb	69	0.12	3.96	15.85	1256.6	45	12	7.81	3.110	15.7	8.30	2.1
DRE01	34.030806	18.724889	CF	Urb	60	3.95	4.04	19.63	62831.9	45	13	7.40	0.222	13.9	7.70	4.1
DRE02	34.034583	18.721556	CF	Urb	61	0.71	4.46	2.24	1885.0	80	11	7.60	0.840	14.8	7.07	44.2
DRE03	34.036417	18.721444	CF	Urb	50	2.39	166.28	68.35	942.5	80	12	7.93	1.158	15.5	6.40	1.8
LOT01	34.058139	18.504556	CF	Urb	63	13.74	5.46	40.80	3010.0	48	10	7.43	1.178	11.2	4.90	1.5

Site code	Latitude (Decimal degrees)	Longitude (Decimal degrees)	Region	Impact category	HDS (%)	$NO_3 + NO_2$ (µg/L)	PO_4 (µg/L)	NH_4 (µg/L)	TSA (m^2)	Depth (cm)	Altitude (m)	pH	Conductivity (mS/cm)	Temperature (°C)	Oxygen (mg/L)	Turbidity (NTU)
LOT02	34.058500	18.503500	CF	Urb	54	12.14	9.45	19.32	13435.0	150	7	7.63	1.362	12.9	6.97	1.6
LOT03	34.058056	18.500139	CF	Urb	59	60.67	34.39	109.57	4519.0	150	7	7.33	1.311	16.0	5.97	12.0
LOT04	34.053972	18.505250	CF	Urb	74	5.87	121.89	14.58	8652.0	48	9	7.66	0.958	15.5	4.00	2.8
LOT05	34.048667	18.510417	CF	Urb	77	2.08	73.89	33.48	8234.0	120	13	7.72	0.911	16.2	3.97	2.0
LOT06	34.038111	18.535639	CF	Urb	67	8241.59	1276.73	1087.33	18153.0	70	18	7.79	1.097	14.7	9.30	3.4
LOT07	34.040056	18.534000	CF	Urb	67	801.02	1407.49	40.53	18153.0	150	18	7.95	1.048	15.9	7.47	2.6
LOT08	34.027389	18.539722	CF	Urb	69	602.28	440.30	24.68	2151.0	83	19	8.00	0.986	13.7	8.60	2.0
LOT09	34.067472	18.494972	CF	Ref	36	1.64	6.36	17.64	8488.0	180	6	8.12	4.023	13.5	8.40	0.9
LOT10	34.071139	18.498278	CF	Ref	21	4.47	2.72	1.47	3000.0	150	7	7.62	0.890	13.4	8.13	0.4
LOT11	34.069556	18.497972	CF	Ref	24	1.72	15.59	21.14	5454.0	150	8	8.31	2.707	14.2	8.60	1.0
KHA01	34.048972	18.717000	CF	Urb	70	3.27	7.76	14.96	1256.6	45	14	8.17	1.100	18.1	9.63	1.4
KHA02	34.045500	18.722583	CF	Urb	67	2.77	37.06	38.51	3927.0	45	9	8.37	0.863	16.4	11.33	4.6
KHA03	34.043528	18.724833	CF	Urb	70	0.94	76.59	31.35	1731.0	80	10	7.63	1.026	15.9	8.57	6.1
MEW01	34.004417	18.643472	CF	Urb	74	0.13	2.10	15.52	2356.2	45	37	8.02	0.948	17.2	6.90	1.3
KEN01	33.999500	18.485417	CF	Urb	50	44.19	8.57	33.23	611.0	28	25	6.68	0.239	11.9	3.70	5.3
KEN02	33.998361	18.485722	CF	Urb	66	5.30	45.79	39.47	298.0	50	24	6.78	0.231	11.4	3.15	3.8
KEN03	33.998111	18.487250	CF	Ref	40	2.12	5.42	19.46	5107.0	30	23	6.55	0.169	12.3	5.00	1.4
KEN04	33.999972	18.486222	CF	Urb	50	0.43	20.97	23.64	543.0	52	25	6.87	0.345	11.3	3.97	3.7
KEN05	34.000361	18.483556	CF	Ref	46	4.55	34.52	42.10	651.0	120	28	6.70	0.281	11.8	5.03	3.4
KEN06	33.998750	18.482000	CF	Ref	36	0.08	1.55	7.09	436.0	18	27	6.61	0.463	12.9	5.80	1.7
KEN07	33.997806	18.482194	CF	Ref	31	6.82	1.55	12.72	214.0	30	29	4.33	0.229	13.6	4.63	1.1
KEN08	33.997111	18.482694	CF	Ref	36	3.28	6.57	11.64	738.0	27	27	4.07	0.141	15.1	6.83	0.9
KEN09	33.996278	18.482111	CF	Ref	34	2.17	3.10	10.23	589.0	50	29	6.82	0.450	15.3	6.87	1.6
KEN10	33.995944	18.483833	CF	Ref	33	13.07	1.66	23.04	1542.0	87	24	4.37	0.234	16.5	5.73	2.0
KEN11	33.994222	18.483556	CF	Ref	36	0.67	2.90	15.26	5631.0	100	27	6.44	0.184	15.1	6.17	1.0
KEN12	33.994472	18.484750	CF	Ref	33	10.18	2.96	21.27	426.0	39	25	4.56	0.216	16.4	8.30	1.0
KEN13	33.996389	18.484833	CF	Ref	37	37.48	1.50	23.57	233.0	62	26	4.48	0.192	12.1	5.20	1.4
KEN14	33.992583	18.487306	CF	Urb	63	6.10	4.51	62.37	486.0	49	26	6.74	0.579	16.2	5.83	2.7
KEN15	33.993194	18.483750	CF	Urb	47	1.01	11.58	34.55	800.0	30	26	7.45	0.764	17.4	6.77	1.0
KEN16	33.993167	18.483222	CF	Urb	51	1.47	86.17	22.24	1081.0	40	28	6.98	0.294	15.5	2.53	0.7
KEN17	34.003833	18.487472	CF	Urb	60	1.33	7.14	11.70	721.0	40	26	8.31	0.114	19.2	11.07	1.5
KEN18	34.004361	18.486806	CF	Urb	61	0.55	75.52	5.50	4355.0	200	26	7.29	0.422	16.9	5.56	1.1
KEN19	34.006000	18.487806	CF	Urb	64	2.29	6.86	15.09	1885.0	200	26	6.72	0.177	16.6	5.15	1.2
DRI01	34.012278	18.663389	CF	Urb	56	5.71	5.13	23.20	1963.5	150	32	8.49	3.840	19.2	9.33	1.5
DRI02	34.011889	18.664278	CF	Urb	63	3.16	14.10	34.33	589.0	50	34	8.11	1.740	18.4	5.53	2.0
DRI03	34.012944	18.664389	CF	Urb	57	0.56	2.81	20.06	3927.0	150	32	9.01	5.093	22.5	10.43	1.5

