Wetland Health and Importance Research Programme

4

Development of a Tool for Assessment of the Environmental Condition of Wetlands Using Macrophytes

Author: F Corry

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WETLAND HEALTH AND IMPORTANCE RESEARCH PROGRAMME

4 DEVELOPMENT OF A TOOL FOR ASSESSMENT OF THE ENVIRONMENTAL CONDITION OF WETLANDS USING MACROPHYTES

Report to the
Water Research Commission

by

Author: F Corry

Editor: B Day

Series Editor: H Malan

Freshwater Research Unit,
University of Cape Town

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OBTAINABLE FROM

Water Research Commission
Private Bag X03
Gezina
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Front Cover: ***Nymphaea nouchalia var. caerulea*** **(Ratels Vlei – Agulhas Plain); Background Phillipi Dune Slac Wetland – Cape Flats**
Photograph: **F. Corry**

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 E. Day, J. 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).

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This work was funded by the Water Research Commission, to whom I extend every thanks for providing the opportunity to gain experience in freshwater ecological research. To the steering committee for this project I would like to extend my gratitude for your patience, input and feedback.

What would the world be, once bereft of wet and wildness? Let them be left, o let them be left, wildness and wet, long live the weeds and the wilderness yet.

from INVERSNAID *by*
Gerald Manley Hopkins

ABBREVIATIONS

BAWWG – Biological Assessment of Wetlands Working Group

CD – Compact Disc

CEC – Cation Exchange Capacity

CLF – Cape Lowland Freshwater vegetation unit (Mucina *et al.* 2006a)

DoA – Department of Agriculture

DWAF – (formerly) Department of Water Affairs and Forestry

FRU – Freshwater Research Unit

HDS – Human Disturbance Score

HDTS – Human Disturbance and Trophic State category

HGM – Hydrogeomorphic

IBI – Index of Biotic Integrity

ICP-OES – inductively coupled plasma-optical emission spectrometer

NDA – National Department of Agriculture

SANBI – South African National Biodiversity Institute

SASS – South African Scoring System

SOM – soil organic matter

UCT – University of Cape Town

VEGRAI – Riparian Vegetation Response Assessment Index

WHI – Wetland Health and Importance (Research Programme)

WIHI – Wetland Index of Habitat Integrity

WRC – Water Research Commission

EXECUTIVE SUMMARY

RATIONALE

Wetlands are recognized as being important ecosystems that not only supply services essential to maintaining biodiversity but also provide resources directly utilized by humans. The plants that grow within wetlands exist as a result of the environmental stresses and opportunities within the habitat in which they occur and therefore aspects of the vegetation have proven useful as indicators of the present environmental state of wetlands (e.g. Whittaker 1962, Karr and Dudley 1981, Karr 1987, Mack 2001, US EPA 2002a). An indication of the environmental condition of a given wetland can therefore be provided by a comparison of the species composition, diversity and functional organisation of plants within that wetland, relative to those of minimally disturbed wetlands within the same region (*sensu* Karr and Dudley 1981). Such a means of assessing the environmental condition of ecosystems using biological assemblages is known as "bioassessment", and the use of plants for this purpose suggests the term "phyto-assessment".

In the often arid and semi-arid climatic regions of South Africa, an Index of wetland environmental condition based on plants has an advantage over aquatic invertebrate bioassessment techniques, in that it can be applied to environments where there is no surface water. To date, no comprehensive phyto-assessment of wetland condition has been developed in South Africa.

STUDY APPROACH

A literature review revealed a dearth of baseline information about the links between South African wetland vegetation and the environmental conditions with which they are associated and within which they occur. A compilation of South African wetland plants (and associated ecological information), as originally prepared from herbarium data by Glen *et al.* 1999 and later updated (Glen *et al.* unpublished), was reviewed and updated in the course of this investigation (see attached CD). From an international perspective, the ecology of wetland plants is considered to be relatively well understood in comparison to other biotic assemblages or communities inhabiting wetlands (Adamus *et al.* 2001, DWAF 2004). In South Africa, however, there is a lack of fundamental ecological information on wetland plants. This makes it difficult to recognise indicator species or other vegetation attributes that could be used to distinguish between, for example, disturbed and minimally disturbed wetland habitat. In this study, therefore, (field-based) methods were developed and used in order to determine the attributes of wetland plant assemblages that best indicate the environmental condition of wetlands.

The use of plants for assessing the environmental condition (i.e. detecting anthropogenic impacts) of wetlands in North America suggests that those attributes of plant communities that indicate differences in, or a change in environmental conditions are often unique to each distinct type of wetland habitat (US EPA 2002c). A combination of climate, geology and hydrology determine the differences between wetland habitat types and hence different regions within a country, which then may have naturally different wetland vegetation communities. The search for attributes of vegetation that reflect changes in environmental conditions can thus be enhanced by studying the separation of natural wetland vegetation into different units. Vegetation units in natural ecosystems may be identified by differences in their adaptive strategies to local environments (functional groups); differences in structure (plant-architecture) and according to the climatic and geological regions (e.g. biogeographical regions) in which they occur, as well as differences in nutrient load, substrate class and hydrology.

In this project a framework is therefore proposed for the identification of different units of wetland habitat type and of different units of wetland vegetation within which the vegetation of minimally disturbed and disturbed wetlands may be critically compared. This framework was based on a combination of the National Wetland Classification Scheme (SANBI 2009), the wetland vegetation units identified in the Vegetation Map of South Africa (Mucina *et al.* 2006a), and the distinction between aquatic, littoral (shoreline) and supralittoral vegetation habitats.

The coastal lowlands of the Western Cape have been identified as a broadly similar phytogeographical region (e.g. Cowan 1995, Kleynhans *et al.* 2005). It is thought that freshwater, inland wetlands with similar hydrogeomorphic character occur amongst the suite of wetlands found in the region (Ewart-Smith *et al.* 2006, SANBI 2009). It has further been suggested that these wetlands contain similar wetland vegetation (Mucina *et* *al.* 2006a). This report therefore explores the potential of using wetland plants for assessing the environmental condition of a specific type of wetland, namely, inland (i.e. no marine influence), freshwater, isolated, depressional wetlands of the coastal lowlands of the Fynbos Biome in the Western Cape. The Cape Lowland Freshwater (CLF) wetland vegetation unit (AZf1: Mucina *et al.* 2006a) dominates many of the non-riverine wetlands of the Cape coastal lowlands. At the outset of this study this wetland vegetation type was considered to have some uniformity and, when mapped, was found broadly distributed across the Cape coastal lowlands (Mucina *et al.* 2006a). The Cape Lowland Freshwater vegetation has some important taxa with widespread distribution within South Africa as well as a number of species with cosmopolitan distribution in analogous habitats (Mucina *et al.* 2006a). The typical landscape features in which this unit of vegetation is found, (flats, seeps and landscape depressions), are also typical of the Eastern Temperate and Subtropical Freshwater Wetland vegetation types found in the Grassland, Savanna, Albany Thicket and Indian Ocean Coastal Belt Biomes (Mucina *et al.* 2006a). The Cape Lowland Freshwater vegetation unit was therefore chosen with the intention of finding information that could be widely applicable to the development and testing of bioassessment in other regions and wetland vegetation types of South Africa.

The determination of vegetation attributes indicative of environmental condition (known as metrics) within units of similar vegetation and wetland habitat, was governed by a protocol developed from a review of the published methods for vegetation assessment (for details see Chapter 2). Internationally, methods for the bioassessment of wetlands are best developed in the USA, where there has been more than 30 years of research in the field. The United States Environmental Protection Agency has published a series of reports on “Methods for Evaluating Wetland Condition” (See US EPA 2002a-2002e). The protocol for the development of metrics suggested by the US EPA (2002c) follows the example of Karr and Dudley (1981) in comparing biological assemblages from disturbed and minimally disturbed wetlands from within a given biogeographical region.

Changes in the abiotic or biotic factors controlling wetland ecosystem functioning can, however, be caused by both natural and unnatural (human) derived stressors. It is specifically the human-derived causes of disturbance that are of importance in this study. The focus of bioassessment is to distinguish between anthropogenically disturbed wetlands and natural wetlands that are minimally impaired by human influence. The ranking of disturbance is therefore based on a human disturbance score determined from the extent and intensity of land-use within and immediately surrounding wetlands (Section 3.5.4). Wetlands were categorized *a priori,* within the study set, as “Reference”, “Moderate” or “Worst” in terms of their environmental condition, depending on the amount of human land-use and disturbance at each wetland.

In the USA, a linear correlational approach was used for comparing the response of vegetation attributes to an assumed single and unidirectional gradient of disturbance within a single unit of wetland vegetation habitat. This approach was not considered feasible in the context of the wetlands studied in the present project. This is due to the lack of clarity about comparable wetland vegetation habitat units, and the consideration that there are multiple stressors impacting wetlands that are potentially synergistic, antagonistic or additive in terms of their impact on the habitat and with regard to the response of plants. A multivariate approach to analysis was therefore adopted.

Within three separate sub-regions of the Cape coastal lowlands, namely the West Coast, the Cape Flats and the Overberg, wetlands were sampled at a number of localities where they were abundant. The spatial hierarchy of this sampling design is presented Table 1.

Table 1: Spatial hierarchy of sampling design within the Western Coastal Slopes wetlands region of the Cape coastal lowlands (n = 60).

Sub-region	Localities in which wetlands sampled	Conditions
West Coast	Darling, Berg River floodplain, Verlorevlei	Disturbed and minimally disturbed
Cape Flats	Kenilworth, Lotus River and Kuils River floodplains	Disturbed and minimally disturbed
Overberg	Hermanus, Ratels River floodplain, Waskraalvlei	Disturbed and minimally disturbed

An extensive array of information was collated for each wetland including the assessment of human disturbance and general data (see Appendices 1 [Human Disturbance] and 2 [General Datasheet]). The general data included information such as geographical position, wetland classification type (Ewart-Smith *et al.* 2006), as well as geo-physical and hydrological characteristics as recorded by observation and measurement on site. Samples of soil and in some instances, water, were taken at most of the wetlands in order to facilitate quantitative analysis of the soil and water chemistry and thus determine nutrient loads (Sections 3.5.5 and 3.5.6). An inventory of all observed plant species in each wetland was collated (Section 3.5.8.1 and Appendices 3).

The vegetation sampling protocol adopted in the USA for wetland phyto-assessment, is based predominantly on multiple adjacent quadrats within the emergent vegetation typical of the littoral hydrological zone of a wetland. The lack of baseline vegetation data available for wetlands in South African and the heterogeneity of vegetation habitat apparent between aquatic, littoral and supralittoral hydrological zones suggested that a sampling method that was best able to capture the full variation of this heterogeneity was important. In the naturally arid conditions that exist in South Africa, a large number of wetlands are represented by ephemeral, or seasonally saturated habitat in the supralittoral hydrological zone of vegetation. The recognition and sampling of this hydrological zone and habitat types were therefore important in the present study.

Within every homogeneous stand of vegetation considered characteristic of a hydrological zone, a 2 x 2 meter quadrat was sampled to determine the cover and abundance of each plant species (Section 3.5.8.2 and Appendix 4). For a given wetland, each of these relevés (quadrats) represented a characteristic stand of vegetation that could potentially provide information for determining the species representative of the ambient environmental conditions within that stand. Combining the data from these relevés provides an understanding of the vegetation community for each hydrological zone and ultimately for each wetland.

The US EPA (2002c) recommends measuring the nutrient load of the sediments and/or water-column within a wetland by combining soil or water samples from three separate locations within the wetland. In the present study, water-column samples were combined from a number of sites within each wetland. Greater accuracy in determining the nutrient range associated with a given species can be obtained by the more standard botanical approach of taking a sediment sample from each vegetation sample plot (Kent and Coker 1992). This standard botanical approach was adopted in the present study in order to maximise the information gained.

MAJOR FINDINGS

Interrogation of the inter-wetland similarity of plant communities of all the wetland study sites revealed:

1. No difference in the vegetation of disturbed relative to minimally disturbed (reference) wetlands when assessed across all Cape coastal lowland study sites (Section 7.1); however,
2. The existence of different groups of vegetation suggests considerable differences between the wetland vegetation of the West Coast, Cape Flats and Overberg sub-regions and also differences in the vegetation of each wetland within a sub-region (Section 4.2-4.4).
3. Examination of the environmental data (based on the measurement of ambient conditions at each wetland) likewise suggested considerable and significant differences in the ambient environmental conditions in every wetland within and between sub-regions (Section 4.5).

This information suggests that the freshwater-inland-isolated-depressional-wetlands (depressional wetlands) of the Cape coastal lowlands contain numerous sub-divisions with regard to their vegetation and natural environmental conditions. Each division should therefore be examined separately in order to determine differences in the vegetation attributes or community of disturbed, relative to minimally disturbed wetlands. This data could then be used in the development of metrics for phyto-assessment.

Note: "freshwater-inland-isolated-depressional-wetlands" will be referred to throughout this report as "depressional wetlands". This is merely for efficacy and the reader is asked to remember that they are, in fact, also freshwater, inland and isolated.

Examination of the vegetation in the different hydrological zones across the whole data set revealed considerable differences between the vegetation in the littoral and supralittoral habitats (Section 7.1.2). This suggests that, in addition to the bioassessment opportunities afforded by differences in wetland vegetation (e.g. community structure, species suites and attributes) due to locality, further differences may be recognised should wetland vegetation be separated into hydrological habitats.

Each of the different localities in which wetlands were sampled was representative of one or more geological substrate and its associated upland or terrestrial vegetation types (*sensu* Rebelo *et al.* 2006). Within localities at which the wetland was on a single geological substrate and surrounded by the same dryland vegetation type, there was considerable uniformity in terms of the wetland vegetation. In such situations, detection of different vegetation in disturbed, relative to minimally disturbed sites was possible. At localities in which wetlands were located on more than one geological substrate and terrestrial vegetation type, however, no such difference was detectable (Section 7.1.2).

One of the most important findings of this project was that, together with the wetland vegetation at each locality being different from that at every other locality, the wetland vegetation representative of different substrates differed within a single locality. This strongly suggests that the geographical distribution of wetland vegetation is determined by many of the same environmental parameters that determine the distribution of terrestrial vegetation. This is contrary to the popularly held hypothesis that the distribution of vegetation of wetlands is azonal; being determined more by hydrology and/or salt concentration than by the climatic and geological elements that determine the distribution of the zonal or terrestrial vegetation (Mucina *et al.* 2006).

The geographical land units used for separating different units of wetland vegetation, namely the wetland regions of Cowan (1995) as adopted by Mucina *et al.* (2006a) and the ecoregions of Kleynhans *et al.* (2005), as adopted for wetland classification by SANBI (2009) are at too course a scale for the purposes of phyto-assessment development. Within the Fynbos Biome the terrestrial vegetation units (i.e. South West Fynbos, Cape Flats Strandveld, etc.) of Rebelo *et al.* (2006: and see Section 3.4) provide a surrogate means of separating different units of wetland vegetation. The Cape Flats Strandveld terrestrial vegetation unit, however, instead of reflecting a single vegetation type, revealed distinctly different units of wetland vegetation when wetlands of the Lotus River floodplain were compared to the Kuils River floodplain. Elements of the terrestrial Fynbos vegetation (namely that the Lotus River wetlands appear to occur within two distinct terrestrial Fynbos vegetation units) do, however, suggest a reason for the difference exhibited between the wetland vegetation of these two localities (Section 4.4.2.ii).

At four localities, the sampled wetlands were located on a single geological substratum. Here, the differences that were discernable between disturbed and minimally disturbed wetlands allowed the identification of species and attributes of vegetation diversity (i.e. metrics) that have the potential to measure environmental condition for phyto-assessment purposes. Metrics developed from these four localities show a strong potential for their use in a multi-metric Index of Biological Integrity (IBI) for depressional-wetlands. Such metrics would, however, only be valid within localities similar to those in which the metrics were developed. Due to the considerable differences shown to exist across the different localities sampled in the Cape Lowland Freshwater vegetation unit, these metrics and the IBI are unlikely to be useful for the phyto-assessment of all wetlands across the entire coastal lowlands of the Western Cape.

SPECIES COVER AND VEGETATION DIVERSITY

Many plant species showed a difference in spatial cover between wetlands of different environmental condition. A few of those species, however, showed consistent and characteristic associations with either minimally disturbed, or disturbed conditions (Sections 7.2 and 7.3). The bulrush *Typha capensis,* the alien grass *Paspalum vaginatum,* and the sedge *Cyperus textilis* all showed consistent and characteristically greater spatial cover in wetlands that were disturbed than those that were minimally disturbed. For wetlands surrounded by Fynbos vegetation, the rush *Juncus capensis* showed the reverse trend, being associated more frequently and with greater cover/abundance in minimally disturbed, than in disturbed wetlands.

A number of attributes of the vegetation were consistently associated with different degrees of disturbance between the sub-regions (see Sections 7.2 and 7.4). Groups of species associated by life history (annuals versus. perennials), growth form (grasses, shrubs or woody species), origin (alien or indigenous) and affinity to the wetland habitat (obligate versus. facultative) were used to search for units of vegetation with consistent responses to disturbance. The following results were found:

- Annual taxa, alien grasses and grasses in general, showed greater affinity (occurring with greater cover) for disturbed than for minimally disturbed conditions.
- Indigenous woody shrubs and small leaved (sclerophyllous) shrubs both showed consistently greater cover in minimally disturbed, than disturbed relevés in Fynbos and Strandveld associated wetlands.
- Lastly, a greater number of obligate and facultative wetland taxa were consistently

associated with minimally disturbed than with disturbed wetlands.

A number of the above attributes have potential as metrics of environmental condition. Care needs to be taken, however, in order to avoid combining metrics that are collinear (measuring or indicating the same change between disturbed and minimally disturbed conditions). For instance, the combination of the percentage cover of alien grasses and of all grasses, or combining the number and the percentage cover of woody taxa, could effectively result in double-counting, as these attributes are collinear.

The grouping of species, based upon their means of acquiring resources, the hydrological habitat within which they are found, and their structural form or architecture, facilitated the development of 36 distinct groups (e.g. rhizomatous grasses with obligate wetland affinity; amphibious succulent herbs (for a full list, see Appendix 8)). These functional groups of plants were developed from field observation in the present study, with the intention of grouping species that would be easily categorised by non-specialists. Unfortunately these functional groups did not show consistent responses to differing degrees of disturbance. Within each functional group, the combination of species, each with different responses to disturbance, is mooted as the reason for their inconsistent response to disturbance. There has, however, been considerable success in the development of metrics from functional groups in North America, as evidenced by the literature on wetland environmental assessment. Further research into determining the responses of South African plant species to disturbance may assist in discovering functional groups with consistent responses to disturbance. Considerably more baseline data is, however, required before this objective can be attained.

TOLERANT OR SENSITIVE SPECIES

A number of plant species were associated only with reference (or minimally disturbed) conditions, whilst conversely, other species were associated only with disturbed conditions. In the development of metrics for phyto-assessment in North America, such species have been termed “sensitive to disturbance” and “tolerant of disturbance” (in short: sensitive and tolerant species) respectively. Some species, such as submerged or floating-leaved plants that are affected by increased competition for light due to shading of the water column, may be expected to be sensitive to disturbance impacts that affect light availability. Thus, increased turbidity or overshadowing by tall emergent plants should be expected to have an impact on this floral group. The ecological tolerances of species therefore predetermine their sensitivity to different types of disturbances. Very little ecological interpretation has been done of the wetland plants of South Africa, however (Cook 2004, Mucina *et al.* 2006a) and ecological tolerances of species are unknown. No single species in the present study occurred in more than four sample wetlands (either disturbed or undisturbed), and many more observations and a far greater sampling effort is required before such species can confidently and consistently be said to be tolerant or sensitive to disturbance. More baseline data on the ecological tolerances of wetland plants generally and/or the frequency of their association with different degrees of disturbance are needed before sensitive and tolerant taxa can be identified with the required degree of confidence to be used for the biomonitoring of South African wetlands.

METRICS

Of the species and/or vegetation attributes found to have consistent responses to different degrees of disturbance, a total of seven have been collated into an Index of Biological Integrity for use in the wetlands of the Cape coastal lowlands. These metrics, however, still require testing to determine whether or not they will consistently indicate the same conditions that they were found to indicate in the present study. The Cape Freshwater Lowland (CFL) vegetation sampled in the present study proved to have considerable natural divisions that prevented the testing of these metrics, and no other dataset with both vegetation and disturbance information is currently available upon which to test them. Field testing of the metrics is thus advised before they are used for phyto-assessment purposes.

CONCLUSION

The present study has generated considerable information that is useful for the development of a phyto-assessment methodology for South African wetlands. Our increased understanding of the ecology of the wetlands of the Western Cape has facilitated the development of a national spatial framework to identify naturally different units of wetland vegetation that may prove ideal for developing metrics for the phyto-assessment of any area of South Africa (Section 9.1). Furthermore, a methodology and protocol have been created for the sampling required for metric development, as well as the testing and subsequent application of IBI's for phyto-assessment purposes. These are presented in Section 9.2.

To enhance the development of phyto-assessment metrics in this country, our knowledge of wetland vegetation, its ecological tolerances and the natural conditions it represents needs to be expanded by gathering baseline data on the distribution and range of wetland plant species. The data collected in the present study, along with that of project WRC K5/1980 (Sieben 2010) go some way to fulfilling this need. With this baseline data and that already captured in the development of the databases of Plants of South Africa (POSA 2009) and the wetland data in the Vegetation Atlas (Mucina *et al.* 2006a), considerable analyses, interrogation and ecological interpretation are possible. The results of these studies will improve our understanding of the tolerance limits of species and their responses to disturbances, and thus ultimately, the identification of indicator species.

Phyto-assessment has considerable potential to assist with the management, monitoring and conservation of wetlands throughout Southern Africa. Much effort will, however, need to be made in order to develop metrics for each area that is comprised of homogeneous units of wetland vegetation. In combination with the wetland classification system (SANBI 2009), the use of the terrestrial vegetation units of South Africa (Rutherford and Mucina 2006), rather than ecoregions (Kleynhans *et al.* 2005), appears to hold the best potential to assist in the identification of units of land with homogeneous wetland vegetation. In the arid environment of South Africa, where wetlands are commonly dry during the dry season, a phyto-assessment technique that could be applied to wetlands that hold no surface water would be extremely useful. The identification of functional groups of plants that show consistent responses to disturbance, and whose species are identifiable by non-specialists, would greatly assist with broadening the applicability of such techniques.

ASSESSMENT OF THE ENVIRONMENTAL CONDITION OF WETLANDS USING PLANTS.

1. INTRODUCTION

1.1. The scope of this project

This document forms part of the output for the Water Research Commission (WRC) project K5/1584 "Wetland Health and Importance (WHI) Research Programme, Phase II of the National Wetland Research Programme". The programme lasted for four years, starting in 2006 and finishing at the end of 2009. A description of the aims, major findings and output of the WHI research programme may be found in Day and Malan (2010). The main aim of the project described in this volume was to develop tools for assessing the environmental condition of wetlands using plants.

This report explores the potential bioassessment value of wetland plants, known as "macrophytes", in palustrine wetlands of the coastal lowlands of the Western Cape (for definition of these terms see Section 1.4). For the remainder of this project, plant-based bioassessment will be referred to as phyto-assessment. This study establishes a protocol for assessing the attributes of wetland plant assemblages, such as the species composition and community structure that reflect the environmental condition of wetlands. Using such attributes, it is possible to develop phyto-assessment metrics and thereby indices that may be used to evaluate the environmental condition of wetlands using macrophytes.

The variability of wetlands both in terms of hydrogeomorphic [HGM] and biotic parameters means that each phyto-assessment metric may not be applicable to all wetlands or even to all hydrological zones within a given wetland type. Although the WHI Research Programme was directed towards palustrine wetlands, there is a natural gradation between riverine, palustrine, lacustrine, lagoonal and estuarine wetland systems. For example, some habitats found in palustrine wetlands (e.g. supralittoral hydrological zones (Section 2.9.3)) are also common to other types of wetlands (e.g. lacustrine and lagoonal wetland edge). Metrics developed for palustrine wetlands may thus have broader application to other wetland types.

Experience in the development of phyto-assessment indices in North American wetlands (US EPA 2002a), however, suggests that individual metrics and indices may need to be developed for each of the areas (bioregions or phytogeographical regions) in South Africa that have distinct types of wetland habitat, with concomitantly distinct or characteristic sets of wetland plant taxa. This important aspect is discussed further in the next section (1.2.1), and is reported on in the literature review (Chapter 2) and tested in Chapter 4.

The distribution of wetland plants is naturally variable geographically, as it is determined by numerous environmental parameters. The challenge this poses to bioassessment is that indicators of a given (natural or disturbed) environmental condition for one area may not be indicators of the same conditions in another area. Allied to this natural variability is a lack of baseline information on the plant species found in South African wetlands and the factors (both natural and human stressors) that influence the distribution of these species. As a consequence, (and with the permission of the Steering Committee), the field component of the project was limited to sampling within the Western Cape in an attempt to confine the assessment of wetland environmental condition to sets of wetlands which exhibit distinct vegetation assemblages. Furthermore, in researching the use of wetland vegetation for purposes of environmental assessment, the focus was placed on material that was pertinent to the lowlands of the Western Cape.

The Cape coastal lowlands hold a heterogeneous microcosm of South African wetlands that may inform our understanding of the national macrocosm. All of the following inland wetlands are common within the planal landscape of the Cape coastal lowlands: Wetlands that are isolated from, or connected to channels; that have ephemerally to permanently saturated and/or inundated soils; that may be either oxic or anoxic; that have brackish to freshwater conditions; that range in size from less than one to more than 100 hectares; that vary in depth from less than 500 to more than 2000 mm and that are vegetated with macrophytes, (Dallas *et al.* 2006). These wetlands represent numerous HGM types, namely: depressions with/or without channelled inflow that have endorheic or exorheic drainage, floodplain depressions and flats with exhorheic drainage, un-channelled valley-bottom flats and depressions with exorheic drainage and wetland flats (SANBI 2009).

Some of the metrics developed for phyto-assessment in this study may have inter-regional applicability. This can, however, only be established with further sampling and a wider application of the phyto-assessment development-protocol (see below) than was possible in the present study.

From this work the major principles of phyto-assessment were distilled and incorporated into:

1. a national framework for the delineation of units of comparable wetlands to assist in the development of phyto-assessment metrics; and
2. a generic metric development-protocol that can be used throughout the country.

In future, the proposed national framework and metric development-protocol may provide guidance for the development of phyto-assessment tools suitable for use in other biogeographical regions that contain distinct suites of wetland vegetation.

1.2. Rationale for the project

The means of assessing "wetland environmental condition" have progressed along with an increased understanding of what this concept entails (Fore 2003). Ecosystem functionality and habitat availability are influenced by both biotic and abiotic components, and the interaction between all of these aspects determines the sustainability and environmental condition of an ecosystem (Karr 1987). A comprehensive assessment of environmental condition therefore implies that the abiotic, functional, habitat and biotic components of a wetland all need to be assessed. The progressive development of assessment methods from physico-chemical constituents, to ecosystem functions, and then habitat condition lead researchers to realise that the biota hold the potential to reflect aspects of ecosystem condition that may not be reflected by any of the individual components and may, in fact, act as an effective summary of all of them (e.g. Karr 1991, US EPA 2002a, DWAF 2004). Since the existence of organisms is determined by the stresses and opportunities within the habitat in which they occur (e.g. Whittaker 1962, Karr and Dudley 1981), aspects of the biotic assemblage have indeed proven useful as indicators of the present environmental state within wetlands (e.g. Karr 1987, US EPA 2002a, Fore 2003).

Phyto-assessment indices of wetland ecosystems have been developed predominantly in North America (e.g. Mack *et al.* 2000, Simon *et al.* 2001, US EPA 2002a, Gernes and Helgen 2002, Lopez and Fennessy 2002, Miller *et al.* 2006). In South Africa to date, no comprehensive method of assessing wetland condition using macrophytes has been developed. The primary objective of this project is the development of a method for assessing the environmental condition of wetlands based on the composition of their macrophyte assemblages.

Plants are a fundamental biological component of many inland wetlands and define many of their biological and ecosystem characteristics (e.g. Mitsch and Gosselink 2000, Keddy 2000). The existence of plants and their community structure is defined by the ambient environmental conditions and their seasonal variation (e.g. MacArthur and Wilson 1967, Walter 1973, Kent and Coker 1992, Keddy 2000). As such, once plant autecology and phytosociology are understood (see glossary in Section 1.6), the existence of certain plants and/or community assemblage patterns can indicate the prevailing environmental conditions (e.g. Fennessy *et al.* 1998, Adamus *et al.* 2001, Mack 2001, Simon *et al.* 2001, Lopez and Fennessy 2002, Gernes and Helgen 2002, Fennessy *et al.* 2004, Mack 2007).

In order to meet South African national legislative requirements (National Water Act No. 36 of 1998), methods of assessing and monitoring wetland condition are required. Worldwide, macrophytes are one of the most popular biotic assemblages used for the bioassessment of wetland environmental condition. This is largely due to the fact that, in countries in which plants have been used for bioassessment, the ecology and functioning of wetland plants is comparatively better understood than other biotic assemblages inhabiting wetlands (e.g. Adamus *et al.* 2001, Butcher 2003, DWAF 2004). The same is not true for South Africa, where considerable effort has been focussed on the study of aquatic invertebrate ecology (Chutter 1972 1994), but little on the ecology of wetland plants (and that only recently) (Cook 2004, Mucina *et al.* 2006a). In the arid and semi-arid climatic regions of South Africa, however, phyto-assessment has the advantage in that it can be used in situations where no surface water exists; a factor that would prevent the use of techniques based on water chemistry or invertebrate assemblages.

1.2.1. National development of phyto-assessment techniques

Phyto-assessment is predominantly a field-based assessment technique, relying on the direct measurement of aspects of the biotic assemblage that are shown to provide information on the present environmental state or condition of the ecosystem under study. The practicality of the phyto-assessment methods described in this report will need to be assessed critically, by end-users with different levels of expertise, in order to determine their applicability in the field. The habitats and regions within which they can successfully be used to indicate environmental condition will also need to be assessed. This implies that field (and, where applicable, laboratory) protocols must be developed to ensure consistency and repeatability between users (e.g. Mack *et al.* 2001, Fennessy *et al.* 2004). Once the development of phyto-assessment metrics and an index are complete for a given ecosystem, international experience suggests that the time-frame required for the assessment of target ecosystems can be relatively rapid, taking less than a day in the field and approximately the same for data processing (e.g. Mack *et al.* 2000, Fennessy *et al.* 2004). In order to facilitate the development of phyto-assessment indices for wetlands throughout South Africa, a national framework is required to establish the following three aspects:

1. the biogeographic regions of South Africa that contain relatively homogenous wetland vegetation;
2. what constitutes a wetland plant assemblage in a natural or "reference" environmental condition; and
3. classification of wetlands into hydrogeomorphic (HGM) types, vegetation habitats and hydrological zones.

The development of this framework for the whole of South Africa was of primary importance in this study as:

1. wetland biogeographic regions of South Africa are poorly understood (e.g. Kleynhans *et al.* 2005, Mucina *et al.* 2006a);
2. the determination of what constitutes an un-impacted or reference wetland ecosystem is undefined; and
3. the HGM-based classification system for South African wetlands (SANBI 2009) was finalised only after the completion of field work for this project and it is still unclear which habitats hold similar wetland vegetation.

A fourth and final aspect of this national approach to phyto-assessment is the development of a protocol to identify the aspects of vegetation assemblages most useful to phyto-assessment techniques.

1.3. Project objectives and the approaches used

The objectives of this study as indicated in the inception report (Malan *et al.* 2009) are outlined in bold as follows. In each case, an indication is given of the extent to which each objective was reached.