Site code	Latitude (Decimal degrees)	Longitude (Decimal degrees)	Region	Impact category	HDS (%)	$NO_3 + NO_2$ (µg/L)	PO_4 (µg/L)	NH_4 (µg/L)	TSA (m^2)	Depth (cm)	Altitude (m)	pH	Conductivity (mS/cm)	Temperature (°C)	Oxygen (mg/L)	Turbidity (NTU)
DRI04	34.013861	18.665583	CF	Urb	56	1.18	20.69	18.36	3534.3	150	35	8.60	6.543	23.3	11.00	2.0
DRI05	34.011889	18.667472	CF	Urb	47	2.33	13.71	96.93	471.2	20	29	8.19	3.553	23.0	5.93	5.5
DRI06	33.984528	18.660611	CF	Ref	43	1.82	7.91	15.30	7854.0	75	38	8.34	3.803	18.9	2.37	1.0
DRI07	33.988889	18.659333	CF	Ref	41	2.28	6.97	27.18	23561.9	80	33	8.06	2.373	21.1	2.00	5.5
AGU01	34.740500	19.679417	AGU	Ref	36	18.50	9.62	73.75	1071.3	63	3	6.71	0.876	15.0	6.53	2.5
AGU02	34.740722	19.678278	AGU	Ref	39	18.54	2.94	41.63	1649.3	70	6	7.74	0.883	16.0	10.23	1.5
AGU03	34.738694	19.640667	AGU	Other	43	5.53	6.34	35.64	392.7	14	5	7.78	2.560	24.9	9.37	2.5
AGU04	34.739667	19.732472	AGU	Ref	40	8.91	9.42	40.99	14137.2	17	7	8.33	2.787	23.7	8.80	12.5
AGU05	34.738250	19.737028	AGU	Ref	29	0.00	1.37	29.77	8136.7	20	6	9.63	48.533	22.3	10.03	45.5
AGU06	34.725667	19.733389	AGU	Ref	36	0.00	11.16	38.35	17592.9	10	13	9.74	13.460	20.5	8.63	5.0
AGU07	34.698056	19.720167	AGU	Agri	53	1.86	11.20	23.40	1963.5	22	100	8.10	0.826	18.8	9.60	9.5
AGU08	34.697000	19.720667	AGU	Agri	50	0.00	3.39	16.41	589.0	42	100	7.22	0.825	18.8	7.87	3.0
AGU09	34.697694	19.727611	AGU	Agri	44	6.73	3.69	21.66	68722.3	70	92	7.05	1.832	21.8	6.77	2.0
AGU10	34.724083	19.751750	AGU	Ref	21	0.00	1.15	13.44	326725.6	70	26	8.71	11.840	23.0	9.07	56.0
AGU11	34.721389	19.756639	AGU	Ref	23	1.99	4.11	8.57	17671.5	80	26	8.80	1.786	22.4	10.33	4.0
AGU12	34.752639	19.801694	AGU	Other	60	0.00	6.36	0.23	1570.8	56	14	8.06	3.093	24.7	8.90	1.0
AGU13	34.641639	19.831361	AGU	Agri	53	3.97	5.23	21.82	1570.8	30	15	7.71	2.573	21.4	8.00	16.5
AGU14	34.602528	19.951361	AGU	Agri	43	0.00	1.83	14.13	3141.6	40	26	9.61	8.053	23.4	9.97	8.0
AGU15	34.595417	19.958917	AGU	Agri	51	0.00	18.80	19.97	7539.8	15	30	10.12	17.880	28.6	12.53	3.5
AGU16	34.642278	19.905333	AGU	Agri	51	0.00	1.25	11.39	88357.3	65	4	9.91	9.130	28.3	11.67	3.5
AGU17	34.676750	19.903694	AGU	Agri	46	0.00	2.03	37.50	1570.8	67	6	9.95	36.967	27.8	10.40	2.5
AGU18	34.682417	19.900556	AGU	Agri	54	10.59	4.74	35.42	3927.0	47	9	8.06	1.867	27.6	6.90	2.0
AGU19	34.670444	19.898139	AGU	Agri	54	0.58	3.57	25.51	314159.3	20	5	10.12	22.300	24.7	7.60	2.0
AGU20	34.750528	19.979361	AGU	Other	61	1.34	1.27	8.86	706.9	60	3	7.23	1.266	22.4	6.27	1.5
AGU21	34.762861	19.906417	AGU	Other	54	0.22	2.69	8.20	6283.2	40	15	9.62	38.000	22.6	11.10	8.5
AGU22	34.763556	19.901750	AGU	Ref	31	0.09	1.37	3.28	14137.2	60	12	8.97	10.783	22.4	10.33	5.5
AGU23	34.710806	19.930611	AGU	Agri	47	0.00	1.58	17.21	29452.4	28	1	8.52	3.143	21.0	10.10	12.0