1. Collation and examination of all available information on the distribution of obligate and facultative macrophytes from South African wetlands and an analysis of their habitat requirements:

A review was done of all existing lists of indigenous and alien plants with wetland affiliations in South Africa in order to determine the affinity of plant species for particular habitats, and to search for species indicative of reference and disturbed conditions. A presentation and mini-workshop were conducted at the National Wetlands Indaba in 2007, and at the Western Cape Wetlands Forum meeting (Sept 2007) to determine the extent of expert knowledge with regard to wetland indicator plants. The objective was to ascertain any unpublished responses of wetland macrophytes to anthropogenic stressors in South Africa. From these sources, a list of all taxa, identifiable as being affiliated to wetland habitat within the winter-rainfall region of South Africa, were collated using the template provided by Glen *et al.* (1999). A brief introduction to this list and the sources used to compile it are presented in Appendix 5. The list of wetland-affiliated flora for the winter rainfall region of South Africa, as determined by this WHI study, is attached to this volume in a CD/DVD.

A list was created of both alien and indigenous invasive taxa and ruderal taxa (opportunistic invaders) in the Western Cape and was added as an appendix to WET-Health (Macfarlane *et al.* 2008) (see Appendix 6 of this volume).

2. A comprehensive literature review of the responses of macrophytes to changes in ecological drivers such as hydrological conditions, sediment fluxes, herbivory, fire and human-induced disturbances:

A synthesis of existing local and international information on the responses of wetland-affiliated plants to changes in ecological drivers is presented in Section 2.6.2 of this volume.

3. Review of the approaches and methodologies employed in the development of macrophyte indices currently used to determine the condition of wetlands and rivers:

i. National and international approaches to wetland and riverine assessment (using physical or chemical aspects and biota) were reviewed with regard to their applicability to the phyto-assessment of palustrine wetlands in South Africa. The results are discussed in Chapter 2 and Chapter 3. A protocol appropriate for the development of phyto-assessment indices for palustrine wetlands in South Africa was formulated.

ii. A generic framework and phyto-assessment index development protocol were created and are presented in Chapter 9.

4. Formulation of hypotheses regarding the relationships between macrophyte taxa or communities and aspects of their habitats:

i. Aim: To indentify an appropriate phytogeographical region and a set of wetlands with similar habitat within the region. These wetlands would then be used to facilitate the development of a phyto-assessment index.

Result: An appropriate phytogeographical region and wetlands were partially identified through the literature review (Section 2.10) and then tested in Chapter 4.

ii. Aim: To facilitate an *a priori* categorization of wetlands, within the study set, as "Reference", "Moderate" or "Worst" in terms of their environmental condition. These would be determined by the amount of human land-use and disturbance at each target wetland.

Result: This objective is addressed in Section 2.8 and Chapter 5.

5. Collection of rooted wetland macrophyte samples to test these hypotheses: this will include all test wetland sites and probably some others, but will be confined to one summer-rainfall region (likely to be in KZN) and one area (possibly the Agulhas Plain) within the winter-rainfall region:

Field sampling (as per proposed protocol) of the vegetation and environmental parameters characterizing comparable palustrine wetland habitats was carried out in 60 wetlands in three sub-regions of the Cape coastal lowlands (namely West Coast, Cape Flats and Overberg). The Cape coastal lowlands region and the collection protocol are described in Chapter 3 of the present volume.

Contrary to this proposed objective (Malan *et al.* 2009), field sampling did not take place in both the summer- and winter-rainfall regions because all international phyto-assessment tools suggest both the need for a bioregional focus and that metrics developed in one area will not necessarily be applicable to another. When discussed with the Steering Committee it was decided that, due to the scale of the project, all effort should be focused in the winter-rainfall area of the Cape coastal lowlands.

6. Employing correlation analyses and multivariate statistics, use macrophytes to develop one or more metrics or indices to assess various aspects of the water quality, hydrology and physical habitat of wetlands. Specifically, develop macrophyte metrics for the phyto-assessment of wetlands in order to assess their environmental condition:

Application of the protocol for the development of a phyto-assessment index within the Cape coastal lowland region resulted in the creation of vegetation metrics suitable for assessing environmental condition, as described in Chapters 6 and 7.

7. Compilation of a key and photographic guide for the wetland plants in different regions and/or for the plants used in the determination of phyto-assessment metrics:

The Steering Committee considered the compilation of field guides and keys for the identification of wetland plants to be unnecessary at this stage, as existing botanical literature was, for the most part, sufficient for identification purposes (Cook 2004). This objective was therefore not attained.

1.4. Terminology used in this report

Section 3.1 and the glossary of the Handbook to the WHI Programme (Day and Malan 2010) provide an explanation of the terminology used in the present study. Other terms used exclusively in this volume for the development of a plant-based assessment (phyto-assessment) of environmental condition are listed below:

Autecology

Autecology is the study of the physiological relationship between plant function and structure and the habitat in which it occurs (e.g. Tansley 1935, Poore 1955).

Classification

Wetland classification is the grouping of **similar wetlands** with homogeneous natural attributes (e.g. hydrological or morphological characteristics) into categories that reflect different environmental habitats (*sensu* Cowardin *et al.* 1979, Brinson 1993, Ewart-Smith *et al.* 2006, SANBI 2009). This is similar in concept to the typing or **classification of vegetation** (*sensu* Braun-Blanquet 1928, Rutherford *et al.* 2006, Mucina *et al.* 2006a) which is necessary for the determination of comparable units of plant habitat. Note that this generally accepted use of the word "classification" is different from the meaning used by the Department of Water Affairs (DWA) and the National Department of Agriculture (DoA), where classification (of rivers, wetlands, estuaries, etc.) is a grading system that uses various categories to describe the condition of a water resource, or part thereof (DWAF 2004).

Ecological versus functional diversity

The concept of diversity as used by Cowling *et al.* (1992) is adopted in this document. Alpha diversity refers to the number of species within a homogenous community. Beta diversity refers to species turnover along habitat or environmental gradients. Gamma diversity refers to species turnover among equivalent habitats along geographical gradients (Cody 1975 1983). It is therefore identical to Whittaker's (1972) concept of delta diversity.

Macrophytes

Macrophytes are large plants, especially associated with water bodies (Merriam-Webster 2009). Often taken to mean a large aquatic plant, the term "aquatic macrophyte" has no taxonomic significance. Macrophyte has previously been interpreted to mean all Charophyta (Stoneworts), Bryophyta (Mosses and Liverworts), Pteridophyta (Ferns and fern allies) and Spermatophyta (Seed-bearing plants) whose photosynthetic parts are permanently or, at least for several months each year, emergent from or submerged in water, or floating on the water's surface (Cook *et al.* 1974, Cook 1996, Glen *et al.* 1999, Cronk and Fennessy 2001). In the current project the term macrophyte was used to describe **all macroscopic plants found within ecosystems defined as wetlands**, including the many terrestrial (non-aquatic) plants whose photosynthetic parts are rarely, if ever submerged, but that are rooted in a substrate that is at least temporarily saturated and are therefore adapted to life in saturated soils. This is in keeping with the South African National Water Act (No. 36 of 1998) definition of wetlands (See section 2.2

below). Macrophytes are therefore essentially the macroscopic component of the taxa that Cowardin *et al.* (1979), defined as wetland plants, i.e. those plants adapted to living in wet conditions or on a substrate that is at least periodically deficient in oxygen as a result of inundation or saturation.

Palustrine

Palustrine systems are inland wetlands dominated by emergent plants (e.g. reeds), shrubs or trees and include a variety of wetland ecosystems commonly described as marshes, floodplains, vleis or seeps (Kotze *et al.* 2005). As defined by Cowardin *et al.* (1979), palustrine systems include any inland wetland which lacks strongly flowing water, contains ocean-derived salts in concentrations of less than 0.05%, and is non-tidal. **It is important to note however:** that this term (and the terms marsh and vlei) are not used in the description of wetlands in the National Wetland Classification Scheme. There, palustrine wetlands are considered to be incorporated into inland wetlands and are distinct from marine wetlands (Ewart-Smith *et al.* 2006, SANBI 2009).

Phytosociology

Plant sociology (or phytosociology) encompasses the study of vegetation and its floristic composition, structure, development and distribution, as well as its classification and the interrelationship of differently classified units (Poore 1955). Phytosociology requires detailed field surveys to identify vegetation associations, which can then be grouped hierarchically into alliances, orders, classes, etc.

2. REVIEW OF THE LITERATURE

2.1. Introduction

The objective of this literature review is to synthesize the existing information on wetland-affiliated plants and methods for the bioassessment of wetlands.

Under natural, unimpacted or Reference Conditions, wherein an ecosystem is unaltered by human disturbances that degrade or impair the natural integrity of the habitat, characteristic sets of biota can be expected to exist within similar habitats (Whittaker 1953). Plant species typically have a range of environmental parameters in which they are able to survive, and changes to any of these parameters can change the assemblage of plants/organisms within a habitat (e.g. Whittaker 1962, MacArthur and Wilson 1967). The presence of certain “indicator” plant species or the overall community assemblage therefore represents a particular set of environmental determinants that exist at a given location. Plant communities are fundamental biological components of most types of inland wetlands, (of which palustrine wetlands are an example) and therefore, they may define the biological and ecosystem characteristics of these wetlands to a large extent (e.g. Mitsch and Gosselink 2000, Keddy 2000). Different types of wetlands and their physico-geographic drivers are important determinants of habitat availability for plants. Along with other environmental determinants of biotic distribution such as climate and soil type, these parameters partition the landscape into areas of different habitat (*sensu* Walter 1973).

Human disturbance, due to various types of land-use may include individual stresses, or large landscape-scale disturbances and often results in the impairment of the unimpacted environmental condition. Land-use causes disturbances that alter the natural environmental parameters that determine habitat availability for the biota. Alteration of habitats by human disturbance can therefore be expected to change the characteristic assemblages found in biological communities. Measurements of the differences in patterns of species assemblages in reference wetlands, relative to disturbed wetlands, can be used to assess wetland ecosystem condition. Biota such as plants can therefore be used to represent the environmental determinants and also the environmental condition of a given wetland. Bioassessment is based on the premise that the distribution and assemblage patterns of the biota are determined by both the underlying natural environmental template and the superimposed anthropogenic disturbances.

All of these topics, as well as information about bioassessment protocols and empirical methods of testing the applicability of these concepts in the context of South African palustrine wetlands (See Section 1.4) are presented in this literature review.

2.2. Wetlands as plant habitats

A wetland is defined in the National Water Act of South Africa (No. 36 of 1998) as: "land which is transitional between terrestrial and aquatic ecosystems, where the water table is usually at or near the surface, or the land is periodically covered with shallow water, and which land in normal circumstances supports or would support vegetation typically adapted to life in saturated soil".

According to this definition, all that is required for an area to be defined as a wetland is for the substrate to be wet for long enough (at least a few weeks in a year) to support plants adapted to saturated conditions. The duration and depth of waterlogging (saturation) and/or inundation are integral determinants of the distribution of wetland plant species (e.g. Keddy 2000, Mitsch and Gosselink 2000, Mucina *et al.* 2006a). Within wetlands, saturated or waterlogged soils (that may be either oxic or anoxic), inundated areas and the water column each represent separate areas of potential growth for vegetation. Each of these growth media are considered distinct habitats and have specific limitations that determine the taxa that are able to grow there. Whilst some species, such as aquatics, will occur only in the water column, other species have broader environmental tolerances and may be present in more than one habitat. Wetlands therefore contain various habitat units which support plant species with different habitat requirements.

Different types of wetlands and their associated habitat units can be classified into units of similar habitat for biota, within which, characteristic patterns of plant assemblage are anticipated to occur (SANBI 2009). It is, however, not yet clear which of these parameters is important in determining the microclimate within each wetland plant habitat.

2.3. The importance of wetlands in the environment

Wetlands are important ecosystems that are under threat from anthropogenic disturbance (Ramsar Convention 1971, IUCN 1980). A large percentage of the world's wetland habitat, and more than half in some areas of South Africa, has been lost or severely degraded due to unsustainable social and development practices (IUCN 1980, Breen and Begg 1989; Kotze *et al.* 1995, Shearer 1997, Dini 2004). Those wetlands that remain are highly threatened due to human population growth and development pressure (e.g. Kotze *et al.* 1995, Adamus *et al.* 2001). In the naturally arid conditions of much of South Africa, the additional water-stress caused by human activities poses considerable threat to the remaining wetland habitat. The ecosystem services provided by wetlands, and their economic value suggest the need for the sustainable management and use of wetlands and the conservation of what wetland habitat remains. Bioassessment can assist in this endeavour.

Wetlands perform various ecosystem services that are considered to have economic and environmental value. These include the purification of catchment surface water, nutrient and pollutant removal; floodwater attenuation and the associated retention of sediment and erosion control; and possibly the recharging of groundwater reserves (Faulkner and Richardson 1989, Johnston 1991, Reddy and Gale 1994, Richardson 1994, Costanza *et al.* 1998, Mitsch and Gosselink 2000, Zedler and Kercher 2005, Brauman *et al.* 2007). All of these wetland functions are considerably enhanced by the presence of vegetation.

Vegetation within and around wetlands tends to slow the flow of water and thus influences water quality by moderating the amount/concentration/volume of nutrients, pollutants and sediment in downstream aquatic ecosystems. Reduction of flow tends to lead to sediments being deposited and/or trapped on the soil surface. At the same time, other chemical constituents (e.g. nutrients and toxins) may also be trapped. Due to the action of the anaerobic bacteria, fungi and protozoa that are present in the sediments and amongst plant roots, these chemical constituents are degraded to simpler molecules (Reddy and Gale 1994). The greater the frictional resistance (roughness coefficient) offered by the vegetation, the more the flow is retarded and the more sediments and associated pollutants may be trapped. Plants also take up nutrients and other chemical constituents, thus removing them from the substrate and the water column. Vegetation can thus improve water and soil quality – hence the use of vegetated artificial wetlands for the amelioration of water-borne waste (Rogers *et al.* 1985).

Wetlands are unique ecosystems that, particularly in arid countries such as South Africa, represent a limited resource within predominantly terrestrial landscapes and are therefore a critical store of biological diversity (Ramsar COP7 1999, Williams *et al.* 2004, Dudgeon *et al.* 2006, Verhoeven *et al.* 2006, Mucina *et al.* 2006a).

Furthermore, many wetlands are highly productive systems, even rivalling rainforests in biomass production and as a result have considerable economic and social value (Thibodeau 1981, Leitch and Shabman 1988, Turner 1991, Gren *et al.* 1994, Costanza *et al.* 1998, Woodward and Wui 2001, US EPA 2002c, Schuyt 2005, Brander *et al.* 2006). Conversely, it is recognised that some wetlands have low productivity because of a limited supply of nutrients (Cronk and Fennessy 2001). In keeping with the high biodiversity of terrestrial vegetation in nutrient-limited systems such as in the Mediterranean floral regions of the world (e.g. Kruger *et al.* 1983), the vegetation of nutrient-limited wetlands may exhibit equally extreme levels of diversity and heterogeneity.

Recognition of the value of wetlands has led to a commitment by all countries that are signatories to the Ramsar Convention to assess and monitor the integrity and environmental condition of designated wetland ecosystems. In South Africa a method of ascertaining the environmental condition of wetlands is required in order for the effective implementation of the National Water Act (1998), as well as for a wider range of activities such as conservation planning and management. Wetland assessment and monitoring, with the aim of determining and managing the impact of human activities on wetlands, will help to facilitate the sustainable utilization and conservation of these important ecosystems (Finlayson *et al.* 2002, DWAF 2004, and Malan and Day 2005a). A strategic overview of the research needs for wetland health and integrity (Malan and Day 2005a) determined that a method of assessing and monitoring the biological integrity of wetland environmental condition was required in order to meet both national and international legislative requirements. This project is therefore one of three in the WHI research programme dedicated to determining methods of biotic assessment of wetland condition (Day and Malan 2010).

2.4. Anthropogenic impacts on wetland ecosystems

2.4.1. Disturbance can be both natural and anthropogenic

It is important to note that disturbances to ecosystem functions and condition may occur as a result of both natural and anthropogenic influences. Both can have a similar effect on the receiving environment; and both may be as a result of the same type of stressors such as fires or floods. Natural stressors such as fire, sedimentation and food procurement by animals have a regular or cyclical, (often seasonally-based) pattern of disturbance in natural wetland ecosystems unaltered by anthropogenic influences. Such events (or their occurrence as a cyclical regime) are termed disturbances, due to their interruption of the successional development of animal or plant communities (Grime 1979). Recognizing that humans have influenced disturbance regimes for a long time, sustainable and small-scale disturbances, such as veld-burning and traditional harvesting regimes, are taken to be natural and considered to be low impact. A change in these natural disturbance regimes, however, such as an increase in the intensity or frequency of fire, flooding, or grazing pressure as a result of unsustainable human development are regarded as unnatural disturbances that cause impairment of the environmental condition (e.g. Kent and Coker 1992, Deacon 1992, Clarkson *et al.* 2004).

In the present study, anthropogenically induced impacts, that result in unnatural impairments of ecosystems or environmental conditions, are considered to constitute anthropogenic disturbance and are often referred to, (for parsimony), as disturbances. Human disturbance essentially alters the physico-chemical environment of a wetland. An event or other stimulus, and/or a land-use that causes stress to an organism or an ecosystem is referred to in this report as a “stressor”. Natural, or non-anthropogenically induced disturbances are not considered as part of the present research. Recognition of the period of time that has elapsed after a disturbance event (whether natural or anthropogenic) is, however, necessary in order to be able to identify comparable units of vegetation for assessment purposes. This is because the effects of disturbance events will change over time and therefore the period of time that has elapsed needs to be similar if the vegetation units are to be compared.

Anthropogenic disturbances to wetlands may be the result of any one or more of a large number of recognized stressors (some of which are listed in Table 1), which alter the ambient environmental conditions within wetlands and thus drive changes in species assemblage patterns. Catchment-wide disturbances such as afforestation or increased surface hardness and localised disturbance in the immediate vicinity of a wetland both affect the environmental condition of a wetland. Physical alteration of wetland habitats occurs as a result of geomorphological and hydrological changes; direct changes in the vegetation species assemblage due to alien species invasion, removal of vegetation and/or land clearance, infilling and human utilization of vegetation. Such geophysical and biological impacts alter the physical landscape and can cause considerable wetland habitat fragmentation (US EPA 2000a, Adamus *et al.* 2001, Fore 2003, Clarkson *et al.* 2004, Rountree *et al.* 2007, Macfarlane *et al.* 2008). Whilst it is obvious that the disturbance caused by complete changes in the physical structure of the landscape will affect the natural functional integrity and environmental condition of wetlands, there are many less obvious stressors that also degrade these ecosystems. Manyland-use practices can result in changes in environmental factors such as the hydrological regime (i.e. water residence time and flow dynamics: quantity, quality and temporal aspects of flow), nutrient and mineral availability, sediment and organic loading and the resultant turbidity and oxygen availability. As it is these environmental parameters that determine species distribution and community assemblage patterns (e.g. Walter 1973, See Section 2.9), if the change to any environmental parameter is large enough, it is likely that natural ecosystem function will be impaired and species assemblage patterns changed.

2.4.2. Common anthropogenic stressors affecting wetlands

A list of anthropogenic stressors to which wetlands are commonly exposed (with examples of the types of land-use causing these stressors) is given in Table 1. All of these stressors can have negative impacts on ecosystem functioning and each should therefore be considered in terms of its potential gradient of disturbance. Individual stressors do not necessarily impact on ecosystems in the same way, however, or elicit the same response from the biotic community. For instance many macrophytes are unaffected by a reduction in dissolved oxygen. The effects of these stressors on wetland plants are presented in Table 2.1.

Anthropogenic stressors cause the degradation of wetland habitat and ecosystem functioning, and thus alter the assemblage of biota that wetlands are able to support. Anthropogenic stressors and their effects on wetland habitat, ecosystem functioning and concomitant changes in biotic assemblages have been the focus of many studies, predominantly in North America. It has been shown that an alteration in the biotic assemblages of a wetland often changes the balance between ecosystem functionality and integrity, thus further altering the environmental condition of the impacted wetland (e.g. Keddy 2000, Mitsch and Gosselink 2000, US EPA 2002a). Very little information exists as to the impacts of these stressors on South African wetlands.

2.5. The history of wetland assessment

Initial trends in the monitoring of aquatic ecosystems (mainly rivers and lakes), followed a physico-chemical approach and were predominantly for the measurement of water quality as defined by/ related to human requirements (Karr and Dudley 1981, Huber 1989, Karr 1991). This approach was criticized firstly for its focus on the provision of services to humans rather than the evaluation of environmental conditions, and secondly for the lack of a clear understanding of the links between the parameters being measured and ecosystem integrity and functioning (Karr 1987, US EPA 2002a). For instance, plants cleanse pollutants from water and soils. This biochemical process is often reflected in the short duration/residence time of certain chemical pollutants in wetlands, making their detection very difficult and suggesting that (broader and complementary) methods of assessment are required (Mitsch and Gosselink 2000, US EPA 2002a).

Table 2.1: Human-related stressors to which wetlands are commonly exposed: (after Adamus and Brandt (1990), Reddy and Gale (1994), Adamus et al. (2001), Clarkson et al. (2004)).

Dehydration: alteration of the hydroperiod due to a reduction in wetland water levels and residence time and/or increased frequency, duration, or extent of desiccation of wetland sediments. • Typically associated with ditching, channelization of nearby streams, outlet widening, subsurface drainage, invasion of wetlands and their catchment areas by plants with high water requirements due to the introduction of alien plants for use in silviculture, global climate change and ground or surface water withdrawals from the wetland itself or in the surrounding catchment for agricultural, industrial, or residential use.
Inundation: alteration of the hydro-period due to an increase in wetland water levels and/or an increase in the frequency, duration (residence time), or extent of saturation of wetland sediments. • Typically associated with the construction of impoundments within, or adjacent to wetlands; or • Changes in catchment land-use resulting in the hardening of surfaces, with

associated increases in the velocity and total volume of runoff reaching wetlands.

- Also associated with increased volume and/or a change in the seasonality of water inflow due to human settlements; for example the increased volume of aseasonal flow in the Kuils River on the Cape Peninsula emanating from the Tygerberg Waste Water Treatment works (Hall 1993).

Eutrophication, organic enrichment, reduction in the availability of dissolved oxygen:

An increase in the concentration or availability of nutrients, particularly nitrogen or phosphorus. Increases ?finely divided carbon, to the point where the decomposition-driven increase in the biological demand for oxygen reduces the availability of dissolved oxygen in the sediments and the water column and increases the concentration of toxic gases (e.g., hydrogen sulphide and ammonia)

- Typically associated with excessive fertilizer application, poor livestock waste-management, overburdened wastewater treatment systems, fossil fuel combustion, unmanaged urban runoff, and other sources of nutrients and finely divided organic matter.

Contamination Toxicity: An increase in the concentration, availability, and/or toxicity of metals and synthetic organic substances.

- Typically associated with agriculture (pesticide applications), aquatic weed control, mining, urban runoff, landfills, hazardous waste sites, fossil fuel combustion, wastewater treatment systems and other sources.

Acidification: An increase in acidity (decreased pH).

- Typically associated with acid mine drainage, agriculture and fossil fuel combustion.

<u>Increased Alkalinity:</u>

Increased pH could be a problem in the Western Cape where Acid Sand Plain Fynbos is recognized as being a relictual vegetation type that has decreased in extent as a result of urbanization (e.g. Cowling and Holmes 1992.)

- Increases in pH are typically associated with the addition of lime to neutralize soil pH, thus increasing turf grass or crop yields or biomass production. Therefore possibly associated with race courses, golf estates and other recreational sports requiring grassed surfaces.
- Liming is also used to counteract the lowered pH resulting from irrigation with acidic water; and the leaching of base nutrients such as calcium, magnesium and potassium from the soil, which occurs more frequently in areas of heavy rainfall or on heavily-irrigated turf.

Salinization: An increase in dissolved salts, particularly chloride, related to parameters such as conductivity and alkalinity.

- Typically associated with saline irrigation return waters, stormwater, seawater intrusion (what? Into rivers and groundwaters?) (e.g., due to land loss or aquifer over-exploitation and slow recharge rates (e.g. Sandveld on West Coast of South Africa, e.g. Conrad et al. 2004, Conrad and Munch 2006), road salt used for winter ice control and domestic/industrial uses.

De-salinization: Equally as important as salinization in arid climates, including South Africa. The process whereby naturally saline (due to the presence of inorganic salts) wetlands become less saline (Malan and Day 2005b).

- Typically associated with urban areas where fresh water inputs to wetlands increase due to increased surface hardness.

Sedimentation/Infilling: An increase in the deposition of sediments, resulting in the partial or complete burial of organisms and the alteration of substrates.

- Typically associated with: changes in land-use, agriculture, disturbance of stream flow regimes and urban runoff.
- Excessive discharge from poorly functioning wastewater treatment plants, deposition of dredged or other fill material and erosion from mining and construction sites.
- Decreased sediment trapping by vegetation due to a reduction in the width of buffer-zones around wetlands.

Thermal Alteration: Long-term changes (especially increases) in the temperature of water or sediment.

- Typically associated with proximity to power plants and other industrial facilities, removal of shading vegetation, lowering of summertime water levels and global warming.

Turbidity/Shade: A reduction in the penetration of sunlight into a waterbody due to suspended sediments, phytoplankton and/or over-storey vegetation or other physical obstructions.

- Typically associated with silviculture, agriculture, disturbance of stream flow regimes, urban runoff, organic-rich discharge from poorly functioning wastewater treatment plants, erosion from mining and construction sites, placement of bridges and other structures and re-suspension of substratum sediment by alien fish such as carp.
- Increased algal volumes/density/concentration in the water column as a result of eutrophication.
- A decrease in the biomass of, or complete removal of submerged aquatic plant species such as *Potamogeton* that by their nutrient procurement process reduce turbidity.

Vegetation Removal: A direct alteration of the vegetation assemblage within and surrounding wetlands, resulting in changes to the natural functioning of the ecosystem as well as influencing the influx of sediments from the surrounding catchment.

- Typically associated with aquatic weed control, agricultural and silvicultural, channelization, bank stabilization, urban development, unintentional defoliation due to crop spraying and unnatural regimes of trampling/grazing/herbivory (e.g. from introduced ungulates, geese, crustaceans and insects) or fire and harvesting.
- Similarly, the removal of buffer vegetation around wetlands reduces water retention. Consequently, the resulting sediment and nutrient trapping and associated conversion by bacteria, fungi and protozoa to constituent molecules and uptake by vegetation are also decreased.

Invasion by non-indigenous plants: Introduced plants can modify wetland function and structure and are recognized the world over as one of the major threats to wetland condition. Alien and invasive species alter nutrient and carbon cycles, light infiltration, water table depth, species relative-dominance and species richness, fire intensity and frequency.

- Typically associated with patterns of human settlement and land-use.

Fragmentation: An increase in the distance between patches, and a reduction in the size of patches of natural wetland habitat, as well as the perimeter to area ratio determine species and community survival and adaptability to human alteration.

- Typically associated with human alteration of natural land for development purposes. Typically associated with the development of unimpacted land.

Physical disturbance: The disturbance of wetland soils during the dry season can denitrify the soils (Ellis and Mellor 1995), increase the dominance of invasive non-indigenous species and destroy much of the viable seed bank of indigenous vegetation.

- Typically associated with activities such as tillage, compaction or excavation.

Destruction of natural seed banks: Seed banks are important for maintaining plant species diversity and can easily be damaged by a variety of human activities. However, little is known regarding the degree to which climate, nutrition, contaminants and other factors influence seed banks.

With the recognition of the important benefits provided by wetlands came the realization that, in order to retain many of those benefits, natural ecological processes and biodiversity also needed to be conserved and properly managed. Thus, later assessment approaches focused on the measurement of wetland habitat structure and functional ability to maintain wetland processes the ability of the wetland to function, and therefore to maintain its ecological processes (Findlay *et al.* 2002, US EPA 2002a, Butcher 2003, Macfarlane *et al.* 2008). The maintenance of wetland processes and ecological integrity is associated with a particular environmental condition that has been likened to ecosystem health. The recently-developed WET-Health tool (Macfarlane *et al.* 2008) provides a method for assessing wetland health, and is based predominantly on the negative impacts of anthropogenic disturbances on wetland habitat and functional processes. Fundamentally, the physical impacts of anthropogenic disturbance (e.g. excavation, reduced water influx, vegetation clearance) on geomorphology, hydrology or vegetative cover are rated, as are the impacts of pollutants on water quality, albeit to a limited extent. The research programme in which the WET-Health tool/system, etc. was developed, described wetland ecosystem health as "*a measure of the similarity of a wetland to a natural reference condition…(in which) … it is appropriate to consider "deviation" from natural reference condition,* …(as)… *the extent to which human impacts* *have caused the wetland to differ from the natural reference condition* ….." (Macfarlane *et al.* 2008).

This concept of a change or deviation from the natural condition stems from the work of Karr *et al.* (1986) in which the reference condition is considered to be a natural state, unaltered by human interference. The reference condition of "full ecosystem integrity" is a state in which the ecosystem has complete ecological and biotic integrity, reflected in its "….*ability to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity and functional organization comparable to that of natural habitat …*" (Karr 1991). In this definition, "*species composition, diversity and functional organization"* can be assumed to reflect the environmental condition, or 'health', of the ecosystem. This in turn spawned the concept of using the present state of the biota to infer an environmental condition and hence the potential for the use of biological assessment or 'bioassessment' in wetlands.

The use of plants or other living organisms to assess the environmental condition of wetlands has developed out of the growing awareness that organisms exist as a result of the ambient environmental conditions in a given ecosystem. And, in certain circumstances can, quite accurately, describe the integrity and sustainability of an ecosystem under these environmental conditions (e.g. Karr 1991).

Human alteration of the prevailing environmental conditions in an ecosystem can affect the biotic and abiotic determinants of species distribution, and thereby change the patterns of species assemblage (e.g. Karr 1981). Attributes such as species composition, or measures of the biodiversity of the plant assemblage characteristic of such patterns (if consistently associated with the environmental conditions responsible for habitat determination), may be considered as indicators of these conditions. Hence, for the purposes of this study, indicator species and attributes are defined as those whose presence in an ecosystem is indicative of particular conditions (such as saline soils or acidic waters). These so-called indicator species and community attributes can thus act as **metrics** (or surrogates for measurement) of the ecophysiological factors (such as water availability, nutrient concentration, sedimentation, turbidity or overshadowing of the biota), that determine the environmental conditions within a particular wetland habitat (e.g. Karr 1981, Adamus *et al.* 2001, Gernes and Helgen 2002, Fore 2003). In this project, therefore (?) metrics are used as a summary measure of species assemblage or composition that shows an empirical change along a gradient of human disturbance. Metrics can therefore indicate human impairment, or disturbance of the natural environmental condition of a given habitat. The combination of multiple metrics based on indicator species and community attributes have been used in the development of Indices of Biological Integrity (IBI's) (Karr 1981). Such indices, composed of multiple metrics, are integrative expressions of the biological condition of a site.

Assessments of different aspects of the environmental integrity and condition of wetlands have thus been developed relatively independently. Four separate approaches can be described:

- *Physico-chemical assessments* – an evaluation of the physical and chemical conditions within a wetland, often with a utilitarian bias toward human needs and demands on aquatic ecosystems (DWAF 1996 and 2002);
- *Functional Assessments* – evaluate the effects of the impacts posed by human stressors on the sustainability of natural wetland functional processes that provide ecosystem services such as the provision of raw materials, flood control and nutrient cycling (e.g. WET-Eco-services [Kotze *et al.* 2008]);
- *Habitat Assessments* – evaluate the quality, quantity and suitability of the physical environment that supports the biota (e.g. Wetland Index of Habitat Integrity [Rountree *et al.* 2007]); and lastly
- *Biotic Assessments* – evaluate the biological condition of a wetland using surveys of the species assemblage and community structure of the resident biota, (e.g. SASS in the riparian context [Dickens and Graham 2002] and the many bio-assessment methodologies outlined by the US EPA [2002a]).