HDS – human disturbance scores; **NTU** – nephelometric turbidity units; **TSA** = total surface area.

APPENDIX 4: AQUATIC MACRO-INVERTEBRATE TAXA SAMPLED FROM ISOLATED DEPRESSION WETLANDS IN THIS STUDY

Certain taxa could only be identified to family level. Chironomids were identified to the level of subfamily.

Table A4.1: Aquatic *macro-invertebrate* taxa sampled from seasonal isolated depression wetlands in this study

Order	Family	Genus (Subfamily for Chironomidae)	Species
Acarina	Arrenuridae	*Arrenurus*	*Arrenurus sp. B*
	Erythraeidae		
	Eylaidae	*Eylais*	*Eylais sp. A-B*
	Hydrachnidae	*Hydrachna*	*Hydrachna fissigera*
	Hydryphantidae	*Diplodontus*	*Diplodontus schuabi*
		Hydryphantes	*Hydryphantes parmalatus*
			Hydryphantes sp. A
		Mamersa	*Mamersa testudinata*
	Limnocharidae	*Limnochares*	*Limnochares crinita*
	Macrochelidae	*Macrocheles*	*Macrocheles sp. A*
	Oribatidae		
	Pionidae	*Piona*	*Piona sp. A*
	Trombidiidae		
	Unionicolidae	*Neumania*	*Neumania sp. A-B*
Amphipoda	Paramelitidae	*Paramelita*	*Paramelita capensis*
			Paramelita pinnicornis
			Paramelita sp. A
Anostraca	Streptocephalidae	*Streptocephalus*	*Streptocephalus dendyi*
			Streptocephalus purcelli
			Streptocephalus sp. A
Coleoptera	Dytiscidae	*Canthyporus*	*Canthyporus canthydroides*
			Canthyporus hottentottus
			Canthyporus sp. A-E
		Cybister	*Cybister sp. A*
		Darwinhydrus	*Darwinhydrus solidus*
		Herophydrus	*Herophydrus capensis*
		Hydaticus	*Hydaticus sp. A*
		Hydropeplus	*Hydropeplus sp. A-C*
			Hydropeplus trimaculatus
		Hyphydrus	*Hyphydrus soni*
			Hyphydrus sp. A
		Laccophilus	*Laccophilus cyclopis*
			Laccophilus sp. A
		Nebrioporus	*Nebrioporus capensis*
		Primospes	*Primospes sp. A*
			Primospes suturalis
		Rhantus	*Rhantus cicurius*
	Georissidae		
	Gyrinidae	*Aulonogyrus*	*Aulonogyrus capensis*
	Haliplidae	*Haliplus*	*Haliplus rufescens*
			Haliplus sp. A
Coleoptera	Hydraenidae	*Hydraena*	*Hydraena sp. A*
		Ochthebius	*Ochthebius extremus*

Order	Family	Genus (Subfamily for Chironomidae)		Species
Coleoptera	Hydraenidae	*Ochthebius*		*Ochthebius pedalis*
				Ochthebius spatulus
		Parasthetops		*Parasthetops nigritus*
		Parhydraena		*Parhydraena sp. A-B*
	Hydrophilidae	*Amphiops*		*Amphiops senegalensis*
		Anacaena		*Anacaena sp. A*
		Berosus		*Berosus sp. A-C*
		Crenitis		*Crenitis sp. A-C*
		Enochrus		*Enochrus continentalis*
				Enochrus picinus
				Enochrus sp. A-B
		Helochares		*Helochares sp. A-C*
		Laccobius		*Laccobius sp. A*
		Paracymus		*Paracymus sp. A-C*
		Regimbartia		*Regimbartia compressa*
	Scirtidae			
	Spercheidae	*Spercheus*		*Spercheus spp.*
Conchostraca	Leptestheriidae	*Leptestheriella*		*Leptestheriella rubidgei*
Diptera	Ceratopogonidae			
	Chaoboridae	*Chaoborus*		*Chaoborus microstictus*
	Chironomidae	Chironominae;	Orthocladinae;	
	Culicidae	*Aedes*		*Aedes spp.*
		Anopheles		*Anopheles coustani*
		Culex		*Culex spp.*
		Culiseta		*Culiseta spp.*
	Dixidae			
	Ephydridae			
	Muscidae			
	Stratiomyidae			
	Tipulidae			
Ephemeroptera	Baetidae	*Cloeon*		*Cloeon spp.*
Hemiptera	Belostomatidae	*Appasus*		*Appasus capensis*
	Corixidae	*Micronecta*		*Micronecta citharista*
		Sigara		*Sigara meridionalis*
				Sigara pectoralis
				Sigara wahlbergi
	Gerridae	*Gerris*		*Gerris swakopensis*
		Limnogonus		*Limnogonus capensis*
	Notonectidae	*Anisops*		*Anisops sardea*
Hemiptera	Notonectidae	*Anisops*		*Anisops sp. A*
		Notonecta		*Notonecta lactitans*
				Notonecta sp. A
	Pleidae	*Plea*		*Plea piccanina*
				Plea pullula
	Veliidae	*Mesovelia*		*Mesovelia vittigera*
Isopoda	Amphisopodidae	*Mesamphisopus*		*Mesamphisopus spp.*
Odonata	Aeshnidae	*Anax*		*Anax spp.*
	Coenagrionidae	*Enallagma*		*Enallagma spp.*
		Ischnura		*Ischnura spp.*
	Libellulidae	*Trithemis*		*Trithemis spp.*
Pulmonata	Ancylidae	*Ferrissia*		*Ferrissia sp. A*
	Helicidae	*Cochlicella*		*Cochlicella spp.*
	Lymnaeidae	*Lymnaea*		*Lymnaea columella*

Order	Family	Genus (Subfamily for Chironomidae)	Species
	Physidae	*Aplexa*	*Aplexa marmorata*
		Physa	*Physa acuta*
	Planorbidae	*Bulinus*	*Bulinus tropicus*
		Ceratophallus	*Ceratophallus natalensis*
Rhynchobdellida	Glossiphoniidae	*Batracobdelloides*	*Batracobdelloides*
Sorbeoconcha	Pomatiopsidae	*Tomichia*	*Tomichia spp.*
Trichoptera	Leptoceridae		

APPENDIX 5: AQUATIC MICRO-CRUSTACEAN TAXA SAMPLED FROM ISOLATED DEPRESSION WETLANDS IN THIS STUDY

Taxa were identified to genus or species level (with the exception of Chydoridae).

Table A5.1: List of aquatic *microcrustacean* taxa sampled from seasonal depression wetlands in this study

Class/Subclass	Order	Family	Genus	Species
Branchiopoda	Cladocera	Chydoridae		Chydoridae sp. A-C
		Daphniidae	*Ceriodaphnia*	*Ceriodaphnia producta*
			Daphnia	*Daphnia barbata*
				Daphnia dolichocephala
				Daphnia pulex/obtusa
			Megafenestra	*Megafenestra aurita*
			Scapholeberis	*Scapholeberis kingi*
			Simocephalus	*Simocephalus spp.*
		Macrothricidae	*Macrothrix*	*Macrothrix propinqua*
		Moinidae	*Moina*	*Moina brachiata*
				Moina sp. A
Copepoda	Calanoida	Diaptomidae	*Lovenula*	*Lovenula simplex*
			Metadiaptomus	*Metadiaptomus capensis*
				Metadiaptomus purcelli
			Paradiaptomus	*Paradiaptomus lamellatus*
				Paradiaptomus sp. A
	Cyclopoida	Cyclopidae	*Acanthocyclops*	*Acanthocyclops sp. A*
				Acanthocyclops vernalis
			Mesocyclops	*Mesocyclops major*
			Microcyclops	*Microcyclops crassipes*
	Harpacticoida	Ameiridae	*Nitocra*	*Nitocra dubia*
Ostracoda	Podocopida	Cyprididae	*Bradycypris*	*Bradycypris intumescens*
			Chrissia	*Chrissia sp. A-D*
			Cypretta	*Cypretta sp. A*
			Cypricercus	*Cypricercus episphaena*
				Cypricercus maculatus
			Heterocypris	*Heterocypris sp. A*
			Paracypretta	*Paracypretta acanthifera*
				Paracypretta sp. A
			Physocpria	*Physocpria capensis*
			Pseudocypris	*Pseudocypris acuta*
			Ramotha	*Ramotha capensis*
				Ramotha producta
				Ramotha trichota
			Zonocypris	*Zonocypris cordata*
				Zonocypris tuberosa
		Cypridopsidae	*Cypridopsis*	*Cypridopsis sp. A*
			Sarscypridopsis	*Sarscypridopsis sp. A-D*
		Limnocytheridae	*Gomphocythere*	*Gomphocythere sp. A*