Bioassessments are complementary to physico-chemical, habitat and functional assessments. As the biotic assemblage that is present in an ecosystem is defined by, and thus also representative of, the determining environmental parameters that exist within an ecosystem, so the biotic assemblage can be used to reflect the cumulative stresses on, and resultant environmental conditions within a wetland.

Internationally, considerable success has been achieved in determining the environmental condition of wetlands by comparing empirical measurements of the biota that a given wetland supports relative to the biota supported under natural or reference conditions for that wetland type; or for a given habitat within wetlands of a similar type (US EPA 2002a, Butcher 2003, Fore 2003).

The development of bioassessment metrics that consistently respond to human disturbance, and the resultant development of IBI's, has taken 20 years in North America (see literature reviews in Barbour *et al.* 1999; Karr and Chu 1999; Karr *et al.* 2000, Fore 2003). Successful biometrics can only be developed when ecosystems are well-researched and their ecological processes and interactions understood. Long-term data collection, modelling, testing and calibration are also necessary. The large array of macrophyte-based IBI's developed for wetlands in the United States bears testament to the natural environmental heterogeneity and the challenges this poses for the development of phyto-assessment techniques. The present study should therefore purely be regarded as the initiation of this development process for South Africa. The development phase is necessary in order to facilitate comprehensive environmental assessment and may well take several years.

In the South African context, although methods of bioassessment have been developed for riverine ecosystems (Dickens and Graham 2002, Kleynhans et al. 2007), there has been very little development of methods for the bioassessment of palustrine wetland ecosystems. In other countries, diatoms, invertebrates, algae, vascular plants, fish, birds and reptiles have all been used with varying degrees of success for the bioassessment of wetland environmental condition (e.g. Adamus *et al.* 2001, US EPA 2002a, Butcher 2003, Fore 2003). The use of plants to assess river reaches in South African is discussed in the following section, in order that any potentially useful findings from this work can be used for the development of phyto-assessment for wetlands.

2.5.1. Existing South African aquatic ecosystem phyto-assessment tools

2.5.1.1. "Riparian Vegetation Response Assessment Index"

The Riparian Vegetation Response Assessment Index (VEGRAI) is a bioassessment protocol that bases ecosystem condition on the departure of the riverine plant assemblage from an anticipated, and qualitatively determined, reference condition (Kleynhans *et al.* 2007). VEGRAI is based upon the perceived ecological importance and sensitivity to disturbance of any given riparian species in the specific region being assessed, and is determined by *a priori* observations of environmentally equivalent river reaches considered to be in reference condition. A quantitative determination of the abundance of these species in a river reach is used to determine the ecostatus of that reach. As such, it is reliant on a thorough knowledge of the riparian vegetation of an area in order to be able to determine, by 'expert judgment', which species are important and sensitive and thereby differentiate the condition of one river reach relative to another (Kleynhans *et al.* 2007). VEGRAI was not based on experimental determination of the characteristic cover/abundance of species under different disturbance conditions or associated ecostatus categories. Rather, it is based on expert opinion and working knowledge of riparian vegetation. If the depth of ecological understanding of wetland vegetation was similar to that which apparently exists for riparian vegetation, then the VEGRAI assessment approach could be adapted for use in wetland ecosystems.

A more exacting measurement of riverine vegetation was developed for the River Assessment Methods adopted in the United Kingdom, where considerable difficulty has been found in consistently relating proposed biometrics to environmental conditions (WFD-UKTAG 2008).

2.5.2. Wetland assessment tools using plants

No vegetation-based assessment protocols have been developed specifically for wetlands in South Africa. WET-Health does include a vegetation module that incorporates disturbance to vegetation and departure from an anticipated reference condition of the plant community as one aspect of the assessment of wetland condition (Macfarlane *et al.* 2008). In much the same way, the Wetland Index of Habitat Integrity (WIHI), incorporates a vegetation module in its determination of the present ecological state of wetland habitat (Rountree *et al.* 2007). The WIHI assesses the intensity and extent of land use with regard to its alteration of the natural vegetation cover. WET-Health and WIHI do not, however, claim to determine the condition of the biological community. The WET-health assessment tool was developed as a representation of the abiotic factors, such as changed hydrology or sedimentation patterns, as a measure of environmental condition. WIHI was targeted as a human-impact-based measurement of wetland habitat integrity. As in the case with VEGRAI, WIHI and WET-Health do not incorporate any experimentally-determined comparisons of the changes in wetland vegetation associated with different levels of disturbance.

2.6. The use of plants in bioassessment

2.6.1 Plant communities as useful indicators of environmental condition

Vegetation is almost always an integral part of any ecosystem and the only way that variations in vegetation and plant species distributions can be explained is within an ecological framework that incorporates an examination of change associated with changes in the environment (e.g. Tansley 1935; Kent and Coker 1992). A plant community is defined as: "*a group of plants characteristically associated together with greater affinity than would be expected by chance and having a predictable affinity to similar environmental conditions that would appear to result in the co-occurrence of a mosaic structure of characteristic plants occurring where environmental conditions within a region are repeated*" (e.g. Whittaker 1962, Kent and Coker 1992).

Early vegetation ecology was highly deterministic, and assumed that the combination of environmental factors at a site was the only control of the plant species growing there. Latterly it was established that properties relating to plant morphology and physiology, including the ideas of plant adaptations and population biology are also very important in determining which species occur at a site. Various interactions between plants influence the coexistence of species at a single site, the most important of which is inter-specific competition, which is known to limit the environmental range across which any species can become successfully established (Kent and Coker 1992). During succession, an important driver of plant community ecology, various plant interactions, such as competition and facilitation play differing roles in the development of a vegetation community after a disturbance. Thus the use of whole communities of plants, rather than the occurrence of single indicator species, has greater validity for the comparison of the environmental condition between two similar habitats in a given ecosystem. However, the most environmentally critical or sensitive species can also be indicative of the existence and environmental condition of a community.

Plant communities are fundamental biological components of many inland wetlands, (of which palustrine wetlands are a limited example) and as such, to a large extent they may be assumed to define the biological and ecosystem characteristics of these wetlands (e.g. Mitsch and Gosselink 2000, Keddy 2000). Increasingly, botanists have searched for patterns of plant community response to biotic and abiotic, as well as natural and unnatural alteration of the environment (Adamus *et al.* 2001). As a measure of environmental condition, direct assessment of biotic assemblages in aquatic ecosystems was initially instituted in rivers where there are international examples of the use of a whole range of plants for this purpose (e.g. Tremp and Kohler 1995, Nixon *et al.* 1996, Thiebaut and Muller 1998, Szoszkiewicz *et al.* 2006, Kleynhans *et al.* 2007, WFD-UKTAG 2008).

More recently, the phyto-assessment of wetlands has been developed, tested and implemented, predominantly through research in North America (e.g. Fennessy *et al.* 1998, Mack 2001, Simon *et al.* 2001, Lopez and Fennessy 2002, Gernes and Helgen 2002, Fennessy *et al.* 2004, Mack 2007). The successful development of phyto-assessment tools in many ecoregions of the USA suggests the potential for the adaptation of this assessment technique for use in other parts of the world including South Africa.

Plants are good indicators of wetland condition for many reasons. They are found in nearly all wetlands, although some extreme wetland environments do not support any vegetation (in South Africa for example, salt pans and deep water habitats contain no vegetation [Jones 2002]). Several plant species may occur together in communities with relatively high species richness but individual species show different stress adaptations, environmental tolerances and life-history strategies. The species composition of the plant community within a wetland can therefore reflect, often with great sensitivity, the environmental condition of that wetland (U.S. EPA 2002c). For instance, different groups of plants may respond rapidly, very slowly, or not at all, to environmental stimuli, thereby suggesting different sensitivities to environmental change. And, as they remain in the same position in the landscape for the duration of their lifetime and have variable response times to disturbance, plants are useful both for ongoing monitoring as well as for assessing the impact of specific events that have occurred, or are occurring at regular intervals (Adamus *et al.* 2001). Collectively, plants reflect the temporal, spatial, chemical, physical and biological dynamics of an ecosystem and as such, they may reflect any long-term, chronic stress acting on the ecosystem. Lastly, sampling techniques for plants are well developed and extensively documented, making them a viable source of information for the determination (via field studies) of changes to the environmental condition of an ecosystem.

Macrophytes are useful indicators of wetland environmental condition as they can be used as indicators of both the structural and functional components of ecosystem functioning, as explained below. The architectural structure of the plant community, be it dominated by trees, shrubs, herbs, or any combination of these structural elements, determines, to a considerable degree, the habitats available for other organisms. From a functional perspective, plants are involved in the energy flow and nutrient cycling within any ecosystem, thereby linking the inorganic environment with the biotic through the photosynthetic process (Keddy 2000, Mitsch and Gosselink 2000). Environmental impacts may change the structure of the plant community as, for instance, shrubs and other terrestrial plant species displace aquatic species due to long-term dehydration (e.g. Butcher 2003, Brock 2003). Comparison of characteristic assemblages of plants from wetlands with similar habitat but different levels of human disturbance should therefore reveal species and assemblage patterns with affiliations to specific environmental conditions. These may then be used as bioassessment metrics.

Despite the many advantages of developing phyto-assessment techniques, the following limitations have been identified:

- A time lag may occur in response to stress, particularly in long-lived plant species, and thus the presence of such species may not indicate the full suite of stressors presently acting on an ecosystem (US EPA 2002c, Butcher 2004). This is particularly evident with woody vegetation such as trees and shrubby species (except in situations of die-back due to excessive flooding), but can also play a role in long-lived herbaceous taxa such as tussock grasses (such as in the Restionaceae, a common family in wetlands in the Western Cape). However, it should be borne in mind that such a time lag can also be considered useful, as wetlands are dynamic ecosystems and long-lived extant vegetation may reflect the prevailing historical environmental conditions in spite of the current environmental conditions.
- Identification to species level can be laborious and time-consuming and requires specialist knowledge. Furthermore, the collection of identifiable samples is largely restricted to the flowering season when the presence of flowers makes identification possible. Field guides that include keys can, however, greatly assist in achieving accurate identification to species level but field guides have not yet been developed for a considerable number of South Africa's wetland plants.

- Sampling techniques for some communities, such as submerged plants, can be difficult; with the result that it is relatively easy to miss a group that could provide strong signals on the condition of a site (US EPA 2002c).
- Published research and literature on plant species responses to specific stressors is considered to be poorly developed in the USA, where a database was compiled from over 200 sources on 1106 plant species that occur in wetlands (about 17% of all U.S. wetland plant species) and 1128 non-wetland taxa and taxa of unknown affiliation that occur in the United States (Adamus and Gonyaw 2000, U.S. EPA. 2002c). Even less is known for South African plants where there is a significant need for research into the ecophysiological tolerances of wetland plants and their distribution (Mucina *et al.* 2006a).

2.6.2 The responses of wetland plant communities to anthropogenic stressors

Many studies have described the changes that occur in wetland plant communities in response to the environmental stressors described in Table 1. Examples of these changes are: hydrological alterations (e.g. Van der Valk 1981, Wilcox and Meeker 1991, Reid and Brooks 1998, Ellery *et al.* 2003), nutrient enrichment (e.g. Rich 1973, Cullen 1984, Templar *et al.* 1998, Craft and Richardson 1998), sediment loading and turbidity (e.g. Sager *et al.* 1998, Wardrop and Brooks 1998), and changes in the concentration of metals and other pollutants (for a summary see Coetzee 1995 and US EPA 2002c). Ultimately, plants respond to alterations in the structural, temporal/seasonal, spatial and biotic determinants of wetland habitats across the landscape by colonizing available habitat, changing growth form or life cycle stage, tolerating the changes or disappearing altogether (Brock 2003).

A number of the species responses and indicators of human alteration (*sensu* Section 2.4.2) of wetland ecosystems in the North American context could potentially be used to develop metrics (that could be adapted for??) for use in bioassessment in South African inland wetlands. These are listed in Table 2.2 It should, however, be recognized that these are generalised plant responses. In any given situation, specific responses are determined by the flora that are present and the types of human impact on the environment.

The above responses of plant assemblages to disturbance (Table 2.2) are examples of observations made by researchers (predominantly) in the United States. These results should in no way be interpreted as directly transferrable indicators of disturbed wetland conditions, and require further investigation. For example, monospecific stands of vegetation or communities dominated by annuals may equally reflect the natural or reference condition for habitats that have, respectively, naturally stable or seasonally fluctuating hydrology. The development of successful metrics for phyto-assessment in the North American States was achieved through empirical comparisons of the plant assemblages of reference and disturbed wetlands in order to extract characteristic vegetation patterns or species assemblages (e.g. Mack *et al.* 2001, Simon *et al.* 2001, Helgen and Gernes 2001, US EPA 2002a).

2.6.3 Vegetation attributes that are not useful for bioassessment

According to the North America literature (US EPA 2002c) the following have produced inconsistent indications of biological change in response to disturbance:

- Forest canopy species – due to their long response time;
- Individual plant health – no clear "dose-response" pattern has emerged with regard to individual plants, although Simon *et al.* (2001, after Karr *et al.* 1986) did attempt to use the presence of disease and deformity in individuals as indicators of disturbance. Field calibration of these conditions proved problematic, however; and

Table 2.2: The following responses of North American wetland plant communities to human stressors may be applicable to similar habitat types in South Africa (Adamus et al. 2001, US EPA 2002, Fore 2003). Additional citations in table.

Hydrological change • Changes in species richness (or diversity), e.g. dehydration may increase/decrease the number of species that can occupy an area; • An increase in the number and/or dominance of invasive and exotic species; • The domination of assemblages by one, (monospecific) or a few species; or by one structural type; • The presence of either very dense or sparse stands of vegetation: (e.g. in response to water levels being stabilized either lower or higher than normal); • The presence of species that move vertically with floodwaters or which grow quickly enough to keep their leaves above water; • Annual (*vs.* perennial) plant species tend to increase proportionately in response to drought and some other severe disturbances, and species richness tends to be lower where wetland communities are dominated by annuals.

Change in trophic state

- Either an increase or decrease in species richness; depending on the initial species mix, nutrient loading rates, season of addition and other factors;
- Over the long-term, nutrient additions to most wetlands tend to:
  - Reduce species richness;
  - Increase the dominance of a few (frequently non-indigenous) species

Increase in sedimentation rate of sedimentation

- A decline in seedling species richness is typical in wetlands receiving 0.25 m of sediment
  - determination of such change requires monitoring over a period of time;
  - Prevents shallow-rooted species from becoming established;
- The proliferation of non-native plants, ruderals (weedy and early succession species adapted to rapidly colonize areas with disturbed soils) and annual species:
  - Weeds become established, especially close to the source of sedimentation;
- A decrease in the biomass of *Carex* species (Cyperaceae or Sedge family) – which is further diminished by high water levels;
- A reduction in seedling recruitment:
  - Typha seedling density and biomass decreased as sediment levels increased from as little as 2-10 mm in depth
  - Germination rates of herbaceous plants are particularly negatively affected;
  - Less than 10 mm of sediment can inhibit the germination of the following genera that are also prevalent in South African wetlands: *Typha, Echinochloa, Leersia* and *Carex* (all grass-like taxa)
- Submerged species have been shown to be reduced by sediment.

Changes in acidity (pH)

- In general, species richness appears to increase with rising pH levels:
  - Increases in acidity (e.g. due to acid mine drainage) are likely to decrease species richness (possibly due to other factors, e.g. elevated levels of metal ions, salts;
- Conversely, the extremely high levels of biodiversity found in the Acid Sand Plain Fynbos on the Cape Peninsula may decrease with the anthropogenic elevation of pH levels (Cowling *et al.* 1992).

Changes in salinization

- Flooding with higher than normal concentrations of saline water causes biomass and seedling survival rates to decrease in some species, resulting in lower species richness;
- Decreased species richness can also occur as a result of increased salinity in saturated sediments (Brock *et al.* 2005)

Changes in turbidity

- Severe turbidity and the resultant reduction in light penetration into the water column typically shifts plant community structure away from submerged taxa towards floating and emergent taxa.

- The use of species that delineate the wetland-edge, as well as the concept of taxa with either facultative or obligate affiliations to the wetland habitat (Reed 1988, and section 2.9 in this Volume) have apparently not proven useful in determining wetland ecosystem condition in North America.

In contrast to the first two findings, the Australian Natural Resources Management factsheet on monitoring wetlands using plants, does suggest using measures of the vigour of individual trees as an indication of wetland condition. Estimations of the living proportion of the canopy foliage, evidence of water stress, chlorosis (the yellowing of plant leaves caused by a lack of chlorophyll pigment – usually due to mineral deficiency or disease) and pathogen activity are all used as indicators of the environmental condition of wetlands. Recent work using trees that depend on groundwater for their moisture needs has also shown that individual plant health has the potential to indicate the environmental condition of ecosystems (Schachtschneider 2010, Schachtschneider and February 2010).

2.6.4 Caveats on the applicability of phyto-assessment

The key hydrological variables that affect wetland plants include water depth, water chemistry, flow rate and connectivity to suitable habitats (Adamus *et al.* 2001, US EPA 2002c, Butcher 2003). These are all affected by natural dynamics (as a result of flow regimes and seasonal fluctuation in water levels), as well as by human disturbance. And thus the cause and effect relationship may not be easy to establish. The rate of change of disturbance is important for plant growth but, as it consists of many interactions, it is difficult to measure. Even under natural circumstances, in a wetland with high hydrological variability, the plant community is not stable over time (Wilcox *et al.* 2002). For these reasons Miller *et al.* (2005), DWAF (2004) and Butcher (2003, after Wilcox *et al.* 2002) suggest that phyto-assessment indices may be best suited to wetlands that have a stable hydrology, and are not exposed to marked natural disturbance such as the flooding regime of riverine wetlands. Due to their static position in a habitat, ground rooted plants are, however, adapted to natural seasonal hydrological variability (Adamus *et al.* 2001), such as is experienced in wetlands of much of the Western Cape (Jones 2002). Any metrics that are developed should therefore be tested for robustness with seasonality as a recognized consideration.

Natural disturbances such as drought, flood and fire, and other disturbances that cause the removal of vegetation or alter the landscape or geomorphology, all cause localized aseasonal changes that can entirely alter the composition of the vegetation over time (le Maitre and Midgley 1992). Localized and aseasonal disturbances may potentially decrease the comparability of sites when attempting to develop an index of wetland condition; as well as for the applicability of the index to sites of different post-disturbance age. Wetlands are dynamic systems that develop and decline both with regard to their hydrogeomorphological structure, or form (due to sedimentation and aeolian excavation), and their vegetation or biotic assemblages; and as such have no fixed state (Jones 2002, Breen pers. comm.). When considering successional change, the comparison of wetland plant communities of different post-disturbance ages poses a challenge in terms of interpreting their present environmental condition (e.g. le Maitre and Midgley 1992). The plant communities that are present at a site reflect the time since last disturbance as well as all the other local environmental determinants and inter-specific competition (Kent and Cocker 1992, le Maitre and Midgley 1992). In order to reduce the effects of such aseasonal (but sometimes completely natural) causes of variation, where possible, comparisons should be made between of wetlands of similar post-disturbance age.

2.7. International approaches to the phyto-assessment of wetlands

International approaches to phyto-assessment have generally used univariate or multivariate statistics for establishing the characteristic responses of the biota to human disturbance. Univariate analyses compare the abundance of an organism along a single environmental gradient; whilst multivariate approaches are able to compare the abundance of many different organisms along multiple environmental gradients simultaneously (see Section 2.10.5 for a further statistical explanation). Both of these approaches combine multiple measures of biotic response into an index of biological integrity (IBI) or of environmental condition.

2.7.1 Statistical methods employed in the development of bioassessment tools

Considerable information is available on the process of developing bioassessment tools in the US EPA "*Methods for Evaluating Wetland Condition*" report series. These reports are aimed at assisting the various USA state environmental agencies in developing bioassessment methods for their wetlands, as well as providing a summary of the information gained by the Biological Assessment of Wetlands Working Group (BAWWG) from many State-wide bioassessment programmes. The BAWWG method is essentially univariate in approach, relying on the identification of homogeneous sets of environments within which to determine characteristic responses by organisms to human disturbance. This approach creates a multi-metric index of environmental condition for wetlands of a particular habitat type in a given ecoregion, based on typical disturbances within that region.

In contrast to the univariate approach, the multivariate approach involves the simultaneous statistical analysis of an array of environmental variables together with biological data; thereby combining many potential gradients of disturbance and often including wetlands or habitats of different types. This allows the determination of those environmental drivers of change that best explain the strongest trends in the biotic community as well as specifying the particular species that are indicative of these trends. Multivariate approaches are considered useful for exploratory data analysis and as such have considerable application in the South African context, where so little baseline ecological information exists. A multivariate approach to bioassessment development was adopted in the Mid-Atlantic region of the United States, providing a useful compendium of information on bioassessment in aquatic environments (Fore 2003; and see Section 2.8 in this report). Although the research programme was based on streams and did not use plants as potential source of metrics, the insights gained from this broadly inclusive and multivariate approach to biological indicator development (which included many habitats and types of disturbances) are extremely applicable to the development of phyto-assessment indices.

The approach to wetland assessment in New Zealand has been limited to an indication of the change from the reference condition as a result of land-use; both within the wetlands themselves and in the area surrounding the wetlands (Clarkson *et al.* 2004). This approach also includes a determination of the dominance of indigenous, relative to alien plant species, and is similar in its approach to the vegetation module in WET-Health (Macfarlane *et al.* 2008) as discussed in section 2.5.1.

The approaches adopted in the various Australian states to wetland assessment do not incorporate a phyto-assessment protocol (Butcher 2003). The BAWWG bioassessment approach has been recommended for the potential development of bioassessment protocols in Australia (Butcher 2003). A plant-based monitoring protocol is published on the Australian government's Natural Resources Management website (NRM 2009). This is intended for use in monitoring wetland environmental condition but there is very limited information with regard to what constitutes assessment, or the measurement of environmental condition. Brock (2003) collated the information available on Australian wetland plant responses to natural and anthropogenic disturbances in an attempt to predict long-term management needs in order to maintain future biodiversity. Changes in nutrient availability, salinity, pollutants and particularly hydrology were included. Brock's work may prove useful in informing the development process of bioassessment protocols. There does not, however, appear to be a well-developed phyto-assessment method in routine use in Australia.

Similarly, wetland phyto-assessment tools appear to be poorly developed in Europe, with a greater focus placed on habitat and vegetation classification and the concomitant determination of indicator species (*sensu* EUR 15 1999 – see Section 2.9). The Mediterranean Initiative of the Ramsar Convention on wetlands (MedWet) also does not appear to have a protocol for the assessment of environmental condition using plants. A conference on Mediterranean temporary ponds (*sensu* EUR 15 1999) revealed that no phyto-assessment tools had been developed for this habitat type, and the primary focus of research was on their description and the collection of baseline data (Fraga i Arguimbau 2009). The limited European studies that focus specifically on phyto-assessment techniques for wetlands are based on a multivariate statistical approach (e.g. Ferreira *et al.* 2005, DeClerck *et al.* 2006).

In stark contrast are the statistical methods employed in the use of macroinvertebrates for bioassessment. Whilst there is a prolific use of multimetric bioassessment of streams in the USA, single metrics are used far more commonly in macroinvertebrate-based stream bioassessment programmes in Europe (Dahl 2004). When comparing the effectiveness of single metrics, multimetrics and multivariate approaches to bioassessment, Dahl (2004) states that this disparity is due, in part, to the early, widespread use of single metrics in Europe. Dahl's work (2004) supports previous conjecture that the multimetric approach gives a broader perspective of the ecological impacts of disturbance (e.g. Fore *et al.* 1996), and that single metrics are often aimed at detecting only a single type of degradation. Advocates of the multimetric approach suggest that reliance on combinations of multiple measures minimises the weakness of individual metrics that focus on only a single stressor (*e.g.* Barbour *et al.* 1999). Furthermore, it is suggested that one of the strengths of constructing a multimetric index is the possibility of ignoring factors that may cause redundancy, or metrics that are not correlated with disturbance. A further strength of the multimetric approach is that it incorporates ecological information on how aquatic organisms feed, reproduce, and exploit their habitats into assessments of water quality (Fore *et al.* 1996). On the other hand, critics of the multimetric method argue that potentially important ecological information may be lost by aggregating individual measures into a single index (*e.g.* Suter 1993). Comparison of the performance of single and multimetric indices and multivariate approaches, however, showed that multimetric and multivariate methods were better at discriminating impairment than single-metric approaches (Dahl 2004).

The Ramsar technical report on biological assessment protocols for wetlands predominantly lists assessments developed for river systems (Ramsar 2009). This report makes particular reference to a report by Nixon *et al.* (1996) that appears to cite only stream bioassessment protocols and does not deal with non-riverine wetlands. A web-based search of European and Ramsar-related websites revealed no information relating directly to the assessment of non-riverine wetlands using plants..

2.7.2. Use of international approaches in South Africa

The "*Methods for Evaluating Wetland Condition*" compiled by the BAWWG (US EPA 2002a) is currently the most comprehensive compendium of information available on the development of macrophyte bioassessment protocols for wetlands, and has been used extensively in the current study. As will be described in the following sections, however, the BAWWG univariate approach to phyto-assessment does not appear to be entirely appropriate for South African wetlands. This is due to the extreme heterogeneity of environmental conditions that occurs both within and among wetlands, even over relatively short distances. The standardized protocol of the BAWWG does, however, offer a useful bioassessment development framework in all other aspects. For the present study the BAWWG approach, together with elements of the multivariate vegetation analysis approach of the European studies of wetland environmental condition, the multivariate phytosociological approach of vegetation description (*sensu* Braun-Blanquet 1928, Westhoff and Van der Maarel 1978) and the lessons learned from the Mid-Atlantic area of North America (Fore 2003) have, where appropriate, been incorporated into the protocols developed in this study.

2.7.2.1. The BAWWG bioassessment development framework

The multimetric bioassessment approach was initially described by Karr (1981) as a means of assessing the biological integrity of rivers using fish. This approach, under the collective name of "Indices of Biological Integrity" (IBI), has been widely adopted as a 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, US EPA 2002c). A broad outline of a typical IBI development process (applicable to any specified biotic assemblage) is presented below. For reviews of this approach see; Karr 1981, 1991, Karr and Chu 1999, US EPA 1998c, and US EPA 2002b).

1. The study sites that are to be compared are established *a priori* based on their classification as wetlands of the same type and reflecting a gradient of human disturbance. The gradient of human influence is based on the relative ranking of sample sites based on an estimation of the degree of human disturbance, such as pollution and the physical alteration of the landscape from a natural state (Section 2.8). Sites should be as similar as possible with regard to their natural state. Thus, in theory, a single type of habitat is represented for the biotic assemblage being assessed. Therefore, in order to separate out sites with comparable habitat for metric development, the sites being compared should be classified as the same wetland type and should occur in the same biogeographical region.
2. Standardised sampling protocols are applied at each site; the protocol used depending on the assemblage being studied. During sampling the aim should be to collect a representative array of the biotic assemblage being studied at each site. If resources permit, the validity of the results is strengthened when two biotic assemblage types are assessed concurrently.
3. The biota should be sampled in the context of the ambient conditions, and correlations made with environmental parameters. Therefore, physical and chemical measurements of all associated habitats should be included in the sampling process. Because of the inter-seasonal variability in many of the environmental parameters within wetlands (such as hydrology and associated chemical changes [Wilcox *et al.* 2002]), all the sites should be sampled within a single season in order to allow comparison of the measurements. Similarly, the dynamics of succession suggest that

sites should also be comparable in terms of the time since their last disturbance events.

4. From a univariate analysis of the species and environmental data, various biotic responses or assemblage attributes, that reflect a change in value in response to a gradient of human disturbance (such as nutrient enrichment), are selected as metrics of environmental condition. For example, a type of plant that has apparently responded to the impact of human disturbance would be consistently more or less abundant, or cover more or less surface area, in wetlands that are more or less impacted. Similarly, species evenness, or diversity and other plant community measures that show a consistent correlation with a gradient of disturbance, are considered to be useful metrics of environmental condition. For example, Figure 2.1 shows a species richness response across a gradient of disturbance in urban relative to natural and agricultural environments in wetlands in Minnesota. The number of grass-like taxa decreases in response to elevated zinc levels (Helgen and Gernes 2001).
5. Biotic responses or attributes, once determined, can be used as surrogate measurements or metrics of ecophysiological conditions such as water availability, nutrient concentration and sedimentation, turbidity or overshadowing of the biota within a wetland (e.g. Gernes and Helgen 2002).
6. The final metrics that are used in the index must give consistent and ecologically relevant signals about the effects of human disturbance.
7. These metrics are combined into a multimetric array as proxy measurements of the environmental conditions within the wetland, and constitute an index of biological integrity (IBI) for the type of wetland under investigation. Increasing reliability is achieved as the number of metrics increases; thus a maximum of 12 and a minimum of 7 metrics is recommended (US EPA. 1998c). No redundancy (collinearity) between metrics should be included. For instance, the number of annuals, expressed as a percentage of all taxa, is usually the inverse of the number of perennials similarly expressed. The use of both metrics would include a redundant measurement in the assessment index. For practical applicability metrics should be reliably and easily quantified from field samples.

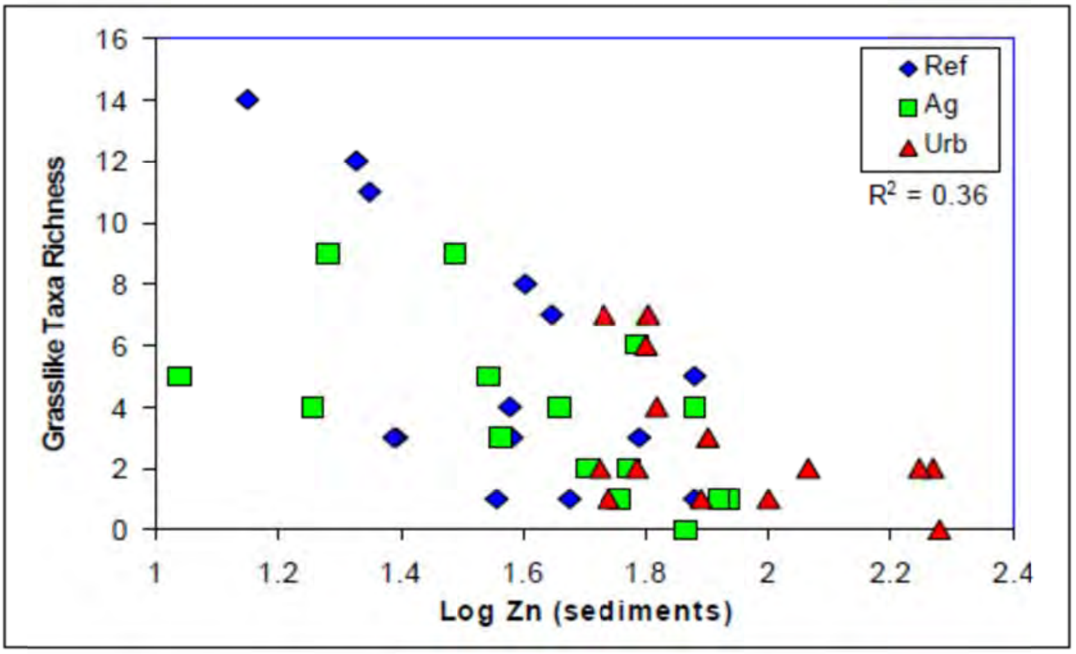


Figure 2.1. Scatter plot illustrating the response of a metric (grass-like taxon richness) to a single variable (zinc concentration), representing the gradient of human disturbance. R2 = Pearson's correlation coefficient; Ref = reference wetlands; Ag = wetlands with agricultural impairment; Urb = wetlands with urban impairment (after Helgen and Gernes 2001).

8. The combination of metrics into an IBI (essentially, an index of environmental condition) is achieved by assigning a numerical value (or a category) to each of the metrics. The following values are used: 5 (minimally impaired), 3 (moderate) or 1 (poor condition) according to how the biota in a test wetland have responded to human impairment of the ecosystem condition.
9. The combination of metric scores then gives a low score to those wetlands that are most impaired by anthropogenic disturbances. This score depends on the number of metrics that are identified for assessing integrity (i.e. 12 metrics x 1 = 12 being the worst possible score for the index) (see Figure 2). Scores are divided into integrity classes, which offer a categorical description of the impairment range for that ecosystem (e.g. reference, moderately disturbed, most impaired). Sites are thus classified into impairment categories based on their IBI scores.
10. In order to check that the metrics accurately reflect the level of environmental condition, they need to be tested on another set of data from the same series of wetlands. The additional test set of wetlands requires the same background

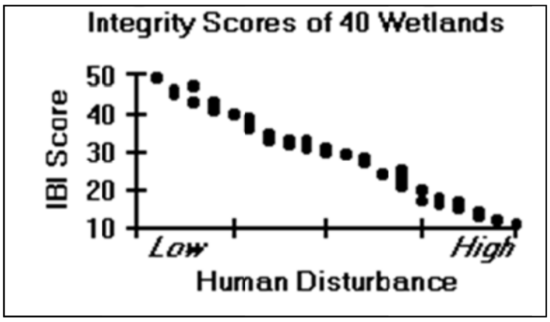
measurements of human disturbance and quantitative measurements of biotic and environmental parameters. An approach advocated by the US EPA (1998c) is to (1) randomly split the data into two halves, (2) develop the bioassessment index on one half of the data, and (3) test the index on the other half of the data. Results should be similar for both halves of the data set. During the development of the IBI process in the USA, it was found that between 30 and 40 wetlands provided a sufficient number of comparative samples for a given habitat to facilitate metric development. Another 30 were required to test the metrics.

11. The BAWWG (US EPA 1998c) suggests that the index scores from a homogeneous set of wetlands should form a relatively straight line when plotted against a gradient of human disturbance (Figure 2.2). Any wetlands that do not lie close to the line suggest:
    - misclassification of the wetland type (or habitat that is being compared), or
    - the failure to identify a stressor/disturbance impacting the wetland and its species assemblage; thereby under or over-scoring the human disturbance score; or
    - an index that is not applicable to all types of impacts.
    - Testing the index against another gradient of human disturbance also serves to determine whether the bioassessment is a robust indication of environmental condition (US EPA 2002b, Lopez and Fennessy 2002, Fore 2003).

According to Karr (1991), the analysis of IBI scores developed under these guidelines were intended to facilitate: (1) evaluation of the current biological conditions at any site within the same defining set of ecosystems; (2) determination of the trends over time at any such site where sampling has been repeated; and (3) comparisons between sites for which data are collected more or less simultaneously.

According to the following authors (Karr 1981, Karr *et al.* 1986, Plafkin *et al.* 1989, Barbour *et al.* 1999, U.S. EPA. 2002a, U.S. EPA. 2002b), the major scientific advantages of an IBI 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 the ability with additional monitoring to determine spatio-temporal dynamics); and
4. it incorporates professional judgement in a systematic and ecologically sound manner.



radient

Whilst the BAWWG approach appears to be statistically straightforward, it is underlain by the following specific assumptions:

1. That it is possible to identify a homogeneous group of wetlands, or habitats within wetlands, that constitute the target habitat for bioassessment.
2. That these wetlands/habitats would have similar communities of biota under reference (or similar) conditions – which implies uniformity within each wetland (assuming that the wetland is the sample unit).
3. That human disturbance (or at least the disturbance type used to develop the gradient of disturbance) is unidirectional in terms of its impact on the biotic assemblage.

The classification of wetland vegetation into separate units of vegetation habitat is no simple task in the Western Cape, however (see Section 2.9.3). It is sometimes extremely difficult, or not possible to determine homogeneous groups of wetlands or habitats using vegetation attributes as a starting point. In these situations, and those in which human disturbances are numerous, an exploratory data analysis based on a multivariate approach is more appropriate for metric and bioassessment development.

2.7.2.2. Advantages of a multivariate determination of biotic response to disturbance

The single region and single HGM-type approach is strongly advocated by the Biological Assessment of Wetlands Workgroup (BAWWG: US EPA 2002b). The experience of Fore (2003), amongst other researchers working on bioassessment in river systems, however suggests that the development of robust metrics across multiple HGM-types and biogeographical regions may be possible. The potential of the multivariate approach for establishing the way in which biotic assemblages reflect the prevailing environmental conditions, is superior to the univariate approach in complex ecosystems, or where multiple variables are being studied. This is due to the simplification or reduction of complexity that is required in univariate approaches in order to control for natural variability when discerning characteristic biotic responses. Inclusion of a wider variety of wetland types and their associated habitats also allows a broader understanding of the ecology of the system, and therefore potentially a more accurate interpretation of the distribution of the biota relative to the abiotic determinants. As the number of wetland or HGM types (and thus the number of environmental variables) increases, however, it is necessary to increase the number of wetlands sampled in order to provide an accurate reflection of the response of the biotic assemblage, as well as to maintain the reliability of predictions (Fore 2003). The multivariate approach therefore requires a comprehensive database of biotic and environmental factors in order to accurately represent a wetland's present environmental condition. Multivariate techniques also have advantages over univariate methods in terms of their predictive power, potential for exploratory data analysis and classification (Gerritsen 1995); their scientific rigour, regional applicability and the ability to distinguish specific stressors (Wright 1995, Reynoldson *et al.* 1997, Boulton 1999, Bonada *et al.* 2006). Exploratory multivariate analyses allow the identification of groups of wetlands and disturbances that can then be interrogated with univariate analyses. Multivariate analysis is, however, complex and often requires specialised practitioners. The results are also more difficult to convey (e.g. Gerritsen 1995, Reynoldson *et al.* 1997).

The stream-based Mid-Atlantic Integrated Assessment Program adopted an approach to metric development that incorporated many different habitat types, from different ecoregions (Fore 2003). Far more research effort has been focussed on the development of cross-regional metrics for riverine, rather than wetland ecosystems, with the result that most cross-regional metrics are riverine. This is evidenced by SASS in the South African context (Dickens and Graham 2002). The Mid-Atlantic region of the eastern United States, which includes New York, Pennsylvania, New Jersey, Delaware and Maryland is a broad area, incorporating many ecoregions, and in which a homogeneous set of wetland sites could not easily be defined. After a thorough testing process, metrics were developed that produced reliable responses across a broad array of environmental parameters, when a heterogeneous array of habitats and ecoregions were included in the data set (Fore 2003). This broadly inclusive metric development process was the culmination of many different research projects at hundreds of sites, performed by hundreds of people, over 6 years. The accuracy of the resulting metrics in reflecting stream condition is due largely to the enormous numbers of sample replicates upon which conclusions were based (Fore 2003).

2.7.3 Optimizing metric choice

The development of a multimetric index involves testing many potential biological attributes (candidate metrics), and then selecting only those that prove sensitive to, and that respond consistently to human disturbances. Although many attributes may need to be researched, there will be many spurious relationships when considering large numbers of disturbance-attribute pairings. Attributes that show considerable variation, (i.e. have a wide range of responses when data from a variety of sites are plotted), will be less useful. Attributes that show a consistent response across a number of independently-derived disturbance gradients suggest a biologically meaningful response. These can then be considered reliable indicators of the biological changes associated with disturbance (natural or anthropogenic), rather than occurring simply by statistical chance (Section 2.8.4) (Fore 2003). When there are a large number of metrics being tested to determine their applicability for inclusion in a phyto-assessment index, metrics may, however, be statistically significant by chance alone (Fore 2003).

Ideally, metrics that are incorporated into a multimetric index should (US EPA 2002b):

- Be relatively easy to measure and interpret;
- Change in a predictable fashion;
- Show a strong response to increased disturbance; and
- Give consistent responses across multiple disturbance measures;

Together the metrics should:

- Be sensitive to a range of anthropogenic disturbances; and
- Focus on more than one aspect of the community (e.g. species richness, diversity and cover or abundance);

Most importantly, it must be possible to use metrics to discriminate between human disturbances and the background “noise” of natural variability.

2.7.3.1. Macrophyte metrics for phyto-assessment

The responses of plants to anthropogenic stressors can be grouped according to the level of biological organization they reflect:

- Community-based (species composition/diversity/evenness or richness);
- Functional groups (based on the taxa acquiring resources by the same functional means; or on structural aspects of growth form, on life history strategies, or on their evolutionary attributes and their affiliation to wetland habitat) and;
- Species-specific metrics (indicator species or species that have high fidelity to a given community due to their sensitivity, limited environmental niche or tolerance range).

These various attributes of the plant assemblage offer different means of detecting the impacts of stressors. Various caveats are suggested by the BAWWG with regard to potential problems with the misinterpretation of such metrics or measures of environmental condition. For instance, ratios and sums of attributes can hide valuable signals, can be difficult to interpret, and are often more variable than individual attributes (Karr and Chu 1999).

The BAWWG proposed the following guidelines for metric development in an attempt to avoid masking valuable signals (US EA 2002b):

- In general, restrict ratios to relative abundance or proportions in which the total number of individuals or taxa is used as the denominator.
- Avoid using attributes that combine different functional or taxonomic groups of species, such as the number of emergent reed taxa divided by the number of aquatic taxa, as their responses to stressors may be very different, or occur for different reasons.

It is for this reason that the initial step in the process of determining whether an attribute will prove to be a useful metric is to test it independently of others, to see whether it shows a consistent and deterministic response to stressors.

For any ecosystem being studied, a fundamental understanding of the ecological processes that drive that system is required, in order to ascertain which plant community attributes will most usefully demonstrate changes to its environmental condition. Not all metrics work equally well for all systems, and thus testing is required for each new wetland type and in each region (Keddy *et al.* 1993). Below are some examples of metrics and indices that have been developed in North America. These are presented here in order to describe the rationale behind plant attribute choice and metric validity.

2.7.3.1.1. An example of the rationale used in the choice of metrics

The following is based on the testing of metrics for large depressional wetlands in a specific ecoregion in Minnesota, where a metric was created based on ‘graminoid species richness’ (Gernes and Helgen 2002). The following is the rationale that was followed, and the results are shown in Figure 2.1.

- The Poaceae, Cyperaceae and Juncaceae taxa (all graminoids) were considered to be structurally similar and also to occupy similar niches in the study wetlands.
- Furthermore, the Cyperaceae appeared to have low ecological tolerance to stress (Galatowitsch 1993) and indigenous taxa in these families are frequently among the first to disappear following human disturbance in North America (Wilcox 1995).
- There was a small but significant decrease in the number of these graminoid taxa with increasing levels of nutrient enrichment of the soil and water in 44 wetlands (Gernes and Helgen 2002). There was a similar decrease in the number of grass-like taxa with increasing zinc concentration (Figure 2.1).

This ‘graminoid species richness’ metric was, however, able to differentiate only the most severely nutrient enriched wetlands from wetlands with natural or reference nutrient levels. Taxa, such as the graminoids, that show limited response to human stressors can thus only be expected to differentiate between extremes of environmental condition.

2.7.3.1.2. Limited knowledge-based metrics

The same Minnesotan assessment determined that a stronger response (than was provided by the graminoid species richness metric described above) to the same nutrient gradient, was provided by a metric evaluating the decrease in taxon richness of the most sensitive taxa, or those most susceptible to human disturbance (Gernes and Helgen 2002). Taxa that were unique to, or occurred in two or more of the reference wetlands and occurred in only one impaired site, were considered sensitive. Non-indigenous species meeting these criteria were, however, excluded. This ‘sensitive taxa-species richness' metric requires no prior knowledge of the vegetation in an ecoregion, other than the identification of alien taxa.

In Minnesota, certain taxa were identified as 'tolerant'. This was based partly on the reported responses of plant species to human disturbance (e.g. Wilcox 1995) and also on field observations which showed that reference wetlands had proportionally fewer tolerant taxa. All non-indigenous taxa were considered tolerant. "Percent tolerant species" values were developed as a proportion of the total number of taxa in the sample. The 'tolerant taxa' metric is thus based on prior knowledge of the species response to stressors, and relies on the field experience of the assessor.

Metrics such as these should work in the South African context, provided that the ecoregional and wetland classification procedures successfully split reference wetlands into distinct physiogeographic units with biotic similarity.

2.7.3.1.3. Knowledge-intensive metrics

A more knowledge-intensive metric concept that will require a greater knowledge base of wetland plants before it can be effectively applied in the South African context, is the Floristic Quality Assessment Index (FQAI). FQAI scores have proved to be successful metrics for detecting disturbance in wetlands in North America (e.g. Andreas and Lichvar 1995, Wilhelm and Masters 1995, Herman *et al.* 1996, US EPA 2002c). The index relies on comprehensive species lists , and a specialist knowledge of the phytosociology of an area, as the fidelity of species to communities needs to be known. The establishment of such a FQAI tool in South Africa would require comprehensive phytosociological studies to be carried out in all the regions or ecosystems in which this index was to be applied. Furthermore, each species would need to be assigned to a numerical class indicating the fidelity of that species to a given community. This would be an enormous undertaking and would take specialist wetland botanists many years to complete. The WRC project K5/1980 of Sieben (2010) in which South African wetland vegetation is being widely surveyed will go some way to providing the knowledge required for this type of knowledge-intensive metric development.

<u>2.7.3.1.4. Indicator species</u>

In South Africa, very little is known about what species indicate in terms of the environmental conditions in wetlands, other than the affinity for saline conditions and for hydrological zones of wetness. That which is known is predominantly due to research into river systems and estuaries (see O'Keeffe 1986, Rogers 1995). A list of wetland-affiliated species and some of their distributions and characteristics is included in the work collated by Glen *et al.* 1999 and updated for the Western Cape (see Appendix 5) in this present volume. Some of the known associations between wetland taxa and habitat are recorded in the 'Vegetation of South Africa, Lesotho and Swaziland' (Mucina and Rutherford 2006) and are divided into 'important' and 'endemic' taxa. The limited amount of phytosociological data and the resultant lack of classification of the wetland flora in South Africa means that classes of wetland vegetation are broad, or coarsely defined and species that are recognized as the indicators of given habitat are few in number and have broad and catholic distribution (Mucina *et al.* 2006a).

2.8. Linking human disturbance to biological change

Phyto-assessment is intended to ascertain the impact of humans on wetlands, facilitating sustainable utilization and conservation (Finlayson *et al.* 2002). Disturbances that result in elevated nutrient concentrations can be quantified by the direct measurement of nutrient ions (e.g. nitrates and ortho-phosphates). Other disturbances such as physical habitat modification may have no chemical signature or may not be quantified by the measurement of constituent concentrations. Such physical disturbances can be quantified by determining the extent and intensity of human activities and land-uses in and around wetlands. Together these quantifications of disturbance provide dual means of ranking wetlands from least to most impaired in terms of their environmental condition. This step is a prerequisite in the identification of metrics. The following section links anthropogenic disturbance to the alteration of the biotic component of wetlands. Furthermore, justification is provided for the use of this approach for ranking wetlands into categories of impairment in order to facilitate the development of phyto-assessment indices.

As is the case for any scientific inquiry, understanding how human disturbance influences, alters, and degrades biological processes and biotic assemblages is an iterative process. In nature it is rare that any two ecosystems classified as being of the same type will be exactly the same. And when the effects of changes in environmental parameters (such as those caused by anthropogenic disturbances) are expected to be large, then investigation into the impacts of single (un-replicated) disturbances may be the only option (Hurlbert 1984). Experience suggests that the same biological parameters tend to correlate with human disturbance in very different geographic settings. For example, a decline in taxon richness is often correlated with an increase in the abundance, and eventual dominance of tolerant taxa and the disappearance of taxa with unique habitat requirements (Fore 2003). Because biological systems are complex and human disturbance is multidimensional, single causes and mechanisms of impairment are difficult to isolate. As a result, much of the evidence for human degradation of natural resources is correlative. For instance, in Minnesota (Figure 2.1) it is apparent that the species richness of grass-like taxa decreases with increasing zinc concentrations typical of wetlands with urban influence. The Pearson correlation coefficient of $R^2 = 0.36$ suggests that this is significant but that only 36% of the variability seen in the richness of grass-like taxa in the wetlands of the different areas is explained by the correlation . Without a direct causal link between stressor and biotic response, concerns have been raised that circular reasoning has been used in the development of metrics. Thus, metrics may be selected as indicators of human disturbance simply because they are correlated with human disturbance (Suter 2001). Metrics proposed as biological indicators of human disturbance may in fact be completely unrelated to the degradation of biological integrity or environmental condition but rather, may purely reflect differences in the availability of natural habitat. A statistical trend does not necessarily indicate a causal effect. It is also possible that the observed patterns of biotic assemblage may be due to variables that were not considered and therefore not measured. Human land-use patterns tend to follow landscape features, thus potentially confounding human activities with environmental parameters. For effective biotic metric development therefore, the challenge is to demonstrate that human disturbance is the most likely causal agent of biological change (Fore 2003).

Fore (2003) provided a variety of safeguards to help reduce the probability of drawing unsubstantiated conclusions when developing bioassessment metrics from data gathered from a number of ecoregions. The safeguards listed below are, however, considered equally useful in the development of metrics within a single ecoregion. The following approaches can be used to help isolate the relationship between human disturbance and biological change from other confounding influences.

1. Randomizing site selection across a large geographic area helps to ensure that the sample is representative of all possible sites. Unbiased selection of sites provides some protection against the effects of human disturbance being confused with other natural features (Stewart-Oaten *et al.* 1986, 1992). Numerous types of wetlands would thus be included in the data set; however, therefore necessitating the use of multivariate statistical analysis. If univariate analysis were to be used, all wetlands would first have to be typed before randomly choosing which ones to sample.
2. The chance of circular reasoning is reduced if the measures of disturbance are selected without a consideration of the apparent changes to the biotic assemblage.
3. Metrics tested on data collected over a number of years, or on a part of the data set reserved for an independent test of the final index, tend to have greater consistency in their response to disturbance.
4. Testing metrics for correlation with multiple gradients of human disturbance allows the development of metrics that are consistently associated with more than one measurement of human disturbance.
5. Factors such as the relative amount of habitat availability, or elevation, which could underlie patterns of both human influence and biological assemblage, should be explicitly tested to look for correlations that would confound metric development. Where necessary these effects should be removed by data partition. In this way, metrics can be selected for their association with disturbance rather than with other natural features (Fore 2003).

When coupled with effective metric testing (Section 2.7.3), these procedures reduce the potential of spurious conclusions being drawn as to the underlying causes of, and possible inferences from, wetland plant assemblage patterns.

Criteria based on logically derived causal inferences (Beyers 1998), were used in developing the Mid-Atlantic States biotic index, in order to avoid circular reasoning in the connections between human disturbance and the biological degradation of fish, invertebrate and diatom assemblages (Fore 2003). Together with the testing of metrics across numerous independently-measured gradients of human disturbance (Section 2.8.4), these criteria assist in reducing the chance that spurious associations are drawn between observed changes in biological assemblage and human disturbances. The criteria used were:

1. **Strength:** when exposed to similar disturbances, a larger proportion of the sampling units should be affected (having similar biological disturbance responses) than the reference samples.
2. **Consistency:** the effects of stressors/disturbances on biological metrics should be consistent with results observed by other scientists in similar situations.
3. **Specificity:** the effect on the biota should be diagnostic of exposure to a specific stressor, e.g. the decrease in *Typha* seedling survival and resultant abundance in a species assemblage as a result of increased sedimentation (See Table 2 in Section 2.6.2).
4. **Temporality:** the exposure to the stressor must precede its effect on the biological assemblage: Evidence of human disturbances tends to persist, thus it can be reasonable to conclude that exposure to a disturbance preceded the biological change (see point 6).
5. **Dose-response:** the intensity of the observed effect is related to the intensity of the exposure, with biotic change responding in proportion to the intensity of disturbance, e.g. Figure 2.1 (Section 2.7.2.1) suggests an increased change in the biotic assemblage with increasing zinc concentrations.
6. **Plausibility:** a plausible mechanism links cause and effect: For example decreased seasonality of dehydration of the Kuils River floodplain as a result of increased water flow from the Tygerberg Waste Water Treatment Works caused an increase in the cover and abundance of *Typha* in these previously seasonal wetland areas (Hall 1993).
7. **Evidence:** a valid experiment provides strong evidence of causation*:* there are many physiological experiments that have correlated wetland plant response to stressors associated with human disturbances, particularly nutrient enrichment (e.g. Boutin and Keddy 1993, Weiher and Keddy 1995).
8. **Coherence:** The hypothesis that human disturbance causes biological degradation does not conflict with existing perception of our ‘knowledge’ or experimental evidence (e.g. Adamus *et al.* 2001).
9. **Exposure:** indicators of exposure must be found in affected organisms: for example ”functional indication of wetland plants exposed to nutrient enrichment include increased leaf tissue nitrogen and phosphorus content and increased biomass production and stem height” (US EPA. 2002d).

2.8.1. The reference condition and pragmatic benchmarking of disturbance

The development of a phyto-assessment index requires the determination of characteristic patterns of response by the biota to human alteration of the natural functioning and environmental condition of a wetland (US EPA 2002a). Patterns of response are due to changes in specific attributes or characteristics, such as an increase in the surface area covered by graminoid plants tolerant of increasing levels of disturbance (for example, Figure 2.1 and other responses in Table 2). If there is sufficient information about plant community composition under natural conditions then measurement of the difference between that natural condition, used as a reference community, and another community from the same habitat type provides information about the environmental condition of the latter community (Yoder and Rankin 1995, Karr and Chu 1999). To discern the response of biota to human disturbance it is necessary to compare examples from reference ecosystems to those altered by human influences. Assessment of many such wetlands provides sufficient samples (*albeit* pseudo-replicates, as in nature no replicate is exactly the same as another) (Hurlbert 1984, Stewart-Oaten *et al.* 1992]) to determine consistent patterns of species/assemblage response to the same stressor (e.g. US EPA 2002b, Fore 2003). Certain types of wetlands may no longer exist in a completely natural state, and thus wetlands that are considered to be ‘least impaired’ are used as the reference condition. To determine useful indicators, the rest of the wetlands in a study group must make up the remainder of the gradient of disturbance and may vary from a moderately impacted to severely impaired (US EPA 2002b).

2.8.2. Quantification of human disturbance

The approach recommended by the BAWWG to IBI development involves the study of plant communities in wetlands with (1) the same environmental parameters that (2) differ only in the magnitude of one supposedly unidirectional anthropogenic impact (or single stressor), such as nutrient enrichment. This approach is adopted with the idea that it would provide a unidirectional gradient of impact along which wetlands of the same type can be aligned and compared (US EPA 2002a) (see Section 2.7.2.1). In contrast, the examples of human disturbance measurement presented by the BAWWG (e.g. Mack *et al.* 2000, Gernes and Helgen 2002) are based upon studies that developed successful IBIs by combining multiple disturbance gradients into a cumulative disturbance score. Similarly, the bioassessment index-development process for streams in the Mid-Atlantic States also combined multiple disturbance gradients.

In wetland assessment, direct and indirect impacts from the whole catchment, as well as from the area immediately surrounding wetlands and within the wetlands themselves, have been used to characterize the extent and intensity of (and thereby rank) the anthropogenic disturbance impacting on wetlands (e.g. Bryce *et al.* 1999, Mack *et al.* 2000, Gernes and Helgen 2002, Fore 2003, Macfarlane *et al.* 2008). Both natural and human-induced stressors and disturbances can occur in a single wetland and at different spatial scales in and around the wetland. Only the anthropogenic stressors, however, should be included in determining the environmental condition of a wetland relative to natural conditions (Finlayson *et al.* 2002). Human disturbance that results in the impairment of the natural environmental condition can be the result of many different types of stresses and ecosystems such as wetlands may be subject to multiple stressors (Fore 2003). Stressors are not necessarily cumulative or unidirectional in terms of their impact on the functions and biota of an ecosystem. Different stressors can act in different ways: they may be synergistic, antagonistic or additive in terms of their impact on the habitat and the response of the plants. For instance, human interventions that change the rates and amounts of water influx (e.g. increased hard surface area in a catchment) or outflow (e.g. drainage in the wetland or of surrounding land) can have different and even opposite effects on depths and on the residence time of water in a wetland. These different types of disturbances have, however, been integrated and used in a cumulative way to rank wetlands in terms of the amount of disturbance to natural function and condition in many assessment protocols (e.g. Bryce *et al.* 1999, Mack *et al.* 2000, Mack 2001b and 2007, Gernes and Helgen 2002, Rountree *et al.* 2007, and Macfarlane *et al.* 2008)

2.8.3. Human disturbance: gradient versus categories

Anthropogenic disturbance is complex and human activities and their resultant ecological stressors are multidimensional, so a challenge exists in integrating disparate measures of human influence into a single axis of human disturbance for metric testing.

Fennessy *et al.* (2004) argue that the most effective methods for measuring environmental condition provide a quantitative measure that describes where a wetland lies on a continuum ranging from full ecological integrity (best ecosystem condition) to highly impaired (poor condition). This is done by means of assigning a numeric score that allows correlational-type comparisons to be made between wetlands (as for example in Figure 2). This approach has been used in the development of all of the IBIsunder the direction of the BAWWG (US EPA 2002a, e.g. Mack 2001, Simon *et al.* 2001, Gernes and Helgen 2002, Fore 2003, Miller *et al.* 2005). On the other hand, in a critique of the concept of indices as effective measurements of environmental states, Suter (1993) cautions against taking the values assigned to such indices too literally. He suggests rather that ecological understanding should be sought to explain the stressor-biota interplay. A numerically-based score of disturbance suggests that there is a single and continuous gradient of change that can be measured. This despite the fact that stressors can interact with each and that the overall impact may be the result of additive, synergistic or antagonistic effects. Nevertheless, a numeric approach is a pragmatic, and widely-used attempt to simplify the complex interplay of environmental stressors and ecosystem responses in order to determine which wetlands are more or less altered from a reference state (e.g. US EPA 2002a). Separation of the gradient of disturbance into distinct categories such as "reference" (least disturbed), "moderate" and "worst" levels of disturbance, reduces the focus on the numerical score and yet still facilitate comparisons between systems with similar amounts of disturbance. Furthermore, some of the complexity of human disturbance can be resolved by testing metrics against both the cumulative amount of human disturbance and also against alternative measures of human impact on these ecosystems (Fore 2003).

The idea of disturbance being a continuous gradient, as considered by BAWWG (Section 2.8.2), requires a univariate, linear search for attributes of the plant assemblage that correlate with disturbance. This correlational approach makes sense only if the assumption of the homogeneity of ecosystem habitat type and biotic assemblage are upheld under reference conditions. Where there is environmental variability among the sites being compared, there is a lack of linearity of the natural drivers of change due to disturbance, and therefore human stressors are equally unlikely to cause linear responses (Fore 2003). In such a situation, a multivariate analytical approach is essential.

Statistically, it is simplest to compare measures taken at impaired sites with the same measures taken at minimally disturbed reference sites (sensu Mack 2000, Gernes and Helgen 2002, US EPA 2002a). When only a few measurements of disturbance are made, statistical testing may involve simple tests of differences in means (ANOVA) or association between one-dimensional measures of disturbance (regression or correlation: Helgen and Gernes 2001, Gernes and Helgen 2002). When multiple, related measures of disturbance are made, however, multiple regression may be used to test the disturbance measures together. Rather than creating a single axis of human disturbance for metric testing, greater statistical rigour can be achieved by testing multiple independently-derived measures of human disturbance (as mentioned in Section 2.7.3) at different spatial scales. The cumulative-type assessments of the intensity of human-induced impacts on an ecosystem (Bryce *et al.* 1999, Macfarlane *et al.* 2008) can also be supplemented by measurement of individual stressors such as physico-chemical measurements.

2.8.3.1. Cumulative measures of disturbance

From the lessons learned from the mid-Atlantic States it was proposed that metrics should be correlated with several different types of disturbance measured at multiple spatial scales (Fore 2003). For streams in the Mid-Atlantic States, specific anthropogenic stressors tended to be more highly correlated with measures of human disturbance that integrated many aspects of disturbance rather than with similar measures that measured only a single aspect of disturbance. For example, correlations were few between substrate particle size, turbidity, riparian vegetation condition and riparian disturbance, but all correlated with a habitat index developed to integrate measures of site condition at the reach scale. Similarly, water chemistry parameters such as total nitrogen, total phosphorus, ammonium (NH_4^+) and sulphate (SO_4^{-2}) showed fewer significant correlations with each other than with integrative measures that summarized human influence at the watershed scale. Uni-dimensional measures of disturbance correlated predominantly with the percentage of disturbed land in the catchment (agricultural, urbanized or mined); suggesting that land-use may be a useful surrogate to indicate overall disturbance (Bryce *et al.* 1999, Fore 2003). Similarly, biotic indicators of disturbance, including multimetric indices for fish, diatoms and invertebrates showed a higher correlation with the cumulative measures of disturbance (that integrated many stressors), than with specific stressors. Thus assessments of disturbance that integrate multiple measures of site condition over multiple spatial scales best captured the cumulative effects of human influence (Fore 2003). This suggests that much of the variability observed in patterns of biotic assemblage that are apparently due to single stressors or aspect of disturbance, (such as phosphorus or zinc seen in Figure 2.1), may, in fact, be associated with multiple levels of disturbance. One-dimensional measures of disturbance (e.g. Figure 2.1), often fail to capture the cumulative influence of human activities on the biota (Karr *et al.* 2000, Fore 2003).

Research in North America and more recently in South Africa has shown that the conversion of land from natural ecosystems to human use is correlated with increased impacts on environmental parameters. Furthermore, there is an increased change in the environmental condition of proximal ecosystems (Bryce 1999, Adamus *et al.* 2001, Macfarlane *et al.* 2008). No single method has been developed to quantify human disturbance specifically, but surrogates such as the percentage of urbanization, mining, silviculture, agricultural activity (U.S. EPA. 2002b, Fore 2003) and percentage cover of alien or ruderal plant species within a wetland (Macfarlane *et al.* 2008) have been used. The use of plant-based measurements of disturbance can lead to circular reasoning when developing a plant based IBI and are best avoided when measuring disturbance. To integrate the many and various human disturbances and thus standardize the evaluation of anthropogenic impacts, a method of determining a cumulative score of disturbance was developed in the Mid-Atlantic States (Bryce 1999), Ohio (Mack 2001b) and in Minnesota (Gernes and Helgen 2002). In Minnesota, this scoring process is known as the Human Disturbance Score (HDS) and (for future reference) this name is adopted in the present project. Similarly in South Africa, the Index of Wetland Habitat Integrity (Rountree *et al.* 2007) and Wet-Health (Macfarlane *et al.* 2008) both integrate many human stressors into an overall score or category that reflects the level of wetland disturbance. In all of these measures of wetland condition, the number, type and intensity of all perceptible human-induced alterations to the target sample site and surrounding dryland areas are assessed (at various spatial scales) and ranked to create a relative index that scores disturbance levels for each site. As described in section 2.7.2.1, in Ohio and Minnesota, as for other BAWWG-based IBI's the HDS, considered as a single gradient of disturbance, is then compared with quantitative measures of various aspects of the biota (e.g. species richness, diversity, percent cover and/or abundance, stem diameter and shrub/tree densities) to search for metrics of biotic response to disturbance (US EPA 2002a).