APPENDIX 6: FULL LIST OF MACROINVERTEBRATE ATTRIBUTES USED FOR IBI TESTING

Table A6.1: Full list of macroinvertebrate attributes used for IBI testing

Attribute	Source
Sum total organisms	Gernes and Helgen (2002)
Sum intolerants ('AAA' - Acarina+Aeshnidae+Amphipoda)	Hicks and Nedeau (2000)
% Intolerants (AAA)	Hicks and Nedeau (2000)
Sum intolerants (All)	Using intolerant taxa defined by Hicks and Nedeau (2000)
% Intolerants (All)	Using intolerant taxa defined by Hicks and Nedeau (2000)
Tolerant Coleopterans	Using tolerant taxa defined by Hicks and Nedeau (2000)
Total Coleoptera	
Corixidae (as % of beetles and bugs)	Gernes and Helgen (2002)
% Dominant taxon	Gernes and Helgen (2002)
Sum Gastropods	
% Gastropods	
Total Hemipterans	
% Hemipterans	
% Dominant 3 taxa	Gernes and Helgen (2002)
Total number of families/taxa	Gernes and Helgen (2002)
Family Biotic Index (FBI)	Hicks and Nedeau (2000)
% Predators	Using Functional Feeding Guilds of Hicks and Nedeau (2000)
% Scrapers	Using Functional Feeding Guilds of Hicks and Nedeau (2000)
% Grazer-collectors	Using Functional Feeding Guilds of Hicks and Nedeau (2000)
% Omnivores	Using Functional Feeding Guilds of Hicks and Nedeau (2000)
% Shredders	Using Functional Feeding Guilds of Hicks and Nedeau (2000)

* All families were also tested as attributes in terms of their percentage contribution to total sample abundance

** All families were also tested as indicator taxa (see section 4.1.3.1)

*** Blank sources imply attributes were developed during this study

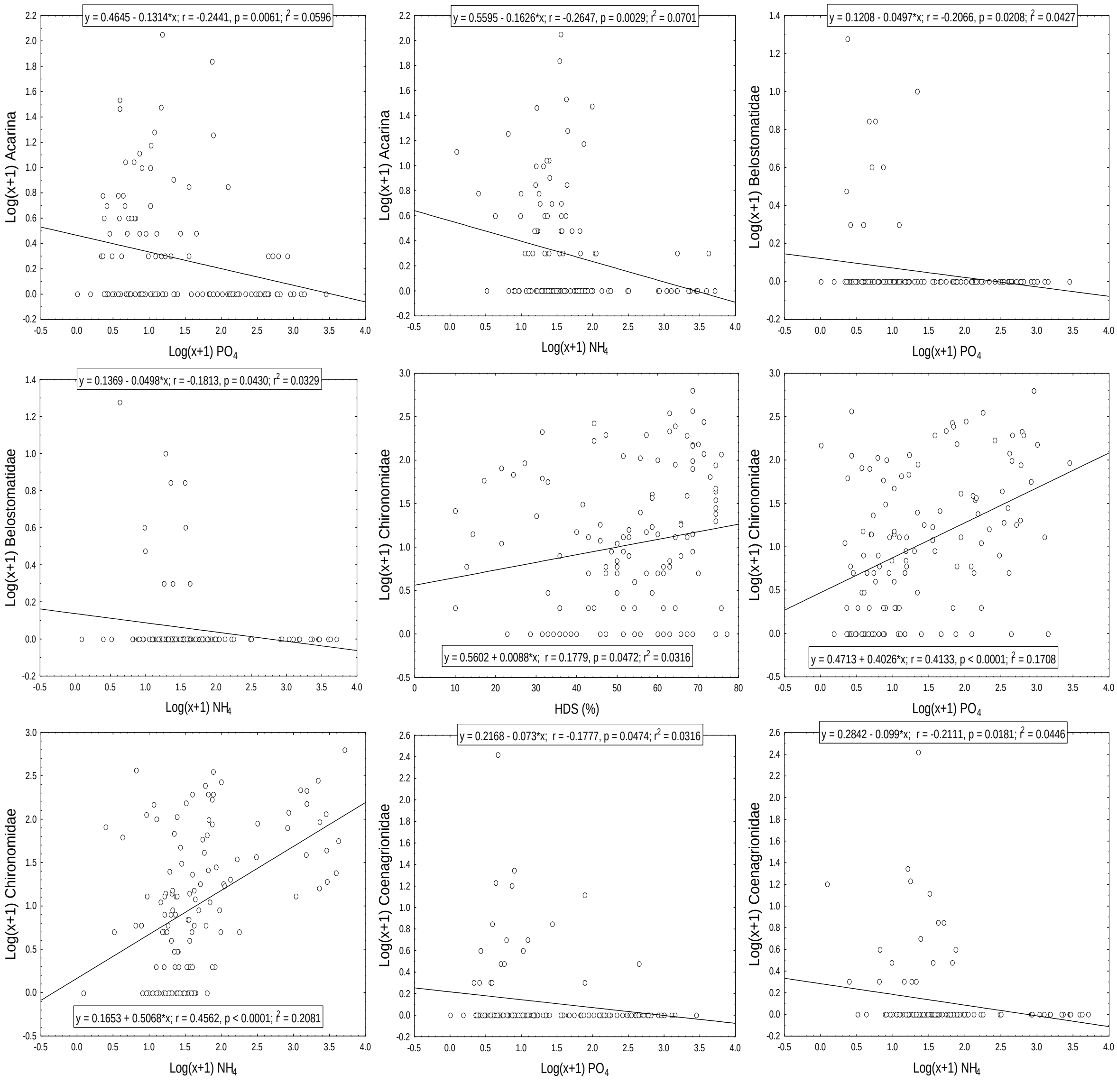
APPENDIX 7: SUMMARY OF STUDY SITE INFORMATION FOR VALLEY BOTTOM WETLANDS SAMPLED IN THIS STUDY