2.8.3.2. Disturbance at the catchment-scale

Disturbances at the watershed or catchment level, rather than in the immediate vicinity of a wetland, can impact on the environmental condition of that wetland (e.g. US EPA

2000a, Fore 2003, Clarkson 2004, Rountree *et al.* 2007, Macfarlane *et al.* 2008). North American research into the development of bioassessment tools suggests, however, that impacts to the landscape immediately surrounding, and within the wetlands themselves, are sufficient to differentiate between reference and anthropogenically-altered wetlands (Mack *et al.* 2000, Gernes and Helgen 2002). When investigating the best method of mitigating human impacts on man-made wetlands with high conservation value in Belgium, Declerck *et al.* (2006) reported that the effects of impacts within the immediate vicinity (<200 m radius) of a wetland had the greatest effect on wetland functioning and condition.

2.8.4. The determination of human disturbance level used in the present study

WET-Health (Macfarlane *et al.* 2008) and the WIHI (Rountree *et al.* 2007) assessment tools require expert judgement, and are used to make qualitative assessments of the intensity of human disturbance to the wetland, as well as of wetland habitat condition. Both processes include an assessment of the broader catchment, the immediate surrounds and the wetland itself. These tools are considered to be 'rapid' (*sensu* DWAF 2004) assessment protocols taking up to two days to complete for each wetland assessed. This was considered too time-consuming for the present study and furthermore, the development of neither WET-health nor WIHI had been completed when this project started. For this reason, it was decided that a new, rapid-assessment method was required for this project that could be used to assess the level of human alteration and disturbance in the immediate vicinity of the study wetlands. The WET-Health and WIHI (draft) methods were amalgamated with those of the Ohio wetland bioassessment program (Mack *et al.* 2000) and the biological monitoring program developed in Minnesota (Gernes and Helgen 2002), which were designed to be completed within a few hours in the field. The Western Cape Wetlands Inventory Datasheet (Dallas *et al.* 2006) was used as a template from which to combine these various human disturbance assessment methodologies (see field sheet in Appendix 1 and Section 3.5.4 for method development).

2.8.5. Nutrient concentrations as indicators of human disturbance

Whilst not all stressors and human land-uses will have a quantifiable physico-chemical signature, nutrient load in sediments or in the water column represent measurable quantities that may indicate human impairment of environmental condition.

2.8.5.1. Primary nutrients limiting plant productivity in wetlands

According to Liebig's Law of the Minimum, the growth/productivity of plants is limited by that factor (usually chemical) that is present in the least amount in proportion to requirements (von der Ploeg *et al.* 1999). Phosphorus (P) and nitrogen (N) are considered to be the primary nutrients limiting productivity in wetlands in many countries (e.g. US EPA 2002d, Dallas and Day 2004). Because phosphorus is generally much less soluble than nitrogen, it is leached from the soil at a slower rate than nitrogen. Consequently, phosphorus is often much more important as a limiting nutrient in aquatic systems (Smith *et al.* 1999). Changes in the availability of N and P are usually responsible for changes in ecosystem function and community composition. Plants that are tolerant of increased availability of these nutrients out-compete intolerant taxa which, over time can result in a change in the wetland species composition. Increased availability of N and P also results in functional changes in wetlands, such as increased storage of these nutrients in the tissues of wetland plants and increased biomass production as species that thrive under elevated nutrient levels come to dominate the habitat. In turn, ecosystem processes such as decomposition, accumulation of soil organic matter and organic carbon exports are affected (US EPA 2002a). Such changes in plant community composition and ecosystem process compromise wetland ecological condition by altering energy flow, nutrient cycling, and niche/habitat characteristics thereby impacting wetland faunal assemblages (e.g. Gernes and Helgen 2000, Mack *et al.* 2000, Fennessy 2004). Elevated phosphate levels in particular are known to be responsible for eutrophic conditions that lead to algal blooms in South African wetlands (Dallas and Day 2004). Elevated concentrations of inorganic nitrogen and phosphorus have seldom been recorded in the water column of un-impacted wetlands in South Africa and this is particularly the case in the Western Cape (Malan and Day 2005b). Algae obtain nutrients directly from the water column and hence are probably more suitable than macrophytes to assessment of water quality. Rooted macrophytes have, however, been used as a means of determining changes in nutrient loading. Floating or submerged aquatic plants, particularly algae, rapidly and efficiently remove nutrients, particularly ammonium and orthophosphate and to a lesser extent nitrate, from oligotrophic waters. Rooted plants, on the other hand, more readily utilise the oxidised form of inorganic nitrogen, namely nitrate (McColl 1975, Wiechers 1985). ‘Indicators of structural change’ or shifts in community composition such as the relative dominance of certain species and the presence or absence of sentinel species (species that act as an indicator of certain conditions) reflect ambient nutrient loading and are known secondary biological responses to a change in trophic level (Keddy 2000). Substrate geology, soil types and associated soil nutrient load are therefore considered potentially informative about the distribution and patterns of community assemblage for substrate-rooted wetland plants in the present study.

Eutrophication, namely the increase in biological productivity, caused by the loading or elevation of nutrient concentrations (Chorus and Bartram 1999), is one of the major threats to the environmental condition of wetlands (Section 2.4). Furthermore, the concept of nutrient enrichment resulting in eutrophication of the water column has long been accepted as a potential impact of human disturbance. More recently (Pullin 2002, Gan-Lin Zhang 2006), it has been recognised that very similar processes impact on soils and that soil eutrophication is also an international problem. The term “eutrophication” and the concept of “trophic states” are therefore applied to the impacts caused by nutrient enrichment in both the soil and the water column. In naturally nutrient-limited ecosystems, the primary effect of elevating nutrient availability is to increase the net primary productivity (biomass) of a few rapidly adaptive species, with a concomitant reduction in overall biodiversity (Coetzee 1995, Keddy 2000, US EPA 2002d). The higher the nutrient loading in an ecosystem, the greater the potential ecological impacts, with specific impacts dependent on which plants are stimulated to grow. Increased biomass of certain emergent vascular taxa such as *Typha* and *Phragmites,* have been recorded at moderate levels of nutrient enrichment (Cowan 1995, Bailey *et al.* 2002). An increase in extent of *Typha capensis* stands at Rondevlei Nature Reserve, near Cape Townwas attributed to eutrophication of the water and sediments of this vlei (wetland), although this conclusion was not unequivocal (Semmelink 1990).

2.8.5.2. An assessment of the nutrient status of wetlands

The largest proportion of nitrogen is insoluble organic nitrogen. Kjeldahl nitrogen is the sum of organic nitrogen plus ammonia (NH_3) and ammonium (NH_4^+). Soluble inorganic nitrogen consists of nitrate (NO_2^-), nitrate (NO_3^{-2}) and ammonium. The forms of inorganic nitrogen present in water are ammonia, ammonium, nitrite and nitrate. This includes the dissolved forms of inorganic nitrogen and those adsorbed onto suspended organic and inorganic matter. Ammonium and nitrate are the forms of nitrogen most readily assimilated by plants. Nitrite is the inorganic intermediate, and nitrate the end product of the oxidation of organic nitrogen and ammonia. Nitrate is more abundant in aquatic ecosystems as it is more stable than nitrite. Inorganic nitrogen concentrations in un-impacted well oxygenated aquatic ecosystems are usually low; as available N is rapidly taken up by the plants (DWAF 1996).

Ammonium is usually a minor component of dissolved nitrogen compounds in natural waters, because it is converted to nitrate and nitrite through aerobic bacterial activity. The relative proportion of ammonium to total dissolved nitrogen increases under reducing conditions, which are common in saturated wetlands with highly organic soils; and in eutrophic, and often turbid conditions in which, the breakdown of large amounts of finely divided organic matter such as decaying algae uses up available oxygen.

Phosphorus occurs in both dissolved and particulate forms. Particulate phosphorus includes that fraction that is bound up in organic compounds, and the fraction adsorbed to suspended matter such as clay and detritus. Under oxidising (aerobic) conditions phosphorus readily interacts with a number of cations (e.g. Al, Fe, Ca) and precipitates out of the water in insoluble compounds. Phosphorus may also adsorb onto soil colloidal particles, organic matter and sediment particles, and in shallow, vegetated wetlands therefore it tends to accumulate, mostly in forms which make it unavailable for uptake by plants (DWAF 1996, Batchelor *et al.* 2002, Dallas and Day 2004, Malan and Day 2005b). In the dissolved form, phosphorus occurs most abundantly as inorganic orthophosphate (PO_4^{3-}), which is directly bioavailable (Walmsley and Butty 1980). The release and adsorption of soluble forms of phosphorus by mineral particles (e.g. clay, silt or sand) plays an important role, influencing the bioavailability of phosphorus. Under chemically reducing, anoxic soil conditions, adsorbed phosphorus is released from the sediments in its soluble orthophosphate form (e.g. Walmsley and Butty 1980, Dallas and Day 2004). In South Africa, as in many other countries, bio-available phosphate (orthophosphate) in waters of un-impacted aquatic ecosystems is usually more limiting to plant growth than nitrogen (Wiechers 1985, Malan and Day 2005c).

For South Africa, limited water quality monitoring data and associated understanding of the ecological functioning of non-riverine wetland ecosystems makes it difficult to associate water quality conditions with desired wetland ecosystem condition (Malan and Day 2005b). Water nutrient concentrations have been used to differentiate between trophic states (oligotrophic, mesotrophic or eutrophic) for South African rivers in which, trophic states were based on “Target Water Quality Range” (TWQR) categories (DWAF 1996, DWAF 2002: see Tables 5.2 and 5.6 in Chapter 5). Although the TWQR (DWAF 1996) were expressed as appropriate for all types of aquatic ecosystems (i.e. rivers, lakes and wetlands), they were based on an extensive time span of river monitoring data (for a review see Malan and Day 2005 b and c). Water quality data of nutrient concentrations in palustrine and endorheic wetland types, predominantly from the Western Cape, were compared to the TWQR categories (DWAF 1996 and DWAF 2002) with the outcome that the categories were considered to require further refinement before they can be considered accurate for inland wetlands in South Africa (Malan *and* Day 2005b). For wetlands, the extremes of largely natural and highly modified ecological conditions are relatively discernable, but the graduation of ecological conditions between these two extremes is difficult to assess (Malan and Day (2005b). In the absence of data for wetlands that suggest what nutrient concentrations reflect natural and impacted conditions “Best Guess” *default water quality objectives* were suggested for certain nutrients, representing conservative boundaries above which, the ecological condition of wetlands could become negatively impacted (Malan and Day 2005b). Along with the “Best Guess” boundaries, the TWQR categories (DWAF 1996 and DWAF 2002) were used in the present study as a guideline to assist in interpretation of nutrient concentrations and to attempt to differentiate between the trophic states amongst sampled wetlands.

2.8.5.3. Characterisation of wetland versus dryland soils

Soil as the growing medium for rooted wetland plants, is an essential environmental parameter for interpreting and understanding wetland vegetation distribution (e.g. Kent and Coker 1992, US EPA 2002c). While plants obtain carbon, oxygen and hydrogen predominantly from water and atmospheric sources, phosphorus, and nitrogen are predominantly derived from the soil. Exchangeable cations of calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}) are also nutrients obtained from soils (Ashma and Puri 2002). The ability of the soil to adsorb (hold) cations is referred to as its cation exchange capacity or CEC (Ashma and Puri 2002). As plant roots remove these soluble inorganic ions from the soil solution, other ionic species are released from exchange sites into the soil solution. The ability of a soil to hold nutrients is dependent upon soil colloids (clay and the humic fraction of soil organic matter) which may buffer chemical changes by taking up and then releasing ions from the soil water solution. Soils with high CEC tend to have high concentrations of colloidal particles and neutral to high pH (>7). A clay-rich soil will have a far higher CEC than an acidic, sandy textured soil. Sandy soils, such as those typical in the coastal lowlands of the Western Cape, have few soil colloid particles and hence have low CEC (buffering capacity or levels of stored nutrients and H^+ ions) relative to shale or clay dominated soils.

An important feature of wetland soils is the interaction between organic matter, moisture levels and nutrients. Soils which are poorly drained, and are perennially or even seasonally wet, typically show appreciable increases in organic matter (and correspondingly in total carbon), especially in the top 20 cm layer, relative to surrounding dryland soils in the same area (Brady 1974, Mitchell *et al.* 1984, Witkowski and Mitchell 1987). This is primarily due to lower oxygen levels and a resultant slowing down in the rate of decomposition of organic matter in wetland soils (Brady 1974). On the other hand, a wetland that is only seasonally saturated is also seasonally aerobic, thus allowing the breakdown of organic matter.

In dryland soils the spaces (pores) between the soil particles are filled with air. Plant roots and biologically active microbes within the soil profile use oxygen in the soil pores which is readily replaced, as they are connected to the atmosphere. In saturated or inundated (hydric) soils, such as in wetlands, the spaces between the soil particles are not connected to the atmosphere due to the presence of water and once available oxygen is consumed by biological activity, it is not replaced (Verpraskas 1995). This shift in the aeration status of the soil induces biological and chemical processes that change the soil from an aerobic (oxic) state to an anaerobic (anoxic) and reduced state via oxidation-reduction (redox) chemical reactions (Verpraskas and Faulkner 2001). A useful indicator of the amount of oxygen in the soil atmosphere (degree of soil aeration) is the redox potential; the difference in electrical potential between the two halves of the coupled oxidation-reduction reaction. Dryland and hence aerobic soils generally have an electrical or redox potential between 300 and 800mV, with that of anaerobic soils being between 300mV and minus 400mV (Ellis and Mellor 1995).

Oxidation-reduction reactions in hydric soils begin when bacteria oxidize organic compounds to release electrons and protons in the form of H^+ cations. The electrons and protons released by the oxidation of organic compounds react with electron receptors to complete the microbial respiration process. In anaerobic wetland soil conditions, oxygen is unavailable and other chemical compounds must act as electron receptors for bacteria if they are to continue their respiration by oxidizing organic compounds. It is these reducing reactions that use compounds other than oxygen, that are the ones most responsible for the major chemical processes that occur in wetland soils and cause the development of the following characteristics (Verpraskas and Faulkner 2001):

- accumulation, in situations of prolonged anaerobia, of organic carbon in the soil surface layers (or A-horizons);
- denitrification – the reduction of nitrate to atmospheric nitrogen;
- mottled soil colouration – due to reduction of ferric to ferrous hydroxide;
- the gray-coloured subsoil horizons – due to removal, by leaching, of ferric iron (Fe^{3+}) by standing water; and
- the production of gases such as hydrogen sulphide (H_2S) and methane (CH_4) – due to reduction.

Both the biological and chemical functions of wetlands are controlled to a large extent by these redox reactions (Mitsch and Gosselink 2000, for a comprehensive review see Verpraskas and Faulkner 2001).

Acidity determines a range of soil characteristics such as nutrient availability, microbial activity and the release of certain toxins such as metals. Soils become acidic through several mechanisms, some natural such as the breakdown of soil organic matter, and others, such as through pollution for example with acidic mine wasted, by humans (Ashma and Puri 2002). The leached, non-calcareous and naturally acidic sandy soils associated with Fynbos vegetation in the coastal lowlands of the Fynbos biome are generally oligotrophic (Deacon *et al.* 1992). In comparison, the alkaline and calcareous aeolian sands associated with Strandveld vegetation are usually less oligotrophic, having higher base ($Ca^{2+}$and Mg^{2+}) content (Deacon *et al.* 1992).

2.8.5.3.1. Soil Nitrogen

Nitrogen is required by plants in larger amounts than any of the other nutrient elements (Whitehead 2000). Plants absorb almost all of their nitrogen through their roots as nitrates and ammonium ions. The greater mobility of soil nitrate, combined with the fixation of ammonium to soil colloids, typically means that more nitrate is available. Soil acidity (low pH) and cold temperatures curtail the rate of nitrification and under such conditions much of total nitrogen uptake is in the form of ammonium (Whitehead 2000).
Typically, in dryland soils, only 5% of soil nitrogen occurs in mineral or inorganic forms in the soil solution and is thus available for plant uptake:

- Dryland, coarse textured, well drained soils tend to leach nitrates and can cause eutrophication of drainage and surface water (Ellis and Mellor 1995) ; whereas
- Wetlands become sinks for nitrogen, sequestering it in accumulating soil organic matter that is not broken down due to anaerobic conditions (e.g. Keddy 2000, US EPA 2002d).

Denitrification, facilitated by anaerobic bacteria, is the primary mechanism for nitrogen removal from wetland waters (Sather and Smith 1984 in Kotze *et al.* 1994). Denitrification may be enhanced further in wetlands which are alternately wet (anaerobic) and dry (aerobic); high levels of nitrogen loss have been shown to occur under such conditions (Patrick and Wyatt 1964; McRae *et al.* 1968; Reddy and Patrick 1984 in Kotze *et al.* 1994).

The global N cycle has now reached the point where more N is fixed annually by human-driven than by natural processes (Vitousek 1994). This additional fixation occurs though human activities such as the addition of fertilizers and the combustion of fossil fuels (Whitehead 2000). When the supply is low, graminoids take up virtually all plant-available inorganic N through their extensive root systems and, in temperate regions with a substantial winter rainfall, there is therefore usually little carryover of inorganic N from one season to the next. In the variable redox conditions typical of seasonal wetlands, nitrates are leached from the soil and also be denitrified via gaseous N_2 loss to the atmosphere, hence nitrate levels can rapidly decline to zero in recently inundated/saturated soils (Cronk and Fennessy 2001). Thus, despite the ability of NH_4^+ to bind to soil colloids, its rapid uptake by plants (Mitchell *et al.* 1987), and its ability to oxidize means it does not accumulate in “un-impacted” soils. Variation in soluble inorganic soil nitrogen with plant uptake, temperature and moisture availability makes it difficult to use this nutrient to assess environmental condition (Whitehead 2000). The measurement of total nitrogen, that includes the inorganic and the organic fraction, was therefore implemented in the present study.

2.8.5.3.2. Soil Phosphorus

Soil phosphorus is derived initially from the minerals of the soil parent material, or from precipitation of atmospheric dust. It is also found in soil organic matter (Whitehead 2000). Phosphorus occurs in the soil in several chemical forms dependent on the soil pH. As a result of various reactions that it undergoes in the soil, P is generally retained strongly, reducing its availability to plants, and loss by leaching (Whitehead 2000). At a neutral pH range, 6-7, more phosphorus is available to plants than in alkaline soils in which insoluble calcium phosphate can form, thus reducing P availability at higher pH levels (Ashma and Puri 2002). Phosphorus is held more tightly to soil particles/colloids in dryland soils or under oxic conditions than under the reduced conditions typical of wetland soil. As discussed above in section 2.8.5.2, in hydric soils, under reducing conditions, soluble orthophosphates may be released from organic material and mineral sediments as a consequence of change in redox potential (Cronk and Fennessy 2001; Keddy 2000).

Furness and Breen (1978), Howard-Williams and Allanson (1978), Twinch and Ashton (1983) and Rogers *et al.* (1985) have all shown that emergent vegetation is responsible for the reduction of phosphorus content in hydric sediments in South African wetlands. In eutrophic conditions, once the vegetation can no longer absorb phosphorus, it is possible that phosphorus may accumulate in soils. Elevated soluble phosphorus concentration is thus likely to be apparent in wetlands with eutrophication causing disturbances and post-disturbance stabilized vegetation communities.

2.8.5.4. Assessment of nutrient status

In attempting to use nutrient levels as an independent measure of human disturbance impacting on wetlands it is necessary to determine at what spatial scale and to what extent both water and soil chemistry vary within and between wetlands. Whilst some studies suggest that water chemistry and related aquatic variables vary more between than within different wetlands (e.g. Mack 2001, Smith *et al.* 2007), experience in wetlands of the Western Cape (Ractliffe, G., Freshwater Consulting Group, Cape Town, *pers. comm*.) suggests that this is not always the case. Similarly with soil chemistry variables, whilst a few conglomerated samples from a wetland are used in the BAWWG approach to categorize the amount of human disturbance (US EPA 2002c, Mack 2007), botanical studies (e.g. Kent and Coker 1992) and research in wetland vegetation in the Western Cape and the Free State suggests conglomerated samples will not provide an accurate measurement for all of the habitats of a wetland (Sieben 2003, Low and Pond 2003, Collins 2005, Ractliffe and Corry 2008).

2.9. Wetland vegetation

The development of reliable metrics for bioassessment is aided by searching within habitat and associated vegetation units that are comparable from the perspective of the species they support under natural environmental conditions. This section describes the means used to classify comparable units of wetland vegetation in the local South African and Western Cape context, along with a description of what are considered to be wetland, as opposed to dryland plants.

Vegetation is perhaps the most conspicuous biotic feature of wetland ecosystems and it provides clues of land that is wet (i. e. of saturated soils) even when surface water is not apparent in the current season (e.g. Keddy 2000, Mitsch and Gosselink 2000). Wetland plants, as defined by Cowardin *et al.* (1979), are any plants adapted to living in wet conditions or on a substrate that is at least periodically deficient in oxygen as a result of inundation or saturation. They are distinguished from other plants by morphological and physiological adaptations that allow their roots to tolerate and grow in oxygen deficient conditions (Sorrell at al. 2000). Plants that are physiologically dependent on water with at least part of the generative lifecycle requiring part or all of their structure to be submerged in, or floating on, water are termed ‘hydrophytes’ (Cook 1996 after Raunkiær 1904). Terrestrial plants that are associated with wetlands and can tolerate submergence for long periods of time but are not physiologically dependent on floating or being submerged for part of their lifecycle are termed ‘helophytes’ (Cook 1996 after Raunkiær 1904). Helophytes are common in seasonally saturated wetlands, or in the supralittoral habitat unit (See 2.9.3.1) of wetlands in which anoxia is infrequent to non-existent, which is a very common habitat unit in the wetlands of arid countries such as South Africa (Prof. Jenny Day, University of Cape Town, pers. com.). Both of these sets of taxa are considered to be wetland plants but have greater or lesser specificity to the wetland habitat. Plant sensitivity to hydrology has led to their use (along with long-lived signs of anaerobic conditions in the soil) as an indicator of the presence of wetlands, to delineate both the current and recent historical position of their boundaries, and as a descriptor of wetland habitat types in many classification schemes (e.g. Cowardin *et al.* 1979, Kotze *et al.* 1994, Tiner 1991 and 1999, EUR 15 1999, DWAF 2003, Ewart-Smith *et al.* 2006 and SANBI 2009).

2.9.1. Plants as obligate and facultative wetland taxa

The distinction between a dryland plant and a wetland plant is a standard indicator used in the DWAF (2003) wetland delineation protocol. It is also used in many other countries for delineating wetland boundaries, in wetland inventories and for regulatory purposes (Tiner 1991). For delineation purposes wetland plants have been split into two groups (Reed 1988):

- Obligate wetland plants – dependent on wet or anaerobic conditions for growth, and
- Facultative wetland plants – capable of growing in anaerobic soils but also reasonably competitive in well-aerated soils.

Observation of plant distributions within wetlands does not facilitate the discrimination of whether a plant is a hydrophyte and thus restricted to wetland habitat and obligated to occur within such environment, or if it is a facultative wetland plant that is essentially terrestrial, or a helophyte and thus able to survive waterlogging and occasional inundation (Cook 2004). Such discrimination would be best provided by examination of plant structure and physiology and extensive and accurate records of distribution and ecological conditions of the habitat in which species is recorded. This information is severely lacking in the South African context (Cook 2004, Mucina *et al.* 2006a). A pragmatic approach to the designation of whether a plant has facultative or obligate association with wetland, as based on frequency of occurrence (correlation) in wetland relative to dryland habitat, was adopted by the US Fish and Wildlife Service (Reed 1988). This is illustrated in Table 2.3.

Given that the discrimination relies on correlation, for any given plant, the accuracy of this designation therefore relies on the number of observations made of that taxon. For South Africa, a list of wetland associated taxa was compiled based on habitat descriptions on labels and vouchers of herbarium specimens (Glen *et al.* 1999, Glen unpublished, See

Appendix 5). This national list of wetland associated taxa does include a designation as obligate or facultative, but does not include the number of observations that this status was based on. The information and lists of wetland vegetation reviewed in the present study (Objective 1 in Section 1.3) were used to update Glen's unpublished list of wetland vegetation for the Western Cape and this database is appended to this volume in a CD as discussed in Appendix 5.

Table 2.3: Classification of plants according to occurrence in wetlands, based on U.S. Fish and Wildlife Service Indicator Categories (Reed 1988)

Obligate wetland species	O	Almost always grow in wetlands (>99% of occurrences)
Facultative wetland species	Fw	Usually grow in wetlands (67-99% of occurrences) but are occasionally found in non-wetland areas
Facultative species	F	Are equally likely to grow in wetland and non-wetland areas (34-66% of occurrences)
Facultative dryland species	Fd	Usually grow in non-wetland areas but sometimes grow in wetlands (1-34% of occurrences)
Dryland species	D	Almost always grow in drylands (>99% of occurrences)

The concept of facultative and obligate taxa has not proved to be particularly useful in determining environmental condition in the ambit of bioassessment (U.S. EPA 2002a). The alteration of hydrological regimes have well documented impacts on species groups: plant zonation patterns shift as plants intolerant of the new hydroregime are replaced by tolerant species and terrestrial species may invade or die back due to drainage or flooding (for a summary see Table 2 and Adamus *et al.* 2001). It is clear, therefore, that a decrease in the number of obligate wetland taxa and an increase in facultative species or non-wetland species can result from hydrological changes such as drainage or excessive water abstraction. As shown in Table 2, hydrological change will impact on plant community composition, and monitoring change of wetland hydrology and plant assemblages over time would develop ecological understanding of species response to hydrological changes. Determining whether hydrological zones have shifted, beyond temporary levels of change, when there are no obvious signs of dehydration (such as soil mottles in a zone that is no longer saturated) is extremely difficult to determine in seasonal wetlands from a single or even multiple field visits (Brooks 2004) and was not attempted in the present study.

2.9.2. Wetlands as habitat for vegetation

There is considerable variability in wetland vegetation, in terms of numbers of species, structural form and functional type (Mucina et al. 2006a). This variability is a consequence of the gradients of environmental conditions that create habitats within wetlands. In South Africa the description of wetland vegetation has predominantly been marginalized with published research being fragmentary and focused on small areas, as botanical research has focused on the dryland vegetation (Mucina *et al.* 2006a). Effective conservation of wetland habitat is very much dependent on knowing the scale of variability in biodiversity of these habitats (DWAF 2004) and yet a comprehensive habitat classification system for wetland vegetation has not been produced (Mucina et al. 2006a). Thus, the degree of variability of these systems is unknown. The classification of wetland vegetation produced for the “Vegetation Atlas of South Africa, Lesotho and Swaziland” (Mucina *et al.* 2006a) is broad-scale, being based on meta-analysis of the limited published and available information for wetlands and recognizes that a more rigorous and data intense classification is necessary (Mucina *et al.* 2006a). In the northern hemisphere, ecological research and conservation practice has identified the phytosociological classification approach as being very effective in identifying patterns of habitat and species variability. Both the classification of wetland plant communities and associated indicator species have been facilitated by this floristic approach to ecological research into wetlands. As a result of this success, a classification system for habitats, which includes the phytosociological typing of vegetation, was adopted in the European Union Habitat Directive (i.e. EUR 15 1999) (Rodwell *et al.* 2002) and has become a powerful tool for wetland conservation and management (Mucina et al. 2006a).

2.9.3. Biogeography and classification of similar spatial units of vegetation

2.9.3.1. Zono-Biomes

Typically, plants have tolerance ranges to various physical and chemical conditions within which they can survive. Along a gradient of change of any of these environmental parameters, community assemblages can also be expected to change (e.g. Walter 1973, Omernik 1987, Olson *et al.* 2001, Mucina *et al.* 2006). In many instances these gradients are a consequence of geography and geology as climate, soil nutrients and soil physical properties determine the growing medium in a given habitat. Hence in general, plant communities from similar habitats within a biogeographical region can be expected to be more similar to each other than communities from similar habitats but a different biogeographical region. On this basis and as a first step in classifying vegetation into broadly typical units, Walter (1973) split land masses of the world into areas of distinct climatic and ecophysiological conditions in what he called zono-biomes. In South Africa, the Fynbos Biome, one of seven such zones (Walter 1973, Rutherford *et al.* 2006), has more complex macroclimatic and geological characteristics than other biomes in South Africa, and contains a considerably greater number of units of dryland vegetation (Rebelo *et al.* 2006).

2.9.3.2. Broad Vegetation types within the Fynbos biome

The Fynbos biome contains three quite different, naturally fragmented vegetation types, Fynbos, Strandveld and Renosterveld, which are further separated into 12 bioregions (See following paragraph). The transition between Fynbos and Renosterveld is predominantly dependent on differences in leaching as determined by annual precipitation, with more leached and oligotrophic soils supporting Fynbos (Cowling and Holmes 1992). The boundary between Fynbos and Strandveld is largely determined by fire dynamics. Sand-Fynbos occurs adjacent to Strandveld and its higher succulent coverage is associated with more nutrients derived from salt spray from the sea. Renosterveld and Strandveld occur on different soil types, predominantly shale and sand respectively, and typically the sand/shale interface is with acid sands supporting Sand-Fynbos rather than Strandveld (Rebelo *et al.* 2006).

2.9.3.3. Bioregions

Based on the ecoregions concept of Olson *et al.* (2001), which focused on terrestrial regions of the world and stressed the central importance of biotas, including distinct assemblages of species, Rutherford *et al.* (2006) created 35 vegetation bioregions for South Africa, based on the floristic composition of the component dryland vegetation types and on climatic differences. These bioregions differ from the ecoregions of Kleynhans *et al.* (2005) and the bioregions of Brown *et al.* (1996) since they are based on different criteria for discriminating between regions. The close proximity and intermingling of very different vegetation types in the Fynbos Biome make it difficult to establish bioregions with strictly distinct floras. As a consequence, the 12 bioregions of this biome include, in some cases, a combination of Fynbos and Renosterveld and were based instead upon differences in climatic conditions (Rutherford *et al.* 2006). At a finer spatial scale than bioregions, the edaphic (soil type and nutrient) dependence of many spatially smaller units of Fynbos vegetation means that substrate type and geomorphic land units were used as surrogates for determining the boundaries of these distinct vegetation units (Cape Flats Sand Fynbos or Cape Flats Dune Strandveld) within the Fynbos Biome (Rebelo *et al.* 2006).

2.9.4. Wetlands as anomalous habitat units within the zono-biome concept

Wetlands represent anomalous environments within the complex of the macroclimatic-determined zono-biome scheme (Walter 1973) because hydrogeological conditions and/or special saline substrates (soil types or bedrock) create habitats that are atypical of the zone. The concentration of salts and/or the hydrological regime created by levels of waterlogging, flooding and tidal influence exert an influence greater on floristic composition, structure and dynamics than the macroclimate. These influences result in vegetation assemblages that deviate from the typical surrounding zonal vegetation and are therefore considered azonal (Mucina *et al.* 2006a [pp 618-619]). Wetland vegetation units in South Africa are predominantly distinct from the surrounding dryland vegetation types. An exception is the Fynbos Freshwater marshes and seeps dominated by endemic elements of the Capensis that are embedded within the shrublands of the Fynbos Biome (Mucina *et al.* 2006a). In most other wetlands within the Fynbos Biome, Mucina *et al.* (2006a) considered wetland taxa as not necessarily Fynbos, Strandveld or Renosterveld affiliates. Where a wetland vegetation unit occurs exclusively within a climatic zone or biome then it is considered to be *intrazonal* and where it occurs irrespective of climatic and vegetation zones it is considered *azonal* (Mucina *et al.* 2006a). In order to map the spatial distribution of distinct units of wetland vegetation, Mucina *et al.* (2006a) classified wetland vegetation into distinct ecological and biogeographical units. The anomalous nature of the wetland vegetation in the zono-biome scheme meant that it needed to be classified independently of the zonal (dryland) vegetation. This wetland vegetation classification approach followed multilayered criteria, driven particularly by the macro-ecological character of *azonality* and the combination of hydrodynamics and/or salt content as azonality-driving ecological factors (Mucina *et al.* 2006a). According to these criteria, firstly the vegetation of “freshwater wetlands” along stagnant or slow-flowing waters (lentic) differs from the second vegetation class “alluvial vegetation” fringing water courses in which the environment is characterized by flow (lotic conditions) and undergoing dynamic change due to a periodic flood regime. The third class, “inland saline vegetation” comprises vegetation accompanying salt-laden intermittent (ephemeral) rivers and salt-pans in which salt is the major ecological determinant. Further division within these categories follows biogeographical (floristic) criteria reflecting correlation with abiotic determinants that are also driving the dryland matrix of zonal vegetation of surrounding biomes (Mucina *et al.* 2006a). The subdivisions of inland freshwater wetland vegetation based on association to zonobiomes for South Africa is presented in Table 2.4.

The vegetation units recognized within these classes were classified with “high certainty” into the four broad geomorphological categories of Cowan’s (1995) regions (Table 2.4), with the notable exception of the azonal subtropical units which are shared by three of the four groups, not occurring in the mountain wetlands group (Mucina *et al.* 2006a).

The terminology used in Cowan (1995) is somewhat confusing as only the Pongola/Mkuze region was considered to be coastal plain whilst the areas such as Agulhas Plain and the Cape Flats, which fall within the Cape Forelands, were considered to constitute coastal slope (See Lambrecht 1984, Kleynhans *et al.* 2005 or Schulze 2006 for description of the generally planar topography of these areas in the Cape).

Table 2.4: Spatial links between the inland azonal wetland vegetation units and the surrounding biome, with reference to the zonality status of the units (After Mucina *et al.* 2006a).

Code	**Freshwater Wetland Vegetation Units**	**Biome**	**Zonality**
AZf 1	Cape Lowland Freshwater Wetlands	Fynbos	Intrazonal
AZf 2	Cape Vernal Pools		
AZf 3	Eastern Temperate Freshwater Wetlands	Grassland	Intrazonal
AZf 4	Drakensberg Wetlands		
AZf 5	Lesotho Mires		
AZf 6	Subtropical Freshwater Wetlands	Savanna; Albany Thicket; Indian Ocean Coastal Belt	Azonal

Cowan’s four broad categories were subdivided, using geomorphology and climate characteristics, including temperature and moisture balance, into 26 regions. These regions contain wetlands with similar topography, hydrology and nutrient regime (as determined by temperature and moisture gradients as well as by geological stratigraphy) and are thus likely to contain similar sets of biota (Cowan 1995) (Table 3.5.). Cowan (1995) anticipated that within the 26 regions, local differences in geology would determine minor groups of plant biota as determined by local nutrient availability. The area of focus for the present study was the Western Coastal Slope Mediterranean region (SWm). The western section of the Western Coastal Slope wetland region (SWm) of Cowan (1995) overlaps with the South Western Coastal Belt ecoregion of Kleynhans *et al.* (2005), other than for the area around Verlorevlei which Kleynhans *et al.* (2005) place within the Western Coastal Belt. However the eastern section of the SWm which is adjacent to the SSa western edge (Figure 2.4) would be incorporated along with the SSa in the Southern Coastal Belt ecoregion (Kleynhans *et al.* 2005). As Cowan's regions were adopted by Mucina *et al.* (2006a) they are used in the present report in discussion of the biogeographical distribution of wetland vegetation.

Table 2.5: Correspondence between the broadest category of separation of wetland regions of Cowan (1995) and the Wetland Vegetation units of Mucina *et al.* (2006a). Unit AZf 6 shown in italics is shared between wetland regions C, S and P, while the other units show exclusive links to a wetland region. (After Mucina *et al.* 2006a)

Broad Wetland Regions (Cowan1995)	**Freshwater Wetland Vegetation Types (Mucina *et al.* 2006)**	
		Code
C: Coastal Plains	*Subtropical Freshwater Wetlands*	*AZf 6*
S: Coastal slope and or rimland	*Subtropical Freshwater Wetlands*	*AZf 6*
	Cape Lowland Freshwater Wetlands	AZf 1
	Cape Vernal Pools*	AZf 2
M: Mountain wetlands	Cape Mountain Wetlands	unmapped
	Drakensberg Wetlands	AZf 4
	Lesotho Mires	AZf 5
P: Plateau wetlands	*Subtropical Freshwater Wetlands*	*AZf 6*
	Eastern Temperate Freshwater Wetlands	AZf 3

*Vernal pools will be described in section 2.9.1.2

Cowan's 26 wetland regions (1995) are typically broader than, and often incorporate, many of the different dryland vegetation bioregions of Rutherford *et al.* (2006) and the dryland vegetation units of Rebelo *et al.* (2006). Within the Western Cape, however, the units of wetland vegetation created by Mucina et al. (2006a) are spatially broader than the wetland regions of Cowan (1995), with for instance "Cape Lowland Freshwater",

Figure 2.4: Cowan's Wetland Regions of South Africa: (After Cowan 1995). Inset from Cowan's Wetland Regions showing the totality of the South Western Mediterranean (SWm) coastal slope region which was the area of focus for the present study.

"Cape Vernal Pools" and "Cape Lowland Alluvial" freshwater wetland vegetation units occurring across both Cowan's Western Coastal Mediterranean region (SWm) and Southern Coast Temperate region (SSa) (See Figure 2.4). Hence, the wetland vegetation units determined by Mucina *et al.* (2006a) to exist in the Western Cape cover very broad areas and constitute a very course classification of wetland vegetation.

2.9.5. The vegetation of Cape Lowland Freshwater wetlands

The Cape Lowland, Freshwater Wetland (CLF) vegetation unit (AZf1) was the unit of focus of the present study because it is the most common vegetation unit in palustrine wetlands of the Western Cape. Whilst vernal pools are not atypical features in the Fynbos Biome, these seasonal or ephemerally waterlogged wetlands, are usually inundated by between 2 to 10 cm at the height of the wet season (in Winter and onward into Spring) and are described by Mucina *et al.* (2006a) as supporting a vernal vegetation unit other than the emergent Cape Lowland Freshwater vegetation typically associated with palustrine wetlands (Kotze *et al.* 2005). The use of the term vernal should not be construed to imply that these wetlands only exist in Spring, but rather that the vegetation is most identifiable in that season. In the arid Western Cape these pools become apparent after the onset of winter rain has saturated the soil pore space and water begins to pool on the soil surface. Whilst the focus of the current research was largely on non-tidal depressional wetlands dominated by emergent plants, namely palustrine wetlands (for definition see Section1.4.). It is important to note that in nature there is a gradation between the different wetland types, be they depressions, micro-depressions typical of vernal pools, lakes, lagoons, estuaries or other inter-tidal marine systems. One of the challenges of the present undertaking is to define the scope of assessment tools that are developed. This is in terms of what types of wetland or habitats within them and under what conditions, they can be used. In addition to the landscape depressions that are typically associated with palustrine wetlands, the CLF wetland vegetation is also to be found in flats, seeps, valley-bottoms, lakes and lagoons. The supralittoral habitat unit is common to most of these wetlands types and metrics applicable to the supralittoral zones of depressions may equally be applicable in the supralittoral zones of these other HGM types.

The CLF Wetland vegetation type has some important taxa with widespread distribution within South Africa and a number of species with cosmopolitan distribution in analogous habitats (Mucina *et al.* 2006a). The Capensis is a region known for high endemism – i.e. species unique to an area (Goldblatt and Manning 2000), but the CLF vegetation unit reputedly contains very few endemics (Mucina et al. 2006a). Most endemics within freshwater vegetation of this region are associated with Vernal Pools, with shrub-dominated ericaceous wetlands (mentioned above in Section 2.9.1.1) and with other such wetlands in the mountains of the Fynbos Biome that were not mapped or are riparian (Mucina et al. 2006a). The typical landscape features in which, the Cape Lowland Freshwater vegetation type is found, namely, flats and landscape depressions, are also typical of where the Eastern Temperate and Subtropical Freshwater Wetland vegetation types are found in other parts of the country (Mucina *et al.* 2006a). These latter vegetation types also have relatively few endemics and many similar species to the important taxa found in the CLF vegetation. The Cape Lowland Freshwater vegetation type was chosen therefore with the intention of finding information that could be widely applied in the development and testing of bioassessment tools in other regions and wetland vegetation types of South Africa.

For the determination of bioassessment metrics, if the vegetation of the Cape Lowland Freshwater Wetlands proves to be uniform under natural (reference) conditions across the entire Fynbos Biome, then metrics and indicator species developed from representative wetlands could be applicable for this vegetation type across the entire biome.

2.9.5.1. Diversity within the Cape Lowland Freshwater vegetation

Within the dryland vegetation of the Fynbos Biome there is an extreme level of diversity due, partially, to the degree of change (turnover) in the species composition of vegetation communities:

- Between the different dryland vegetation types – as determined by beta diversity namely, the change in species assemblage along habitat or environmental gradients within local landscapes; and
- Between the dryland vegetation of equivalent habitats separated by different geographical distances – or gamma diversity namely, the change in species assemblage of equivalent habitats along geographical gradients such as distance between habitat units (*sensu* Cody 1975 and 1983).

The high diversity in the dryland vegetation is due to the extreme turnover of macroclimatic factors and soils within the Biome (e.g. Lambrechts 1978, Cowling *et al.* 1992, Rebelo *et al.* 2006).

The geographically broad distribution of Cape Lowland Freshwater Wetland vegetation suggests relatively low gamma-diversity among the wetlands progressing across the Western Coastal Slopes. This suggests that there is generally a low level of geographical diversity or environmental difference in the habitats containing Cape Lowland Freshwater vegetation across the region it covers. Metrics and indicator species determined from a representative set of wetlands should therefore be applicable across all of, or a considerable number of, the wetlands with this type of vegetation. Such metrics should work particularly well in those wetlands that are geographically close to the representative wetlands from which the metrics were developed. It is recognized, however, that further division is possible within this vegetation unit along biogeographical lines and corresponding with the abiotic determinants (climate and soils) that also drive the divisions in the dryland matrix of zonal vegetation (Mucina *et al.* 2006a). The limitations of the available data for lowland freshwater wetland vegetation may therefore account for the broad scale of this unit of wetland vegetation and the resultant coarse scale at which the vegetation mapping of this intrazonal unit was performed (Mucina *et al.* 2006a). Within the Western Cape the consideration that Cowan's (1995) Western Coastal Slopes region will hold a uniform set of wetland vegetation appears to contradict the acknowledged diversity and extreme turnover in the dryland vegetation of the Fynbos Biome. The present study serves as a potential source of information about both the biotic turnover across the landscape and associated environmental parameters amongst the wetlands of three different sub-regions in the Western Coastal Slope region in the Fynbos Biome. Far greater uniformity in dominant species, including some species of sub-cosmopolitan distribution, occurs in freshwater wetlands outside of the centres of regional endemism (represented by the Cape and Drakensberg). This suggests lower gamma diversity outside of these centres (Mucina *et al.* 2006a) and therefore greater potential for the identification of indicator species and phyto-assessment metrics with broad spatial application. The development of phyto-assessment metrics from a representative set of wetlands from one area of the Western Free-State, an area of considerable homogeneity in wetland vegetation, may therefore be expected to provide metrics that are widely applicable in other areas within the Free-State. This would considerably reduce the cost and time required for development of widely applicable phyto-assessment tools.

2.9.6. Classification of structural forms and functional groups of vegetation

Further divisions of vegetation units can be based on broad structural form (US EPA 2002c [as discussed below in Section (i)]), plant morphology (e.g. Hutchinson 1975, Cook

1996 [Section (ii)]) and functional groups [Section (iii)]. Functional groups are plants that have similar traits associated with the physical means of exploiting resources (Grime 1979, Boutin and Keddy 1993, Weiher and Keddy 1995, Weiher *et al.* 1999). These groupings all provide sets of taxa with similar adaptations to habitat that suggest they may have similar response to drivers of change. These different units of vegetation are therefore potential sources of metrics of environmental condition and are discussed in more detail below.

Rutherford *et al.* (2006) point out that there are several failings to the structural approach to classifying communities. These failings may also be pertinent when attempting to use this approach for determination of phyto-assessment metrics. Firstly, the same structural phenomena can be encountered under very different habitat conditions. Secondly, even if there is an ecological “message” in convergent structures/growth forms, it is as yet very difficult to establish a specific link between vegetation structure and function. Equally though, even less is known about the autoecological requirements of particular species (e.g. Rutherford *et al.* 2006 and Mucina *et al.* 2006a). The use of structural form for the development of functional groups of wetlands plants may therefore combine species that do not respond in similar manner to environmental change or disturbance. Such groupings will not make useful phyto-assessment metrics.

2.9.6.1. Structural forms of wetland vegetation

Stands of vegetation with distinctly different plant architecture can occur in wetlands (US EPA 2002c). Vegetation with obvious structural differences and affinities to different habitat are (Mucina *et al.* 2006a):

- submerged or aquatic vegetation,
- emergent herbaceous vegetation typically dominated by graminoid taxa,
- scrub-shrub vegetation, in the South African context, more often associated with saturated rather than inundated situations, and
- forested vegetation (including mangroves).

Such structural vegetation units can entirely dominate a wetland or may occupy different hydrological zones within it. The different habitats that these structural vegetation units occupy expose them to different environmental determinants and natural or anthropogenic stressors. Furthermore these vegetation units have different spatial arrangement and thus require different sampling techniques to capture community composition effectively (e.g. Kent and Coker 1992, Krebs 1999). These vegetation structural units reflect naturally distinct habitats that are not comparable and should be considered independently for the development of bioassessment metrics (e.g. US EPA 2002c, Mack 2004). Where combinations of these structural units of vegetation occur together in one wetland, it is suggested that each is sampled independently. The focus of the present study was on wetlands dominated by emergent herbaceous vegetation as this vegetation unit typifies vegetated wetlands in the lowlands of the Western Cape (Mucina *et al.* 2006a).

2.9.6.2. Morphological adaptations to habitat

There have been many attempts to classify wetland plants according to their growth form or morphology and the interaction of these forms with habitat. The life-forms of Raunkiær (1904) divide species based on the location of the plant's growth-point (bud) or propagule (seed/rhizome/bulb/tuber, etc.) during the seasons with adverse growing conditions (frost, drought). The helophytic group of Raunkiær include any plants with resting buds in marshy ground, whilst hydrophytes survive adverse conditions with propagules that are submerged under water (Raunkiær 1904).

Cook (1996, 2004) provided a classification of plants associated with wetlands based on the interaction of structural parts (roots, stems, leaves and propagules) with water, soil and air, thus providing a means of grouping wetland plants, based on their responses to these milieu. Such groupings were based on morphology and its interaction with the growing medium and the way a plant survives adverse conditions (*sensu* Raunkiær 1904). For instance the helophytes as terrestrial plants that tolerate submergence are a group of taxa that are likely to occupy areas in a wetland that are seasonally rather than permanently inundated (Cook 1996).

Plants with similar morphological adaptations to their habitat may potentially ‘respond’ in the same way to human disturbance

2.9.6.3. Functional groups of wetland plants

In bioassessment techniques based on invertebrate taxa, separation into different genera, families or into functional feeding groups is often adequate (e.g. Gernes and Helgen 2001, Bird 2010). The hierarchy of plant taxonomic groups, however, does not lend itself to a similar use. For example, the genus *Senecio*, contains plants that range from small, fleshy herbs to large woody shrubs adapted to a range of habitats, from wet to arid. *Senecios* are a member of the family Asteraceae, the other taxa of which, are also extremely morphologically diverse and occur in a vast array of habitats. Similarly, whilst invertebrates can be separated into feeding guilds (Root 1967) based on the type of food they consume (Pianka 1983), plants typically all compete for similar resources (Harper 1977, Grubb 1977). Different methods of separating plants have been developed, based on the different means by which they acquire resources (e.g. Grime 1979).

Early, simple functional group classification schemes were based on traits which can be determined upon inspection of plant morphology (often single structural characters), methods of vegetative propagation and life history strategies such as annual *vs.* perennial life cycles (e.g. Hutchinson 1975, Campbell 1985). More recently developed functional classification groups are based upon characteristics associated with nutrient acquisition, interaction with other plants and ability to withstand flood, drought and fire (Boutin and Keddy 1993, Weiher and Keddy 1995, Weiher *et al.* 1999). Functional groups are defined with the intention that taxa within them can be expected to respond to change in resource availability and other such stressors/disturbances in similar ways (Hobbs 1997 in US EPA 2002c). Functional traits have thus been used to explain group response to ecological conditions and anthropogenic stress and there has been much effort to identify morphological traits that allow rapid categorization of species according to sensitivity to all kinds of impacts, as elaborated by Keddy (2000).

The categorization of species into “functional groups” could greatly expedite the development of successful multimetric indices. The assumption that there is an ecological “message” in convergent growth forms (i.e. a link between form and function), makes the use of morphological traits plausible for the derivation of functional groups. Rutherford *et al.*’s (2006) caveat that there is limited ecological understanding of the link between form and function is very pertinent here; and any data collected in the present study will assist with development of this understanding. Notwithstanding this caveat, functional groups can and have been used as indicators of certain environmental conditions within wetland habitats (Adamus *et al.* 2001). Functional groups that illustrate such differences in wetland taxa are tussock-forming graminoids (bunch grasses) *vs.* matt forming graminoids (lawn grasses), and clonal or vegetative (rhizomatous or stoloniferous) *vs.* seed-based spread or reproduction (Boutin and Keddy 1993, Weiher and Keddy 1995, Weiher *et al.* 1999).

For an enormous number of wetland plant species, essential prerequisite autoecological information about life history and habitat requirements and therefore the tolerance limits to alteration of environmental determinants is unknown (Adamus *et al.* 2001). In North America a database of tolerances of wetland species to excessive nutrients and hydrological condition was established to assist in autoecological determination and functional group creation (Adamus and Gonyaw 2000, See Section 2.6.3). Very little has been published about the life histories and habitat requirements of South African wetland plants (Goldberg and Van Nieuwenhuizen 2000, Cook 2004, Mucina *et al.* 2006a), let alone functional traits. Autoecological studies of nutrient tolerance and other habitat-specific requirements of wetland plants and associated ecotoxicology studies are equally lacking (Cook 2004, Mucina *et al.* 2006a).

2.9.7. Separating wetlands into comparable units for bioassessment

Different types of wetlands and associated habitat units can be classified into units of similar habitat (SANBI 2009) within which, characteristic patterns of plant community assemblage are anticipated to occur. This section describes the means of classifying wetland into comparable vegetation-habitat units and the implications of this classification for bioassessment development.

A multitude of natural and anthropogenic factors work together to create the habitats and environmental conditions that occur within a wetland and these factors in turn determine the constituent taxa of the plant community that can exist within it (e.g. Keddy 2000). A primary step in the development of successful multi-metric bioassessment indexes is the separation of comparable groups of wetlands or habitats within them (Mack 2001, Simon *et al.* 2001, Gernes and Helgen 2002, US EPA 2002a). This is done in order that any variation in plant communities that occurs along a gradient of anthropogenic disturbance is not confused with that naturally occurring (1) between regions as a result of differences of macroclimatic and physiogeographic factors or (2) between different habitats created by the different hydrogeomorphology of different wetland types and (3) different habitats created by hydrological zones within wetlands. Major determinants of natural variability between comparable units are thus controlled for, making the detection of differences in ecosystem condition between wetlands more apparent and the determination of indicator attributes easier. The separation of wetlands into comparable units is done by the categorization of ecoregions, wetlands and habitats into classes (termed classification in this report). Classification is a way to account for the effects of natural environmental influences on wetlands and helps avoid comparing wetlands or habitat units of unlike classes. Excessive emphasis on classification, or inappropriate classification, can however impede development of cost-effective and sensible assessment and monitoring programs. Using too few classes fails to recognize important distinctions among ecoregions, wetlands or habitats and can produce insensitive metrics; using too many adds unnecessary costs to the development of biocriteria (metrics) (US EPA 2002b). For metric development purposes, the challenge is to create a classification of ecosystems with only as many classes as needed to represent the range of relevant biological variation in a region and yet at a level appropriate for detecting and defining the biological effects of human activity (Karr and Chu 1999). The incorporation of more than one class within a dataset does facilitate the testing of whether metrics may be applicable to several classes.

Based on physico-geographic and hydrological or hydrogeomorphic (HGM) parameters, a number of different types of wetlands can be recognized (Brinson 1993). These HGM types are also important parameters (e.g. lotic *vs.* lentic conditions) that determine different habitat for plants. Geological and climatic constraints are recognized as other important determinants of habitat type and character and the resultant geographical distribution of plants (*sensu* Walter 1973). Collectively, these HGM, climatic and geological parameters are thought to partition a landscape into areas of different habitat for wetland vegetation (e.g. Mucina et al.2006a). Which of these parameters is the most important in defining the microclimate of the habitat in which wetland plants grow is not yet clear. The focus of the current study is thus predominantly restricted to wetlands of a particular HGM type as well as a few similar habitats from other wetland types. These are all from within a similar landform and region that presents similar climatic, hydrogeomorphic and geological constraints. This is considered as a starting point from which to determine the important criteria for subdivision of different wetland vegetation habitat.

The national wetland classification system (NWCS) for the South African National Wetland Inventory (Ewart-Smith *et al.* 2006, SANBI 2009) classifies different types of wetlands and associated habitat into comparable vegetation-habitat units. This classification system provides a useful summary of what were considered to be palustrine wetlands, of how this wetland type is now classified and how they are distinguished from other wetland types. The structure of this classification system is initially hierarchical, with primary discriminators distinguishing between wetlands of significantly different ecosystem type in regard to hydrogeomorphic and ecological character and the functions that wetlands perform. The wetlands that were the focus of the present study were inland freshwater wetlands with a hydroregime of lentic conditions characterized by a range of ephemeral to permanent saturation and predominantly seasonal inundation, as well as by endorheic drainage. These wetlands were from the South Western and Southern Coastal Belt ecoregions, predominantly from a planal landform and with a depressional HGM type.

2.9.7.1. Vegetation classification and the issue of scale

It is important to keep in mind the spatial scale used to differentiate between “habitat units” in the process of classification of wetlands for developing phyto-assessment tools. Whilst wetland represents a different habitat from dryland, within either of these divisions there are many further subdivisions of different units of land that present different habitat for organisms. Within a wetland, the combined variations in flow, drainage, depth and periodicity of the hydroregime are some of the hydrological parameters that determine which organisms can exist. Different combinations of environmental parameters can occur within a wetland each presenting different habitat units for vegetation. Thus, many habitat units of a wetland do not hold the same types of vegetation (US EPA 2002c). Within each wetland a critical level of habitat separation that is dealt with by secondary discriminators at level 5 of the NWCS (SANBI 2009) is the differentiation brought about by hydrological zonation. The nomenclature used for differentiation between hydrological zones in wetlands within the NWCS (temporary, seasonal, permanent) is not considered useful for differentiation of vegetation habitat and an alternative nomenclature of vegetation habitat zonation is presented below. Essentially, land can be permanently waterlogged but never inundated, whilst another section of habitat may be permanently waterlogged and occasionally inundated, and yet another may be permanently inundated. Whilst each of these sections of land is commonly referred to as permanently waterlogged they constitute very different habitats for vegetation as described below.

2.9.7.1.1. Hydrological zonation in wetlands creates different habitat for plants

Ground-rooted individual plant specimens do not move; therefore they have to be able to deal with all environmental conditions that exist at a given location throughout the various seasons of the year. Wetlands, or the hydrological zones within them, can be permanently, seasonally or temporarily wet, depending of the season, presenting habitat that ranges from being permanently inundated to ephemerally saturated. The exact delineation of such habitat is not simple because water levels tend to fluctuate. Depth of the water column in aquatic environments, or in the soil, varies with rainfall, infiltration and evapotranspiration rates and hence the spatial hydrological zonation changes with the seasons. From the perspective of the vegetation, three broad hydrological habitat categories, namely; supra-littoral, littoral and aquatic, are distinguishable as a result of the different hydro-dynamics or residence time of water in and/or inundating the substrate of wetlands (Figure 2.3) (US EPA 2002c, DWAF 2003). These three hydrological habitats, depending on the availability of water and the type of substrate may not be present in all wetlands.

Initial field surveys, discussions with wetland specialists, and the National Wetland Classification System (SANBI 2009), suggested that significant hydrological zonation and concomitant habitat differences were apparent in wetlands of the Western Cape. In the present study the following habitats were recognized *a priori* as representing different hydrological zones:

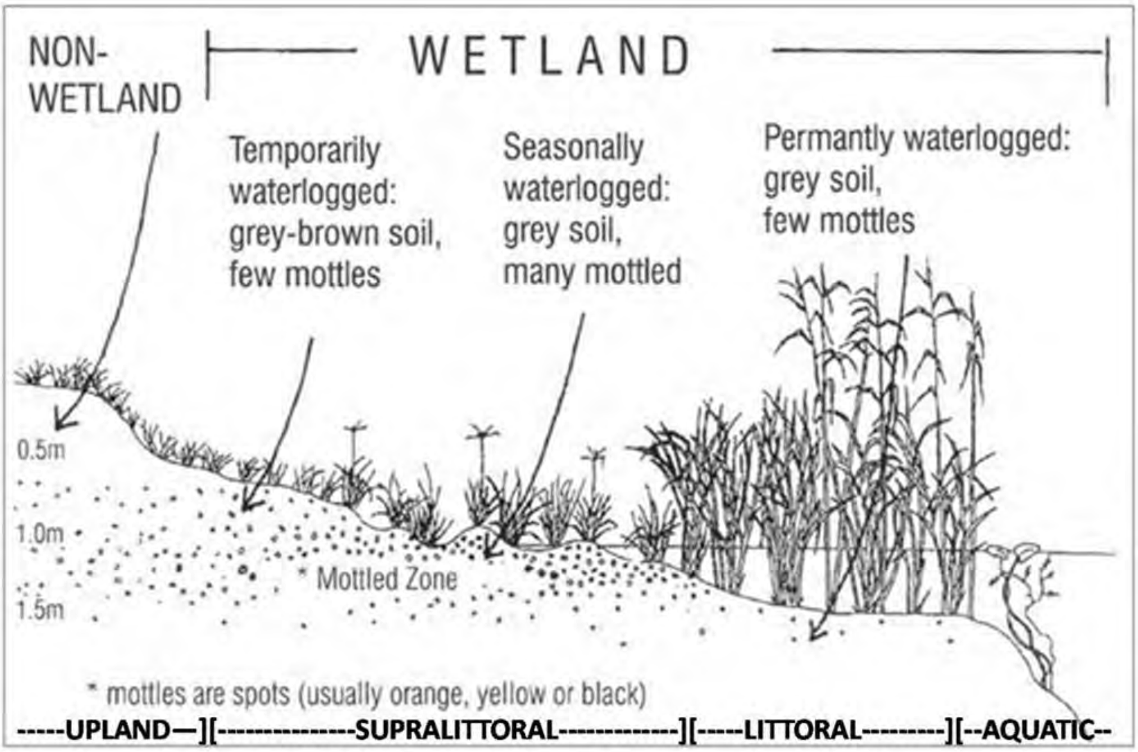


Figure 2.3: Cross section through a wetland, indicating how soil wetness and vegetation indicators change along a gradient of decreasing wetness, from the middle to the edge of the wetland (After DWAF 2003 and Kotze 1996). The change in hydrological habitat for vegetation from aquatic habitat associated with permanent and/or long term inundation to shoreline or littoral habitat associated with emergence from waterlogged or inundated land and supra-littoral habitat is depicted in association with the more commonly known zones of permanent, seasonal and temporary waterlogging.

i.) Supralittoral zone

Land that is typically **ephemerally to seasonally saturated** has, if at all, only short periods of shallow inundation and is therefore dominated by vegetation that does not emerge from water standing above the surface of the ground but whose root systems or propagules do at times have to cope with anoxic conditions. This habitat is typically situated above the line of seasonal to permanent inundation and is thus called **supra-littoral** for the purposes of this study and covers both the temporarily-saturated and part of the seasonally saturated or waterlogged sections depicted in Figure 2.3. Supralittoral land supports plants that are adapted to waterlogging (hydrophytes) including perennial taxa typical of marshes or vleis (helophytes), and many annual plants which survive the unfavourable season in the form of seeds and complete their life-cycle during favourable seasons for growth (therophytes) (e.g. Raunkiær 1904 and Cook 1996 [Section 2.9])

ii.) Littoral zone

Land that is **seasonally** to **permanently saturated or inundated**, that is dominated by so called **emergent vegetation** and is typically associated with the shore line in situations where inundation does occur, supports **littoral vegetation.** This zone is therefore referred to for the purposes of this report as the littoral zone. Littoral land supports helophytes, tenagophytes (plants with the juvenile phase submerged in or floating on water and the adult [flowering] phase terrestrial) and hyperhydates (plants with leaves and or stems emerging above the water surface) (Section 2.10).

iii.) Aquatic zone

Land that is **permanently to seasonally inundated** may support aquatic vegetation and is referred to for the purposes of this report as the aquatic zone.

Although some species may be able to tolerate all zones, these distinct habitat units generally support very different plants as a result of the different environmental conditions within them.

2.9.8. Summary of comparable vegetation units

In terms of the phyto-assessment of environmental condition, habitat specificity of plants allows comparison between different wetlands (Section 2.9.1). The total complement of a wetland plant community is a reflection of all the different available habitats within a wetland. This suggests that comparison of the entire community of plants between two or more wetlands only makes ecological sense when the wetlands being compared contain the same habitats. In essence, even in the absence of anthropogenic stressors, the occurrence of different habitats within a single wetland, mean that different plant communities would be present within it. For instance:

- the comparison of supralittoral (seasonally saturated) to littoral (seasonally inundated or waterlogged) or aquatic (permanently inundated) habitat (Section 2.9.3.1 and partially dealt with in Level 5 of NWCS, SANBI 2009) will in all likelihood reveal different plant communities, even in the absence of human impacts;
- In the same vein, comparison of plants representative of seasonally saturated clay habitat with those from seasonally saturated sandy habitat would not be ecologically meaningful in terms of determining the relative environmental condition of either habitat, other than simply one is sand based and the other clay (Level 6 of NWCS, SANBI 2009); similarly, comparison between different climatic regions reduces the likelihood of comparable vegetation (biomes and bioregions Section 2.9.1) and
- The comparison of emergent herbaceous to scrub-shrub, or aquatic to emergent herbaceous vegetation (As mentioned in Section 2.9.2(i)).

Hence substrate classes (sand, silt, clay) and associated nutrient levels (oligo-, meso- and eutrophic), hydrological habitat situation (lentic or lotic and supralittoral or littoral) and structural vegetation types (aquatic, herbaceous, scrub-shrub and forested) are all essential parameters for separation of comparable units of wetland vegetation.

In summary, the separation of vegetation into units from different nutrient and substrate classes and hydrological habitats, with different adaptation to their local environment (functional groups) and different ‘structural’ (plant-architecture) units in different climatic and geological regions (e.g. ecoregions) can provide units of taxa that can be expected to respond similarly to a given anthropogenic or natural stressor. Whilst species and associated assemblages of communities may differ between wetlands of different HGM types and localities, functional groups should be common to most wetlands. The use of functional groups to represent the species in wetlands may therefore assist in the endeavour to develop metrics applicable over multiple-HGM and habitat types.