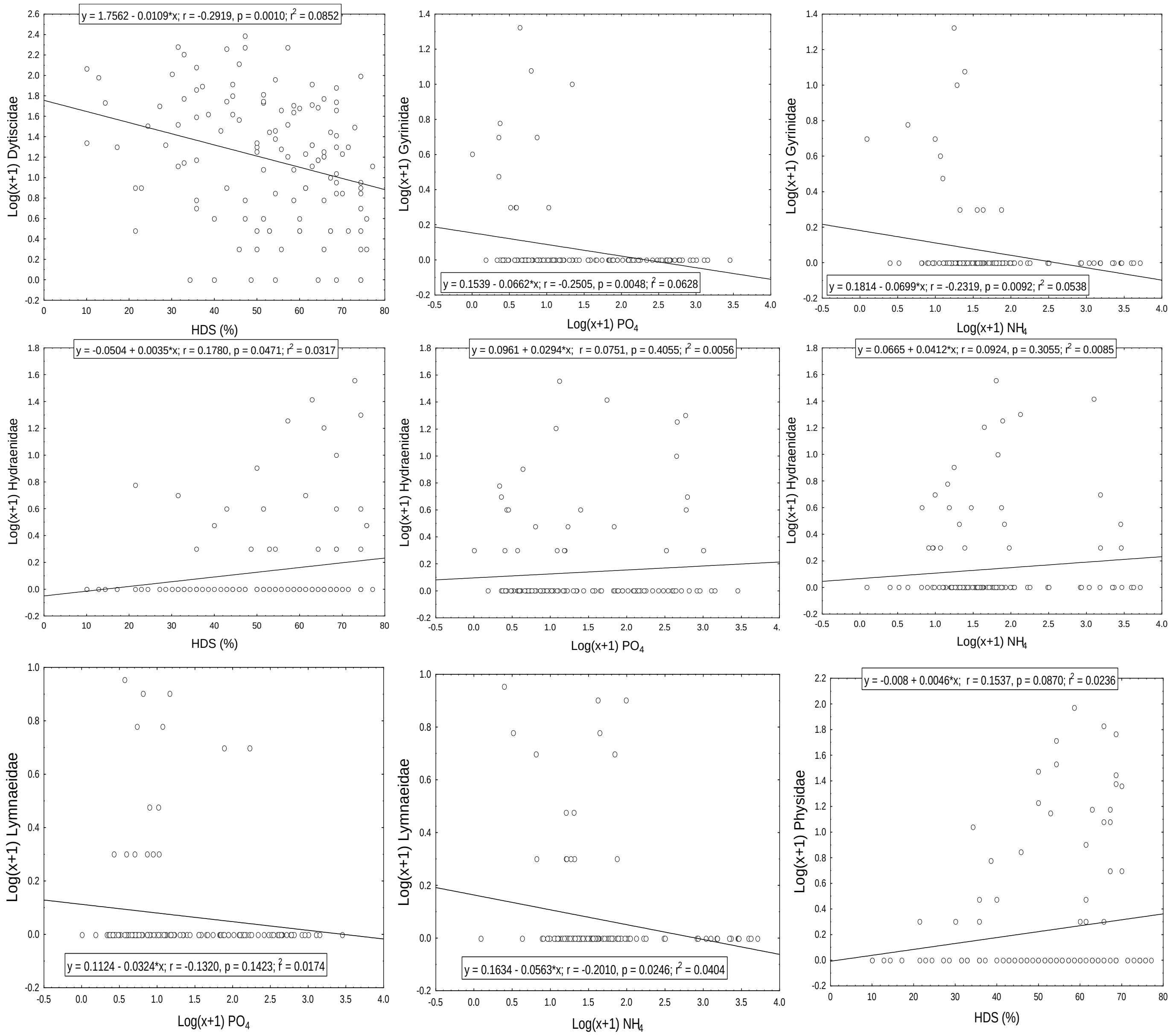
For sites where replicated samples were taken, ASPT scores, conductivity, temperature, pH and oxygen are presented as mean scores across replicates.

Table A7.1: Summary of study site information for valley bottom wetlands sampled in this study

Site	GPS co-ordinates	Impact category	NO_3+NO_2 category	PO_4 category	NH_4 category	NO_3+NO_2 (µg/L)	PO_4 (µg/L)	NH_4 (µg/L)	pH	SASS ASPT	Conductivity (mS/cm)	Temperature (°C)	Oxygen (mg/L)
VB 01	S32 46.034 E18 49.337	impacted landscape	low	medium	low	8.77	7.67	1.35	7.15	3.50	0.439	23.9	6.31
VB 02	S33 10.157 E18 40.863	impacted landscape	low	low	medium	4.72	5.75	26.56	8.04	4.57	9.860	22.4	6.32
VB 03	S33 09.652 E18 40.437	impacted landscape	high	low	high	332.54	4.79	59.64	8.35	3.75	9.210	23.2	7.92
VB 04	S33 08.625 E18 39.691	impacted landscape	high	high	high	581.43	64.24	172.11	7.83	3.44	6.290	24.0	8.50
VB 05	S33 31.634 E18 36.035	impacted landscape	medium	high	high	37.89	67.11	399.93	7.51	3.83	1.122	29.5	1.42
VB 06	S33 33.300 E18 36.942	impacted landscape	high	high	high	10577.57	45.06	72.02	7.81	4.25	0.914	25.9	1.61
VB 07	S33 34.385 E18 37.085	impacted landscape	high	high	medium	9585.36	50.81	40.42	7.97	3.56	0.916	26.2	1.98
VB 08	S34 09.360 E18 25.166	impacted landscape	medium	medium	medium	166.97	16.30	48.26	8.02	3.00	0.704	18.4	4.07
VB 09	S34 09.380 E18 25.520	impacted landscape	high	low	medium	171.53	0.00	31.77	7.75	3.67	0.722	19.5	3.52
VB 10	S34 12.131 E18 22.459	nature reserve	medium	low	low	12.95	5.75	21.33	6.17	6.25	0.480	23.5	3.76
VB 11	S34 14.422 E18 25.355	nature reserve	medium	high	high	80.56	215.72	54.27	4.55	5.50	0.468	17.7	10.30
VB 12	S34 19.610 E18 50.878	nature reserve	low	medium	low	4.72	6.71	5.09	5.76	3.00	0.105	19.3	7.18
VB 13	S34 19.581 E18 50.368	nature reserve	low	low	low	10.01	4.79	17.09	4.49	5.38	0.195	18.1	6.72
VB 14	S34 19.352 E18 57.980	nature reserve	medium	medium	medium	11.98	7.67	22.31	4.55	6.00	0.097	20.6	3.44
VB 15	S34 20.356 E18 50.579	nature reserve	low	medium	low	6.60	6.71	9.85	6.04	4.50	0.124	18.3	7.58

APPENDIX 8: ABUNDANCE DISTRIBUTIONS OF THE POTENTIAL MACROINVERTEBRATE INDICATOR FAMILIES IN RELATION TO HUMAN DISTURBANCE FACTORS




y = 1.7562 - 0.0109*x; r = -0.2919, p = 0.0010; r2 = 0.0852
Log(x+1) Dytiscidae
HDS (%)
y = 0.1539 - 0.0662*x; r = -0.2505, p = 0.0048; r2 = 0.0628
Log(x+1) Gyrinidae
Log(x+1) PO4
y = 0.1814 - 0.0699*x; r = -0.2319, p = 0.0092; r2 = 0.0538
Log(x+1) Gyrinidae
Log(x+1) NH4
y = -0.0504 + 0.0035*x; r = 0.1780, p = 0.0471; r2 = 0.0317
Log(x+1) Hydraenidae
HDS (%)
y = 0.0961 + 0.0294*x; r = 0.0751, p = 0.4055; r2 = 0.0056
Log(x+1) Hydraenidae
Log(x+1) PO4
y = 0.0665 + 0.0412*x; r = 0.0924, p = 0.3055; r2 = 0.0085
Log(x+1) Hydraenidae
Log(x+1) NH4
y = 0.1124 - 0.0324*x; r = -0.1320, p = 0.1423; r2 = 0.0174
Log(x+1) Lymnaeidae
Log(x+1) PO4
y = 0.1634 - 0.0563*x; r = -0.2010, p = 0.0246; r2 = 0.0404
Log(x+1) Lymnaeidae
Log(x+1) NH4
y = -0.008 + 0.0046*x; r = 0.1537, p = 0.0870; r2 = 0.0236
Log(x+1) Physidae
HDS (%)