2.10. Considerations for Sampling Design

The wealth of experience of previous research facilitates the adoption of many existing methodologies in the development of an approach to testing bioassessment in South Africa. There is a wealth of previous research that can be used to inform the development of bioassessment in this country. This section summarises some of the sampling methodologies that were considered to be appropriate.

Wetlands contain a heterogeneous array of habitats that create considerable environmental and concomitant plant diversity within the confines of a single wetland (Section 2.9.3). The human stressors that change plant distribution do not act evenly across all of the habitats and it is therefore unclear in which habitats to sample in order to detect the impacts of these stressors (Section 2.9). Sampling the full array of habitats and assemblages of species in every wetland of a study group facilitates determination of which plants, in which habitats, show a characteristic response to human impairment (Sections 2.7.2.1 and 2.11.1.2). As comparison among wetlands of reference and impaired conditions is only ecologically plausible when the same habitat type is being compared (2.6 and 2.7, 2.9); the comparison of the collective floral complement of all habitats of different wetlands is usually not valid. Only when all the habitat units are the same, is it valid to compare the collective floral complement of one wetland with another. This issue was a key challenge to the present project in terms of determining which habitats and concomitant floral assemblages were comparable for the determination of metrics for phyto-assessment. The comparison of like habitats does, however, offer the opportunity to compare plant assemblages from wetlands that may not be of the same HGM type. The supralittoral zone, for instance, is ubiquitous between different wetlands, differing perhaps only in periodicity and seasonality of saturation.

In the context of the palustrine wetlands of the coastal lowlands of the Western Cape the plant communities are dominated by emergent herbaceous and scrub-shrub vegetation. Trees, where they do occur in wetlands of this study area, are generally alien invasive species with the occasional exception of trees associated with perched coastal seeps and zones of seasonal to ephemeral waterlogging. The forested structural unit of wetland vegetation, and the associated sampling techniques, were therefore not considered in the present work. Many of the vegetation attributes most successful in showing responses to human stressors, and therefore of use as phyto-assessment metrics, in the North American context, were based on diversity and cover data for the herb and shrub layers and density data for the shrub (and tree) layers (US EPA 2002c). Characterization of the herb and shrub layer was thus of paramount importance in the present study.

2.10.1. Comparison of different spatial sampling techniques

Vegetative cover in wetlands can vary from less than 5% to 100%, potentially co-occurring at different height strata, from submerged aquatic taxa and ground-covering herbs to canopy-forming trees. A sampling method that is flexible enough to account for this horizontal and vertical variation in vegetation is therefore required for phyto-assessment (e.g. Mack 2004 and 2007). Both quadrat and transect approaches were assessed by the BAWWG for their applicability to the development of bioassessment protocols (US EPA 2002c).

Within the science of vegetation description and comparative analysis, various sampling techniques have been devised to ensure an adequate inventory of the sociological relationship of flora (cover and abundance data), and the diversity and heterogeneity (number of taxa and functional groups) of the vegetation. A brief summary follows of three spatially different methods of surveying that integrate considerations of plot location, shape, size and number.

2.10.1.1. Braun-Blanquet quadrat sampling

The Braun Blanquet method of vegetation description recommends that, in order for a complete floristic description to be made, sample surveys are carried out for each homogeneous stand of every vegetation unit within the plant community of interest. This approach recommends and offers a means of sampling every potential habitat of a wetland. Any area that contains a unit of vegetation that has no obvious internal boundary separating different units is considered to be homogeneous (Westhoff and Van der Maarel 1978). An initial rapid overview of the wetland to be assessed provides the researcher with an *a priori* concept of distinct stands of homogeneous vegetation based on dominant and abundant species. A stand of vegetation is only sampled if it is considered representative of the plant communities present at a site. Stands with 100% cover (88% median cover) by a single species (e.g. of *Typha capensis*), being relatively similar at different sites within the same wetland are typically only sampled once in a vegetation survey. Such homogeneous stands may represent a considerable percentage of the wetland surface. Having only a single representative sample of such vegetation stands, however, could lead to the misconception that the species within them have low fidelity to the vegetation type or habitat unit being assessed.

Within each distinct unit of vegetation, a plot, typically in a square or quadratic layout, is assessed, providing a relevé (list) of the cover and abundance of each species using cover classes (Braun Blanquet 1928, Barkman *et al.* 1964, Van der Maarel 1979 [Table 3.6, Chapter 3]). The convention of the Braun Blanquet School of phytosociology is to restrict the size of the quadrat in which a relevé is made to the size that fits into the 'homogeneous' and 'characteristic' stands of plant communities (Westhoff and Van der Maarel 1978). The size of the quadrat should be large enough to contain all the species that are characteristically representative of that stand. The optimal sample area appropriate for floristic comparisons of different vegetation units found in wet conditions has been determined through the experience of many vegetation ecologists. In equivalent wetland vegetation to that found in the Fynbos Biome, herbaceous vegetation plots of one to four square meters are recommended (Westhoff and Van der Maarel 1978, Peet *et al.* 1998, Sieben *et al.* 2004).

The Braun Blanquet (1928) cover/abundance scale as adjusted by Barkman *et al.* (1964) is regarded as very effective in estimating the cover and abundance values of species (Westhoff and Van der Maarel 1978, Kent and Coker 1992). The first four classes are an abundance scale, thereafter and above five percent cover, the values represent percentage cover classes, i.e. 2a represents the 5-12.5% cover range. These values represent an estimate on an ordinal scale (categories) and thus calculations using these are not possible. The scale facilitates the rapid capture of large amounts of data. This scale employs a 'doubling principle' in which the difference between each level of the scale of measurement is large enough, so that each is easily distinguishable. This is considered to provide a smoothing influence when several researchers are recording data that ultimately must be comparable and thus it is appropriate for a phyto-assessment protocol (Peet *et al.* 1998, Mack *et al.* 2000).

The relevés (lists of species with cover/abundance values per sampling plot) provide a representative estimation of the characteristic species composition for a homogeneous unit of vegetation within the area of study. Wetlands with more heterogeneity in habitat, typically have greater vegetation heterogeneity, and consequently have a larger number of relevés representing them. The sampling effort should encompass all hydrological zones. Hence, similar sampling effort should be made in the supralittoral zones (of ephemerally and seasonally waterlogged soils) as in the littoral and aquatic zones harbouring the emergent, floating or submerged vegetation habitats of a wetland. In this way a representation of the diversity and community structure is recorded for each distinct homogeneous assemblage of plants corresponding to each habitat and hydrological zone.

The intention of this type of sampling is to describe an area of vegetation that is considered to be a distinctive community of plants and that all plots can be allocated to a community type. This process facilitates the classification of plants into "differential", "constant companion" and "dominant" species (Westhoff and Van der Maarel 1978) from which phytosociological classification and naming of the vegetation unit is made possible. A "differential" species has a varying frequency of occurrence or association among different communities and can thus be used as an indicator of difference between communities at the same syntaxonomic level (Westhoff and Van der Maarel 1978). A guideline that has commonly been suggested (Kent and Coker 1992) is a difference in frequency of occurrence of more than two cover or abundance classes (to as little as 40% cover difference) and. A "constant companion" is a species that occurs in more than 60% of all the relevé samples from a community. A "dominant" species is so called when it is constantly associated with a community and has an average cover of more than 25% (Westhoff and Van der Maarel 1978). It is possible using multivariate statistical analysis to classify plants in a sample dataset as characteristic of, and discriminatory between, different habitats or environmental conditions (Clarke and Warwick 2001). Whilst the phytosociological nomenclature may be useful for vegetation description and classification, for phyto-assessment a determination of characteristic or discriminatory species would seem to be sufficient.

2.10.1.2. Single plot and nested quadrat sampling

Based upon successful phyto-assessment development programmes, the sampling technique adopted by the BAWWG, compares the flora of only one or two large sample plots per wetland, typically recommending the emergent or littoral zone as the most useful for determining metrics and largely ignoring all other zones (US EPA 2002c). In the temporary and more seasonal wetlands typical of the arid to semi-arid South African environment the littoral zone, when it does actually exist, may be considerably smaller than the supralittoral zone of vegetation. While needing to sample only one or two plots per wetland is an enviable situation, it is the endpoint of considerable investigation effort in determining where best to sample in order to find characteristic plant responses to disturbance. A key recommendation of the BAWWG, is that at the initial stage of the development of bioassessment protocols, sampling should 'over-stratify' in both the vertical (different height strata of vegetation) and horizontal dimensions (multiple hydrological zones) until it can be determined which vegetation and hydrological strata are "responding" most strongly to human disturbance or to the impairment of environmental condition (US EPA 2002c). The State of Ohio found that the herb and shrub vegetation responded strongly, although some intermediate tree size classes (e.g. 10 to 25 cm diameter at breast height) also appeared to be responsive (Mack *et al.* 2000). Ultimately, the decision to sample habitat zones independently (splitting distinct homogeneous communities) or collectively in one plot (clumping communities) depends on whether this is necessary in order to detect plant responses to stressors. 'Homogenizing' community types by placing a quadrat or transect across them (e.g. stretching from the aquatic bed to emergent to waterlogged shrub zone) can be appropriate if clumping does not obscure the plant responses to disturbances. It is not possible to separate clumped data sets after the collection process is complete. If only one stratum of the plants appears to be responding to human disturbances then having clumped the data may obscure this response.

Different sampling approaches exist in the literature, each with combinations of multiple adjacent quadrats that form a single large sampling unit (e.g. Mack 2001, Gernes and Helgen 2002). These techniques vary in the number and size of quadrats, some having intensely measured, internally nested-quadrats (Peet *et al.* 1998). In these rectangular quadrats, in order to sample a homogeneous plot the long axis is aligned to reduce the environmental heterogeneity within the plot (Peet *et al.* 1998). This alignment is believed not to be important when the heterogeneity in question would not affect the goal of characterizing the vegetation of different habitats; a situation described in Ohio as occurring most frequently with mixed emergent marshes (Mack 2004). The description of 'mixed emergent marshes' bears considerable resemblance to the palustrine wetlands that are the focus of the present study. In mixed emergent marshes, water depth generally decreases towards the dryland boundary and the vegetation is zoned in narrow to broad bands. Typically, a narrow external shrub zone gives way to a broad emergent zone which grades into a floating-leaved marsh to open water zone. In this situation, a sampling plot should be located such that intensively measured nested-quadrats are located within the emergent zone but the "tails" (ends) of the plot include portions of the shrub and aquatic bed zones. It is considered important to include the presence and percent cover of the species in the shrub and floating leaved zones, but the main focus should be on the emergent zone (Mack 2004).

The use of a single large, but subdivided, plot may prove to be a useful sampling technique, should it be found that the emergent zone is of the greatest importance in determining plant response to disturbance in the South African context.

2.10.1.3. Transect sampling

Numerous transect methods exist for sampling vegetation, with the location of transects determined in several ways.

- Randomly using a random-number generator;
- Systematically located at fixed intervals perpendicular to a baseline; and
- Using a stratified random design in which different portions of the site are targeted for sampling to ensure that the habitat complexity of the wetland is represented, but within these zones transects are located randomly.

The first two transect methods homogenize the vegetation at a site to some degree as they clump together supralittoral, littoral and aquatic stands of vegetation. For the present study these methods were therefore considered to be inappropriate for the determination of the impact of stressors that might affect only a sub-set of the vegetation community of the whole wetland. The stratified random approach would address this problem to a degree; however, the zones of waterlogging in the wetlands of the present study are in many cases small, making the use of transects unwieldy in comparison to quadrats.

2.10.2. Choice of sampling technique

The most effective method of vegetation description for bioassessment was investigated in the State of Ohio (Mack *et al.* 2000). Different spatial techniques using transects, quadrats and a composite quadrat method developed by Peet *et al.* (1998), were compared. The Ohio EPA found that transect, quadrat and composite quadrat methods yielded equivalent results when the data resulting from these methods were used to calculate a vegetation-based index of environmental condition (Mack *et al.* 2000). Whatever method is selected, it must be amenable to sampling with sufficient completeness such that plant responses to human disturbances are detectable. The Braun-Blanquet method of sampling separate quadrats in each homogenous vegetation stand offers the most precision for comparing the vegetation of different habitat units within a wetland. This method provides the most detailed phytosociological and autecological information for the species associated with distinct habitats. This sampling approach facilitates the distinction between homogenous stands of vegetation and between hydrological zones (Section 2.9.3.1) but also allows the information to be amalgamated into a concept of plant sociology, or vegetation unit per hydrological zone, or per wetland should the need arise. The flexibility afforded by this approach was deemed appropriate for the initial feasibility studies that this research represents. The quadrat-based Braun Blanquet method of vegetation sampling was chosen as it represents the most flexible and therefore appropriate technique at this primary stage of assessment protocol and metric development. A hydrologically stratified approach to vegetation sampling was employed in the present study, in order to avoid obscuring plant responses to human disturbances that occur in one zone, but not in another. Single large quadrats were considered likely to obscure important habitat differences between supralittoral (shrub dominated), littoral and aquatic zones. Transect sampling methods were also considered to be inappropriate for the determination of the impact of stressors that might affect only a part of the vegetation. Whilst the stratified random approach would address this problem to a degree, the zones of waterlogging in the wetlands of the present study are in many cases small, making the use of transects unwieldy in comparison to quadrats. Using the Braun Blanquet sampling approach, it is also possible to split or clump samples of different hydrological zones of a wetland (e.g. supralittoral, littoral and aquatic) thus offering considerable flexibility with this sampling regime. In order to compare the complete flora between wetlands using the multiple-quadrat based Braun Blanquet sampling technique, the results from individual relevés of a wetland can be combined to provide an average cover and/or abundance value per species per wetland. A similar procedure was adopted in Minnesota to combine the results from disjunct plots most representative of the different stands of vegetation of a wetland, into a summed value per species for the whole community or habitat (Genet *et al.* 2005).

For the purposes of developing a wetland phyto-assessment index and thereafter for wetland condition assessment, the goal is to correlate a wetland's aggregate vegetation characteristics to measures of human disturbance and environmental condition. Since the goal is not just plant community classification but also biological assessment, deciding where to place a survey plot should be based on both of these goals (Mack 2004). The use of randomized placement of quadrat plots to characterize vegetation reduces researcher bias and allows inference to un-sampled locations within the vicinity of those sampled (Fore 2003). An entirely random approach to vegetation sampling would result in transects and quadrats that extended across the boundaries of supralittoral and littoral zones and therefore combine vegetation with different environmental drivers, particularly hydrology. A stratified random sampling approach, would avoid the transgression of these hydrological strata, and have the advantage, as a randomized approach, of allowing the extrapolation of findings to other parts of the same strata in the same and other wetlands. Such extrapolation is considered to be invalid in a non-random model (Fore 2003 – See Section 2.11.3), however, the time required to first reconnoitre all potential sampling sites to allow a random generation of sample choice is prohibitive and equally will not always facilitate the best choice of indicators of plant response to human stressors. The Braun Blanquet School of phytosociology is based on a non-random, targeted, expert-opinion based choice of sampling sites to best represent the vegetation being surveyed (Westhoff and Van der Maarel 1978) and this approach was adopted in the present study.

2.10.2.1. Vegetation data – quadrat or wetland scale

From the discussion above, it is clear that plant responses in the distinct quadrats could be expected to provide clearer signals than would be derived from the average response within either the hydrological strata or the entire plant community of the wetland. However, the method of determination of environmental condition for wetland vegetation in the Ohio and Minnesota States of North America was based at the wetland scale. To this end, after the major plant communities were identified, a location was determined where a sampling plot(s) would best capture or represent the vegetation types found in the whole wetland (Mack *et al.* 2000, Gernes and Helgen 2002). As determined by the BAWWG, in wetlands expected in the natural/reference condition to be dominated by emergent herbaceous vegetation, typically the position that best represented the vegetation community of the whole wetland, and that provided a response to stressors, was found to be at the emergent/aquatic vegetation interface (US EPA 2002c).

In the lowlands of the Western Cape, it is apparent from site observations that there is considerable heterogeneity of the community in each wetland. The lack of existing data on this wetland vegetation, however, suggests the need for a finer-scale approach of vegetation description to facilitate the development of strong metrics. Individual quadrat sampling (2.11.1.1) does not preclude the ability to lump all of the relevés from a wetland into a single representation of the wetland plant community, at a later stage, thereby facilitating wetland scale comparisons. To this end, a weighted-average value per species per wetland can be developed, whereby the relative area that each hydrological zone occupies in the wetland is used to weight the average cover value of each species per zone which can then be summed over all zones. The area covered by each habitat is therefore taken into account when determining wetland average cover for any given species. This is akin to the concept of stratified sampling whereby different strata of an ecosystem are sampled independently to increase precision in the estimates of the parameters for the entire community (Cochran 1977, Krebs 2003). The assessment of quadrats lumped together within each separate hydrological stratum (supralittoral, littoral and aquatic) could facilitate the discovery of plant responses that would not be apparent in the response of the entire community. Lumping all of the data in this fashion facilitates the comparison of the flora of hydrological zones or of the whole wetland. This may not, however, make for valid comparison where one or a number of wetlands within the data set do not all contain the same habitats.

2.10.3. Haphazard vs. random sample design – which wetlands to sample?

In a study designed to determine the impact of human disturbance it is necessary to choose which wetlands (sites) to sample. The decision as to where to sample within the wetlands was discussed above in the introduction to section 2.11.2. The site-selection process requires a number of decisions to be made.

- Targeted sampling selects sites based on some known aspect, for example sites might be sampled close to a point source of pollution to evaluate the extent of its influence; or some scientific or observational criteria such as amount of human disturbance;
- Convenience sampling selects sites where sampling is possible, e.g. private access may restrict the choice of sites;
- Haphazard sampling*:* Selection of sampling units may be casually described as “random”; however, if the selection process is not formally random, that is, based on the use of random number tables or a randomization algorithm, the method of selection is actually haphazard.

From a statistical point of view, any sampling approach other than census (sampling every site) or random sampling will be technically “haphazard” (see Overton and Stehman [1996] or Olsen *et al.* [1999] for further discussion). Haphazard, or targeted sampling designs, are not inherently bad or wrong. Their primary drawback for monitoring, is that the results from sampled sites cannot be extended to any neighbouring un-sampled sites, since generalizing from a non-random sample to the larger un-sampled population of sites typically yields biased conclusions (Overton and Stehman 1996, Fore

2003). This view is, however, considered to be rather statistically rigid and unrealistic for field based studies. Sampling a large degree of the variability within the study area considerably increases the ability to generalize from targeted sampling designs to the un-sampled population of sites. From the perspective of the development of phyto-assessment indices, random sampling often fails to capture enough least-impaired (reference) wetlands or severely degraded wetlands (US EPA 2002b). Random sampling may not consider important factors such as accessibility to sites and the potential for selection of reference and disturbed sites. Fore (2003) reports that for a multiple-year bioassessment development study, random sampling did not produce sufficient reference sites in the first year. For these reasons targeted sampling was the method used for wetland site choice in the present study.

2.10.4 Sampling season

The establishment of a standard sampling window or phyto-assessment index period, helps to ensure that representative and comparable data are obtained at each site, in order to ensure that valid comparisons can be made between different wetlands (US EPA 2002b and c). Both identification of the species, and comparison of those taxa visible in a wetland at a given time are important to bear in mind; many annual and geophytic plants are not easily detected in some seasons. The appropriate season for development of the index and the appropriate assessment sampling season or index period are not necessarily the same. Seasonal variation in nutrient levels also needs to be taken into consideration when comparing between wetlands when developing phyto-assessment metrics.

2.10.4.1. Floral seasonality

Temporal variability in maturity of the plant community is a function of climatic and thereby of geographic location (Kent and Cocker 1992). With sampling typically limited, due to time and cost, to a single visit per wetland, identification of wetland plants can pose a challenge because different species reach maturity or flower at different times during the growing season. For instance members of the Cyperaceae, Restionaceae and Poaceae, which are important components of wetland vegetation, tend to bloom late in the growing season, suggesting that an early summer sampling period would be best. Sampling at this stage runs the risk of missing the many annual and geophytic taxa, however, as they flower in early spring (Goldblatt and Manning 2000). Identification of the species of *Ficinia* and *Isolepis* genera in the Cyperaceae often requires seed set to facilitate identification (Dr Muthama Muasya, Botany Department, UCT pers. comm.), suggesting that even later sampling would be useful.

From the perspective of phyto-assessment, being able to identify the greatest number of species possible would provide the greatest opportunity for the development of diverse and non-redundant metrics. It is possible, however, that once an index has been developed for an area, the metrics may not require identification to species level. This would increase the potential length of the sampling season . As plants persist often throughout the year, even after seasonally inundated wetlands dry out, the sampling season is not necessarily restricted to when water is present, suggesting an advantage of this group of biota over invertebrates for assessing the condition of seasonal wetlands.

Defining an index period involves trade-offs because no one sampling period is able to capture all species. Particularly if the goal is to assess a wetland in a single visit, an index period that corresponds to the peak maturity of the community as a whole is generally considered most appropriate (US EPA 2002c). The period chosen will ultimately depend upon when in the year is the easiest time to identify the species used in metric development.

2.10.4.2. Seasonal variation in nutrient levels

As a result of dilution of nutrients in the water column by seasonal rainfall and changing conditions of oxidation and reduction in the soils, and uptake and release by plants, nutrient concentrations in wetland are seasonally variable (Malan and Day 2005c). From the point of view of metric development, comparability of nutrient concentrations is important in order to accurately rank wetlands according to nutrient load. Comparison of water nutrient concentration from the time of greatest inundation depth, coinciding in the Western Cape with early spring, with levels in early summer when wetlands are often drying, reveals different concentrations within the same wetland (Malan and Day 2005c). Seasonal variation in phosphorus availability in soils in the Fynbos Biome has also been demonstrated (Mitchell *et al.* 1984). These potential sources of natural variation in nutrient concentrations need to be considered in the developmental phase of phyto-assessment. If categories of nutrient availability are used as a means of separating sampled vegetation quadrats, or wetlands, into different groups for determination of plant response to nutrient availability, then a broad season of data collection with considerable fluctuation in water levels would be likely to reduce the accuracy of ranking of like systems. This would cause considerable variability or noise in the different groups, which in turn would decrease the accuracy of interpretation of plant response to nutrient availability.

2.10.5 Statistical Analysis

A description of the statistical procedures commonly used for the development of phyto-assessment metrics and the methods used during this study are presented below.

Linear correlation was recommended by the BAWWG (US EPA 2002c) for comparison of vegetation responses along single gradients of disturbance. This was shown for s increasing abundance of grasslike taxa relative to disturbance (as represented by zinc concentration in Figure (2.1). When samples come from a uniform habitat (*sensu* wetland and vegetation classification units), then the vegetation response to a single stressor with increasing magnitude can be expected to be relatively linear. In a data set in which many gradients influence natural biotic assemblages, and several stressors are at work, then biotic responses to even a single gradient of disturbance may be diverse. In a 2-dimensional scatter graph of this data, such as shown in Figure 2.1, the diverse response of the biota will show as a scattered arrangement of the samples (as apparent at the left hand edge of Figure 2.1) with limited linear correlation to the gradient of stress. A multivariate analysis of this same collective of samples, recognising the diverse stressors, facilitates classification of like samples and comparison sample response to multiple stressors. The patterns of response are no longer likely to be linear, so interpretation is more complex but there is greater potential for autoecological interpretation coupled with broader geographic applicability if units from a diverse set of locations are included (e.g. Fore 2003, Declerk *et al.* 2006). The multivariate analysis approach is believed to be essential in the present study due to the extreme heterogeneity of environmental parameters (Cowling *et al.* 1992) and variability in disturbance effects (as evidenced by site reconnaissance) that are known to be at play in the Western Cape.

Multivariate statistics analyses the collective amount of difference based on all environmental or biotic parameters being compared between samples. This difference is calculated by calculating how similar one sample is to another based on the cover/abundance of each species or measure of each environmental parameter that affects each sample. “Raw” data matrices of the cover/abundance of each species in each sample and the measured value of each environmental variable in each sample are collated. A similarity, or resemblance coefficient (Legendre and Legendre 1998, Clarke and Warwick 2001), then converts this raw data matrix into a triangular resemblance matrix that compares wetland to wetland or sample to sample. The Bray-Curtis measure of (dis)similarity, or resemblance coefficient, was used to compare samples (wetlands) on the basis of species and functional group cover/abundance for the macrophyte data. Euclidean distance was used to compare samples (wetlands) on the basis of environmental parameters. The amount of Bray-Curtis dissimilarity or Euclidean distance between samples can be used to graphically display the samples as data points in a two- or three-dimensional approximation of the multiple-dimensions (multi-dimensional space) that makes up the differences between samples. Further details of the graphical means of displaying similarities will be provided below. Euclidean distance is the "ordinary" distance between two points that one would measure with a ruler, and is given by the Pythagorean formula. By using this formula as the measurement of distance within multidimensional space, Euclidean space (or even any inner product space) becomes a metric space. The use of the Bray-Curtis formula is more appropriate for the determination of difference between cover/abundance of organisms than Euclidean distance (Field *et al.* 1982).

Groups of samples of similar vegetation units, that come from habitat with similar environmental determinants, that represent the reference environmental condition, have characteristic species assemblages (Whittaker 1962) and will thus be relatively homogeneous in their distribution in multi-dimensional space (e.g. Clarke and Warwick 2001). Groups of samples that have similar dispersal of their samples in multidimensional-space, as can be represented by similar dispersal of data points in two dimensional or three-dimensional graphical ordinations, are said to have homogeneous dispersion. In analyses that compare the amount of variation of the samples of one group to another, groups with homogeneous dispersion are more accurately comparable than groups with heterogeneous or different dispersion. Both characteristic (uniform) and uncharacteristic responses by the biotic assemblage to disturbances or stressors are likely decrease homogeneity of group dispersion (increased multivariate differences) thereby increasing the spread or scatter of such samples in a lower dimensional ordination of their multivariate distribution (Anderson *et al.* 2008). Comparison of the multivariate dispersion of reference as opposed to disturbed sites would reveal greater dispersion (wider scatter of data points in an ordination) in the disturbed group (Anderson *et al.* 2008).

2.10.5.1. Techniques for visual display of multi-dimensional data

Principal Co-Ordinates analysis (PCO), non-metric multidimensional scaling (MDS) and cluster analysis are all useful and complementary techniques that assist with the interpretation of the distribution of samples in multi-dimensional space (Clarke and Warwick 2001, Quinn and Keough 2003, Anderson *et al.* 2008). The distribution of samples in multi-dimensional space, relates to how similar one sample is to another; be it a simple measure of geographical distance, or complex measure of plant assemblage, or of multiple environmental variables. These different techniques are methods of unconstrained ordination, in which, no imposition of *a priori* grouping is imposed on the samples and any groupings that emerge are considered to be the result of similarities between samples. Constrained ordination uses *a priori* grouping of samples and tests whether these groupings are accurate for all samples of a group. Examples using the single parameter of geographical distance are presented below, in order to explain the difference between the tools used in the present study.

2.10.5.1.1. Principal Co-Ordinates analysis

Principal Co-Ordinates (PCO) analysis places samples onto Euclidean axes (i.e. so they can be drawn) using only a matrix of inter-point dissimilarities (Legendre and Legendre 2003). Principal Co-Ordinates analysis is very flexible as it can be based on any (symmetric) resemblance matrix (i.e. one generated using Bray-Curtis, or Euclidean, or any other resemblance coefficient). However, as for principal components analysis (PCA), PCO is a projection of sample points onto axes that minimize residual variation in the space of the resemblance measure chosen (Anderson *et al.* 2008). Principal components analysis is a specialised case of PCO and is limited to using Euclidean distance. Principal Co-Ordinates analysis shows the amount of information that is explained by the two axes that data are projected onto, an example of which is shown in Figure 2.5. In this example, the two axes in a PCO of the geographical coordinates of the sampling points from the present study explain 100% of the variation in the data. Being a Euclidean measure, geographical distances are easily and completely described in this 2-dimensional format. In the depiction of geographical positions shown in Figure 2.5, the 32 wetlands of the Cape Flats were so close together, relative to the sites on the West Coast or in the Overberg, that they are superimposed. The distance between the various samples represents the geographical distances by which they are separated in the field as PCO attempts to preserve the Euclidean distance measurement. PCO has the advantage over PCA, in that it can be used to represent non-Euclidean distances in a 2-dimensional (Euclidian) format/ordination. For instance, the similarities of the floral assemblages among sites are best measured with a distance measure that is not Euclidean and thus is not as easily represented in two dimensions. In this instance it is typical that only a fraction of the multi-dimensional variability will be explained by the two axes of the PCO ordination and the percent that each axis explains gives some measureable concept of how much of the variance or difference among samples the ordination explains. This percentage thus explains how successful the PCO is in describing the difference between groups as displayed in the ordination.

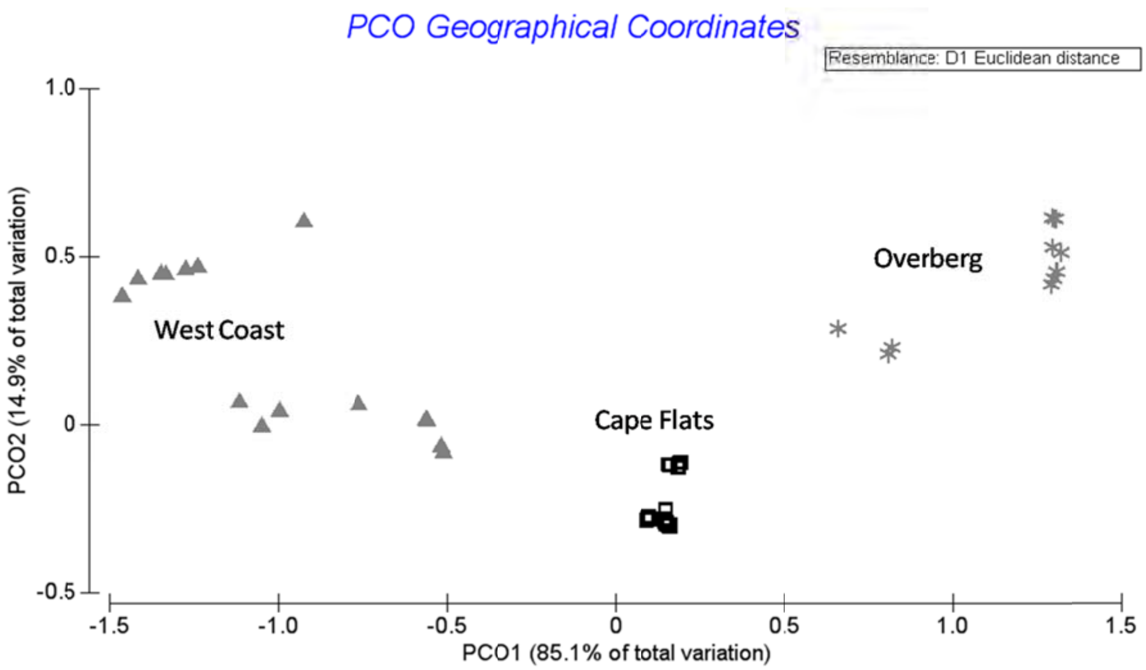


Figure 2.5: An example of Principal Co-Ordinates Analysis (PCO) using the geographical position coordinates of the wetlands from the present study. The two axes in combination explain 100% of the variance or distribution of the wetlands in this multivariate space as would be expected in a representation of Euclidian distances such as geographical coordinates.