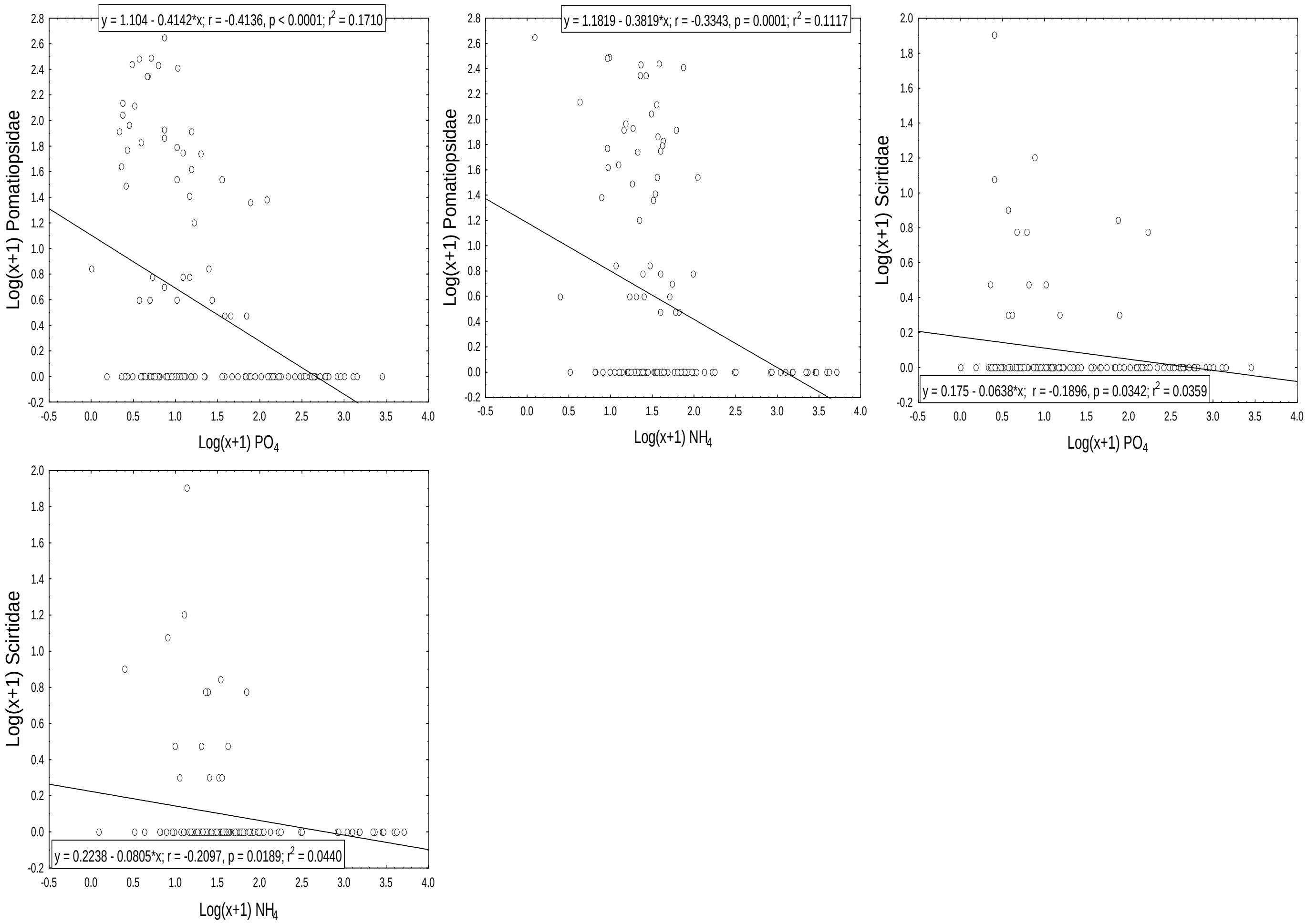


Figure A8.1: Abundance distributions of the potential macroinvertebrate indicator families in relation to human disturbance factors. Logged abundances are in no/m^3 and nutrient values in µg/L. Regression equations, Pearson correlation coefficients (r), coefficients of determination (r^2) and significance values (p) are provided.