<u>*2.10.5.1.2.* Multi-Dimensional Scaling</u>

Multi-Dimensional Scaling analysis is similar to PCO in that it is very flexible and can be based on any (symmetric) resemblance matrix. MDS ordination diagrams present samples as points in a two- or three-dimensional projection such that the relative distances between the points is in the same rank order as the relative resemblance of the samples, as measured by some appropriate similarity matrix calculated on the raw data (i.e. sample 1 is more similar to 2 and will therefore be placed closer to it than to 3). The interpretation of MDS is therefore straightforward: points that are close together represent samples that are very similar (i.e. similarly ranked in terms of spatial or environmental parameters, or in community composition) and points that are far apart correspond to very different values of the variable set (Clarke 1993). Ordination by MDS is therefore an appropriate technique for displaying similarity amongst floral assemblages represented by Braun Blanquet (1928) data, because it primarily relies on the rank order of the full set of similarities, rather than relying on some distance measurement that it is trying to "preserve" in a lower dimensional ordination of the multi-dimensional data (*sensu* PCO: Clarke and Warwick 2001). As an example, an MDS ordination of the geographical position of the wetlands of the present study is presented in Figure 2.6. Once again, as was the case in the PCO (Fig 2.5), ordination using MDS depicts the wetlands from the Cape Flats as being very tightly clustered due to their close geographical proximity to one

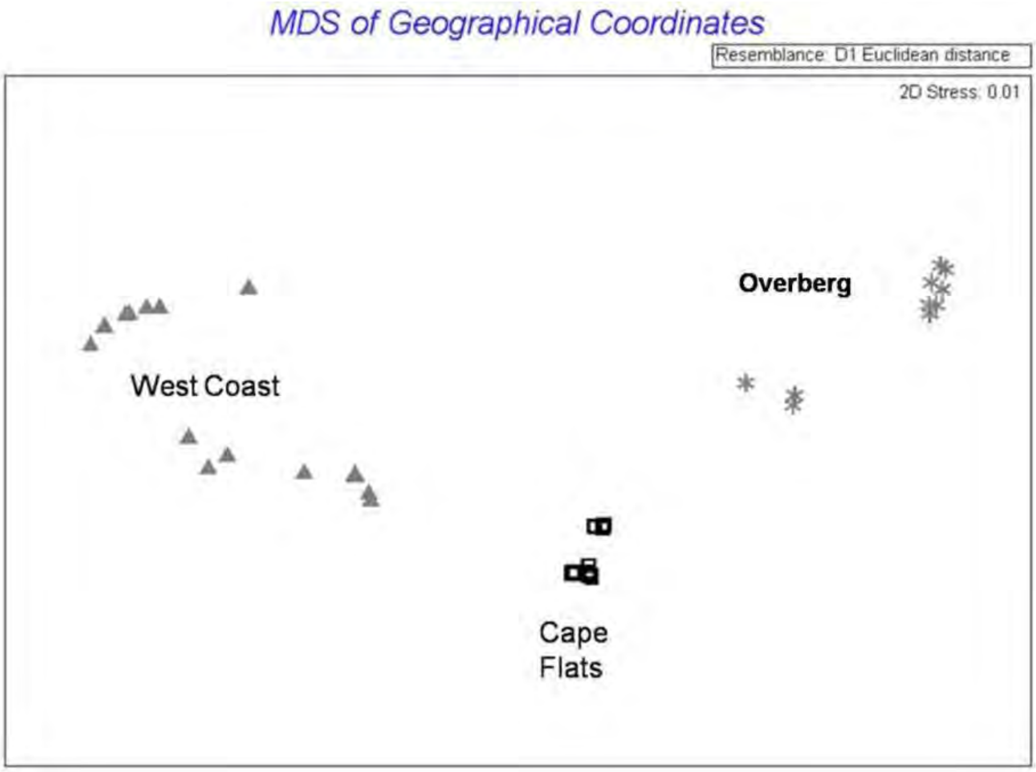


Figure 2.6: An example of Multi-Dimensional Scaling (MDS) using the geographical position coordinates of the different wetlands sampled. Wetlands and sampling areas that were geographically further apart from each other in the region are further apart in this ordination.

another. The West Coast wetlands are further apart from each other, accurately reflecting the greater distances between wetlands assessed in that area than on the Cape Flats. Ordination of the Bray-Curtis similarity of the wetland floral assemblages, rather than the Euclidean similarity of geographical position, would show how similar the flora of one wetland is relative to that of another.

The accuracy of the MDS ordination can be increased by re-running the algorithm that determines where in the 2-dimensional ordination the points representing each wetland will be positioned, the greater number of re-runs of the algorithm increases the number of random permutations of the data set, thereby increasing accuracy (Clarke and Warwick 2001). The amount of stress reported in the final ordination is the difficulty of fitting the points to the 2-dimensional format depicted, as opposed to the multiple dimensions that the data actually represent. In the ordination of geographical distances in Figure 2.6, the stress level of 0.01 is understandably very low, as only a single parameter (Euclidean distance measure of geographical position) was used to create the ordination. The stress value is useful to determine whether the MDS is adequately capturing the multi-dimensional distribution in two dimensions (Clarke and Warwick 2001, Clarke pers. com. 2008). The stress is a measure of the inability to plot the MDS in 2-dimensional space based on the scatter around a Shepard diagram as derived from the Bray-Curtis dissimilarity (Clarke 1993). Stress is similar to the percentage of total variation explained by the PCO axes, except that smaller stress values suggest more variance is visually apparent in the ordination image. A rule of thumb for stress value interpretation is: stress <0.05 is excellent, <0.1 is good, <0.2 is potentially useful, >0.3 is possibly a random placement of points and by the time stress reaches 0.35 the samples are effectively random (Clarke 1993). Stress tends to increase with an increasing number of samples, thereby limiting the number of samples one should attempt to include in an MDS ordination. Examination of the samples that contribute most to this stress value can reveal wetlands that are considerably different from others in the data set, if this is not already apparent from the ordination. Essentially, if a data set has too much internal variability, stress levels will be high and it will therefore be apparent that it is necessary to first split the data set before useful ordinations that can assist our understanding of a data set can be created.

2.10.5.1.3. Clustering dendrograms

Dendrograms, or cluster trees, are another means of determining how similar one sample is to another, for grouping like samples, and for searching for outliers from a data set (see summary below). Clustering dendrograms require a clustering algorithm that computes how similar one sample is to another and an agglomeration criterion to determine how these clusters should be joined together in this 2-dimensional relationship tree (example in Figure 2.7). Different clustering algorithms result in different associations but none are as simple or intuitive as the rank order association of MDS ordinations (Clarke and Warwick 2001). The group average clustering algorithm was used in the present study (Clarke and Warwick 2001, Clarke and Gorley 2006). Using this criterion, samples are joined into a *cluster* based on the similarity of their values. The magnitude of similarity between the "group averages" (of all samples within a cluster) of different clusters is then used by the hierarchical agglomeration criterion, to arrange how groups are joined in the dendrogram. Agglomeration starts by joining clusters with the highest mutual sum of group average, then gradually lowering the similarity level at which clusters are joined.

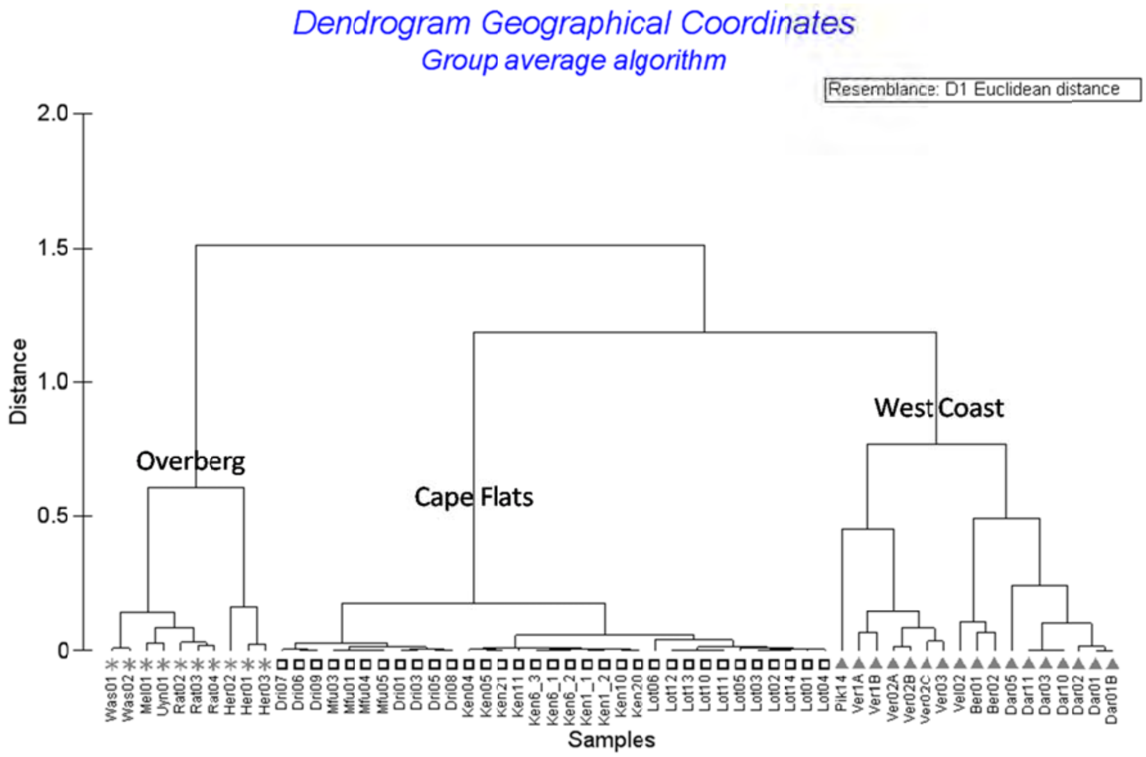


Figure 2.7: Example of a clustering dendrogram of the sampled wetlands in the present study as based on the geographical coordinates of each wetland. As would be expected, wetlands closer together in geographical space are clustered in similar groupings.

The small distance between Cape Flats wetlands, as depicted on the y axis of Figure 2.7, accurately reflects the information also shown in Figures 2.5 and 2.6, namely; that the Cape Flats wetlands are geographically closer together than those from other sub-regions. The wetlands (samples) of the Overberg, West Coast and Cape Flats sub-regions are all separated from samples of the other sub-regions as is the case in all of these Figures (2.5-2.7).

2.10.5.1.4. Constrained ordination

Canonical Analysis of Principal coordinates (CAP), is a constrained ordination technique, that by discriminant analysis can define a linear combination of the (Bray-Curtis or Euclidean) resemblances of the biotic or environmental data of different groups of samples (Anderson and Willis 2003). This technique maximizes the between (or inter-) to within (or intra-) group variation of biotic/environmental data within a dataset, making differences more apparent between distinct groups with similar resemblance values. Using the geographical coordinates of the wetlands sampled in the present study an example of a CAP analysis is presented constrained by *a priori* separation of wetlands into different sub-regions (Figure 2.8).

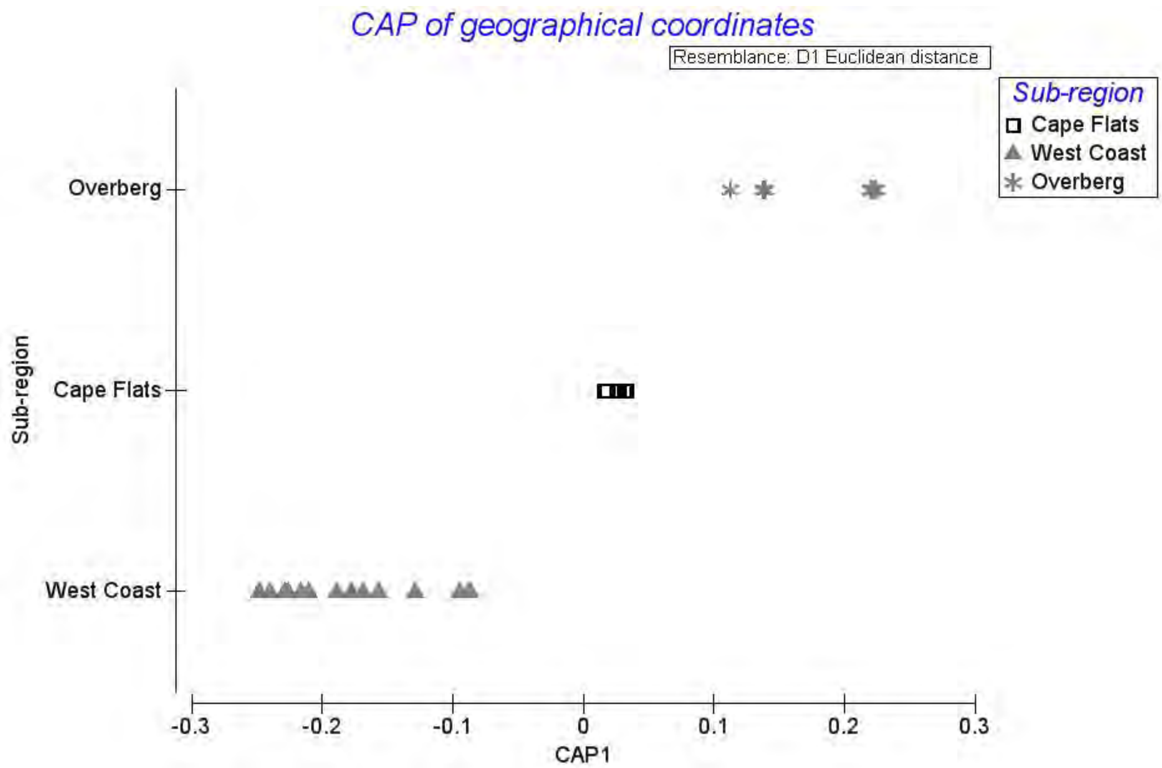


Figure 2.8: Example of a constrained ordination produced with canonical analysis of principal coordinates of the sampled wetlands in the present study as based on the geographical coordinates of each wetland. As would be expected, wetlands closer together in geographical space are clustered in similar groupings. The squared canonical correlation of sample position relative to the first axis (CAP1) = (δ_1^2) = 0.9134, represents the accuracy of the ordination (and in situations where only one axis is used, also indicating the trace statistic for the magnitude of difference between groups).

In order to assist with interpretation of the CAP analysis, a table of diagnostics is presented, based on the number of samples that were correctly "classified" or allocated to any given group by the resemblance values that each sample represents (Table 2.6). A test-statistic (tr(Q_m'HQ_m) based on permutational determination of significance as calculated on resemblance values is also presented.

Table 2.6: Diagnostics for discriminant analysis between species assemblages of different sub-regions using Canonical Analysis of Principal coordinates.

tr(Q_m'HQ_m)=0.9134, p=0.001; No. of permutations used: 999

Total correct: 59/60 (98.33%): Misclassification error: 1.6%

	Classified as:				
Original group	West Coast	Cape Flats	Overberg	Total n***	%correct
West Coast	17	0	0	17	100
Cape Flats	0	33	0	33	100
Overberg	0	1	9	10	90

***n = number of wetlands

In this CAP analysis, it is apparent that the coordinates of each sub-region are significantly different and the classification/allocation was more than 90% successful; suggesting that the constrained ordination is an accurate rendition of the differences between the geographical positions of wetlands from each sub-region. Since with a possibility of three groups to choose from an allocation of 33.3% can be expected by chance alone, the 90% success rate for the least successfully classified sub-region (Overberg), suggested the CAP trace test (tr(Q_m'HQ_m) can be considered as accurate for making predictions. This test suggested that the position of wetlands from each sub-region was significantly different (tr(Q_m'HQ_m)=0.9, p=0.001).

2.10.5.1.5. Summary of visual techniques of displaying multidimensional data

Ordinations are a visual representation of multi-dimensional data in a low dimensional format (2- or 3-d). Comparisons among vegetation samples from single units of habitat, or hydrological zones, using ordinations and dendrograms would predominantly need to be carried out in isolation from other units/zones if a response between reference and disturbed condition is to be visually discernable. Otherwise, the multi-dimensional “noise” of different habitat/hydrological zones would obscure the separation that would otherwise be visible between samples with different assemblages or disturbance levels in a (typically) 2-d ordination. Multivariate analysis of all of the habitats together can be accurately achieved, but visual depiction is less easy; reflecting Gerritsen’s (1995) caveat about the difficulty of describing results of multivariate analyses (Section 2.7.2.2).

Depiction of multidimensional data in two dimensions can be used to identify discontinuities or outliers in multivariate data sets. Distances between main clusters of observations and isolated samples (or objects) (i.e. those which are different from the group as defined by the multivariate data) are likely to be expressed by minor axes which are orthogonal (i.e. perpendicular in the multidimensional space) to those axes of the major plane that are explaining the distribution of the major clusters in an ordination (Legendre and Legendre 2003). In a (low) 2-dimensional ordination, such isolated samples may be superimposed over the main cluster but they are in fact outliers from the main cluster, being very different (or having a discontinuous multivariate relationship) from them. Ordination by multidimensional scaling (MDS), is more efficient than other methods of ordination at flattening multidimensional data into a user-determined, small number of dimensions and will often reveal such outliers/discontinuities. Visual comparison of dendrograms and ordinations and overlaying MDS ordinations with contours that represent links between members of a dendrogram derived group, provides a means of determining such outliers or discontinuities (Clarke and Warwick 2001, Legendre and Legendre 2003).

2.10.5.2. Analyses within multivariate space

Once ordinations and dendrograms have revealed discontinuities and outliers from *a priori* determined groups, other statistical tools must be used to test the multivariate association between objects (e.g. wetlands or quadrats) within groups (e.g. habitat types and disturbance categories). For instance, the determination of the affinity of members to a group, or the amount of difference between the floral assemblages of reference and disturbed sample sites, must ultimately be determined in multivariate space. Analysis of similarity and analysis of variance are tools for comparing samples from different groups. These tools can assist in the determination of how similar samples from each group are to one another.

2.10.5.3. Analysis of similarity

The Analysis of Similarity (ANOSIM) is a means of determining the amount and significance of difference between *a priori* determined groups; and is compatible with both a univariate and multivariate approach. ANOSIM is a simple, non-parametric permutational procedure, applied to the (rank) similarity matrix (species or environmental variables *vs.* samples) underlying the ordination or classification of samples. This is combined with a general randomization approach to the generation of significance levels

(Monte Carlo tests, *sensu* Hope 1968). ANOSIM is similar to the Kruskil-Wallis ANOVA as performed on variables without normal distribution. The ANOSIM test statistic (*R*) reflects the observed differences *between samples in different* groups contrasted with differences among samples *within* groups; as based upon the rank similarities between samples within the space of the resemblance matrix.

ANOSIM produces a global "*R* value" which varies from -1 to +1. Where for example, 0 indicates no difference in intra- and inter- sample (i.e. habitat/wetland) or group (i.e. disturbance level or location) variability. On the other hand, a positive value of 1 indicates complete difference between groups (i.e. all samples for a given group are more similar to each other than to any samples from different groups). *R* will usually vary between 0 and 1, indicating some degree of discrimination between groups. An *R* value substantially less than zero, is unlikely, since it would correspond to similarities among samples from different *a priori* classified groups (habitat/wetlands) being *higher* than those within groups. This is more likely to occur as a result of misallocation of a number of samples to a group, which can occur due to a major environmental discriminator or disturbance being unconsciously encompassed in the sampling array, and then not recognized in the classification of samples to different groups. The *R* statistic is a useful and absolute measure of the degree of separation between groups, and its value is argued as being at least as important, if not more important, than its statistical significance (Clarke 1993). As with standard univariate tests, if there are many replicates in each group being compared, it is possible for *R* to be significantly different yet inconsequentially small; suggesting little actual difference between groups.

The calculation of the significance level of the *R* statistic is performed by referring the observed value of *R* (i.e. value of compared samples in the resemblance matrix) to the range of *R* values achieved from 999 random permutations generated by reshuffling the sample labels. If only *t* of the *T* simulated values of *R* are as large (or larger than) the *observed R* then the null hypothesis (H_0) that group A is different from group B can be rejected at a significance level of (t+1)/(T+1), producing a *p*-value or percentage of significance that the difference shown to exist between A and B would be chance alone (Clarke and Warwick 2001). The probability of such (Type 1 statistical error) chance selection equals the p-value selected for the test. The permutational determination of significance therefore assumes that the samples are exchangeable under a true null hypothesis. Hence spatial autocorrelation (or correlation resultant from affiliation to a variable with spatial influence), or significant multivariate dispersion(the inclusion of two entirely different ranges of variables such as wetlands with only 5% cover in one group and only 50% cover in the other group), could make the test invalid (further explanation below).

2.10.5.4. Multivariate analysis of variance

Permutational Multivariate Analysis Of Variance (PERMANOVA) is a technique that facilitates comparison between *a priori* determined groups of samples (Anderson et al.2008) such as between disturbance categories within a habitat zone. PERMANOVA compares the total variation within one group relative to the total variation within another. This is performed by comparing the total variance, or dissimilarity of all samples within each group, relative to the total variance within each other group being compared. If total variance of groups is different, then the samples of each group are considered to be different. The determination of significance is computed in the same permutational manner as in ANOSIM described above. This analysis approach is essentially a Multivariate Analysis Of Variance (or MANOVA) performed using permutational generation of significance (PERMANOVA).

PERMANOVA tests the dissimilarity values generated by the 'sample by species' resemblance matrix on which permutations are based, through an analysis of variation of the estimates of pooled within-group variability, generating a test statistic of pseudo-*F* (or pseudo-*t*, for *a posteriori,* pair-wise, t-tests between subsets within groups) (Anderson *et al.* 2008). *A posteriori* t-tests are used to determine how similar the sub-sets of a factor, or group that is being compared with PERMANOVA actually are. For instance, the difference between Reference and Worst (most impacted) wetlands within or between sub-regions of the Western Coastal Slope (refer to appropriate section). These statistics are given the prefix *pseudo* as they are similar to the ANOVA-*F* statistic and Students' t-test in most ways, other than the generation of significance, which is carried out by means of permutation. The only assumptions about the data that are made in the use of PERMANOVA are that samples are exchangeable under a true null hypothesis; in order that exchanging the labels of the samples to generate significance levels can be performed. This assumption is tantamount to assuming that the multivariate observations (samples) are *independent and identically distributed* under a true null hypothesis. Exchangeability of multivariate observations (samples) is assured, if a random allocation of sample units (wetlands) to groups (disturbance categories and/or sub-regions) has been carried out *a priori* (Fisher 1935). For observational studies such as the present one, in which groups already occur naturally distributed in nature and a random sample is drawn from that, exchangeability under a true null hypothesis is an assumption that is made (Kempthorne 1966). If the samples in different groups are not independent, for example if they are spatially correlated, or if their multivariate dispersion is not homogeneous, then they are not really exchangeable and randomly shuffling (permuting) said samples will destroy this inherent structure (Legendre 1993, Anderson *et al.* 2008). Results from such an invalidated analysis could lead to the incorrect acceptance that groups are significantly different from one other. The determination of homogeneity of dispersion is dealt with in the PRIMER statistical analysis package PRIMER-E v.6 and its add-on package PERMANOVA+ (Clarke and Warwick 2001, Clarke and Gorley 2006, Anderson *et al.* 2008) and is briefly explained below (i). Spatial correlation is not dealt with in PRIMER but it can be avoided by the separation of data sets into groups that are spatially independent (ii).

2.10.5.5. Identical distribution and multivariate dispersion

PERMANOVA and ANOSIM procedures are sensitive to differences in dispersion of variance among groups (Clarke 1993, Anderson *et al.* 2008). The construction of pseudo-*F* ratios (the test statistic) in PERMANOVA uses pooled estimates of within-group variability. Thus, homogeneity of multivariate dispersions is implicit in the partitioning between groups (Anderson *et al.* 2008). A separate test for homogeneity of dispersion, using the PERMDISP (Permutational Dispersion) routine can be performed to determine differences in dispersion of the variance between groups (see Anderson et al.2008). PERMDISP uses permutation of residuals (i.e. the permutation of samples among groups after centering all groups onto a common location) in order to generate *P*-values. PERMDISP detects differences in dispersion that, in many cases, are not substantial enough to inflate the error rates of the PERMANOVA test. This is analogous to univariate analysis of variance, which is quite robust to many forms of heterogeneity of dispersion, especially in situations of large sample size (Box 1953). If significant heterogeneity of multivariate dispersion were detected by PERMDISP and differences among groups were also detected using PERMANOVA, then the latter could have been caused by difference in location (i.e. greater association with one group), differences in dispersion, or some combination of the two. Thus performing a test using PERMDISP, as well as examining the average within and between group dissimilarities (using ANOSIM) and the position of samples from different groups in unconstrained ordination plots such as MDS or PCO will help to uncover whether samples of different groups are similarly dispersed (have homogeneous dispersion).

2.10.5.6. Spatial autocorrelation

Spatial autocorrelation is loosely defined as, "the property of variables, which take values at pairs of sites a given distance apart, that are more similar or less similar than expected for randomly associated pairs of observations" (Legendre and Legendre 2003, Rosenberg 2009). Autocorrelation refers specifically to the lack of independence among the error components of pairs of observations of field data, due to spatial proximity (Cliff and Ord 1981, Legendre 1993, Legendre and Legendre 2003). This occurs because variables can change as a function of space (e.g. temperature or constituent elements of soil), or are impacted by another variable that is spatially structured (e.g. altitude, rainfall, pH, soil type, etc.) (Legendre and Legendre 2003). Spatial autocorrelation causes over-estimation of the number of degrees of freedom in an analysis (Legendre and Legendre 2003). This over-estimation artificially narrows confidence intervals and leads to Type I errors, i.e. the false rejection of null hypotheses. For example, when positive spatial autocorrelation is present in the smaller distance classes, the usual statistical tests too often lead to the decision that correlations (Cliff and Ord 1981), regression coefficients or differences among groups (i.e. ANOVA) (Legendre et al.1990) are significant, when in fact they may not be. It is therefore necessary to modify the statistical method in order to take spatial autocorrelation into account (Legendre and Legendre 2003). When the presence of spatial autocorrelation has been demonstrated, or where it is anticipated to exist due to significant spatial structure such as the emphasis of climatic or geological differences in two disparate areas, splitting the data into sets that are spatially independent makes it possible to compute the usual statistical tests or permute the labels independently for each set.

2.10.5.7. ANOSIM versus PERMANOVA

Whilst the *R* statistic of ANOSIM is an absolute measure of difference between groups and is thus directly comparable between different studies, the pseudo-*F* (or pseudo-*t*) of PERMANOVA is:

1. Necessarily reliant on the degrees of freedom of the analysis, and so cannot necessarily be compared in value between groups or studies that are based on a different number of samples (for a given dissimilarity measure). A greater number of samples (increasing the degrees of freedom), increases the reliability and strength of the pseudo-*F* statistic.
2. The inter-group variability is always scaled against the intra-group variability. Thus, the intra-group variability plays an important role in the value of pseudo-*F* or pseudo–*t*. Where intra-group variability is considerably higher for one of a number of groups

being compared (i.e. greater dispersion in variability of samples from worst-disturbed than from least- and moderate-impaired ecosystems), then comparison of this group with any of the other groups will result in a lower value of pseudo-*F* or pseudo-*t.*

Pseudo-*F* or pseudo-*t* cannot be compared between studies based on different dissimilarity measures.

In PERMANOVA, the *p*-values (significance level) should be used as a measure of *strength of evidence* with respect to any particular null hypothesis being tested (i.e. a null hypothesis (H_0) is that: Reference and Worst wetlands hold different plant assemblages). The option of determining *p*-values using Monte-Carlo sampling (Hope 1968) as typically utilized when the number of permutations are less than 100, means that the power of the test need not especially rely on the number of possible permutations (Manly 1997, Anderson *et al.* 2008).

2.10.5.8. Percentage similarity of species assemblages

When a difference has been shown to exist between two groups of a data set, such as between species assemblages of reference and worst disturbed wetlands, then a formal determination of which species contribute most to these differences is needed. Analysis of the percentage of similarity (SIMPER) is a means of determining which variables (species) are characteristic of, or discriminatory between, groups or sub-sets of a data set and thereby contributes most to the difference.

In the SIMPER analysis, between all pairs of samples (one of each belonging to a different group, e.g. reference *vs.* worst), the average (Bray-Curtis) dissimilarities for species data are broken down into percentage contributions from each species, listing the species in decreasing order of such contributions (Clarke and Warwick 2001).

Characteristic species can be defined as those that have consistently high cover/abundance throughout the samples of a group, such as throughout the samples of one level of disturbance, or a sub-region. The consistent high cover/abundance means that such a species will have a low standard deviation in contributing to group similarity and therefore the ratio of group average similarity (group average squared distance) to standard deviation of group similarity (Sim/SD) will be high. The high Sim/SD of characteristic species does not guarantee that these species are a good discriminator between two or more groups as it may occur with equal cover/abundance in both or many groups and therefore not distinguish between them (Clarke 1993). It is also possible by the same procedure to determine the species that are discriminatory between different levels of disturbance from the ratio of group average dissimilarity to standard deviation of group average dissimilarity (Diss/SD). Species that are typical of each group, but occur with equal cover/abundance or value in each, are not discriminatory between them. Species that contribute much to the difference between the groups will be of low discriminatory use when they do not occur consistently enough (or do not have high enough fidelity) across the samples of either group (i.e. have high standard deviation and thus low Diss/SD) (Clarke 1993). Therefore, the higher the Diss/SD ratio the more reliable the species is as a discriminator. A measure of influence of each species is dependent on species cover/abundance. The greater the percentage contribution a species makes to a group, the greater the magnitude of influence in the group, or between groups.

2.10.5.9. Multivariate analysis of biotic affiliation with environmental variables

Distance-based Linear Modelling (DistLM) (Legendre and Anderson 1999, McArdle and Anderson 2001) was used to determine which environmental variables were most responsible for the difference in the (Bray-Curtis) resemblance of the vegetation assemblages of Reference and Worst disturbed wetlands. The DistLM, is an exploratory tool that matches subsets of biotic assemblage patterns to environmental variables to determine which variables explain most of the variance in the assemblage pattern between two groups, i.e. between disturbance categories (Anderson *et al.* 2008). The DistLM partitions the variation in biotic data distribution according to a multiple regression model, based on the linear combination of predictor environmental variables that fit the biotic distribution. Which environmental variables to select are determined by a "*selection criterion".* Two *selection criteria* were used in this study.

1. The Adjusted R^2 predictor variable selection criterion (Anderson *et al.* 2008) which excludes a variable if its inclusion does not add more to the explained sum of squares (correlation) than the inclusion of any other random variable.
2. A modification of Akaike's (1973) "An Information Criterion" (AIC) adjusted for situations in which the number of biotic samples is low, relative to the number of environmental variables (AIC_c) (Anderson *et al.* 2008). This criterion determines typically more parsimonious solutions than the Adjusted R^2 criterion.

The linear combination of selected variables is determined by the chosen *"selection procedure"*: all specified / forward selection / step-wise / best fit, etc. The "Best" environmental variable selection procedure was chosen in this study, which examines and selects the highest “sum of squares” value explained for all possible combinations of predictor variables. The distance-based linear model determines which variables independently explain a significant percentage ($p<0.05$) of the variation between biotic communities (vegetation assemblages) from different groups (Reference and Worst disturbed wetlands) and reports these variables, in addition to the significance level, as Marginal tests. Collectively, looking at the multivariate interaction, DistLM determines the linear combination of variables that best explain the association of biotic assemblages to different groups (called the best solution). All potential best solutions to the model, with less than two AICc units, or adjusted R^2 units, difference from first solution are viable solutions that describe the linear combination of environmental variables that best explain the difference in association of biotic samples with one, or another group.

Distance linear modelling is susceptible to the influence of outliers; hence ordinations and dendrograms were also used to search for any obvious environmental or biotic outliers in the predictor and response data sets. The DistLM procedure is also susceptible to skewness and multi-collinearity in the predictor variables. Predictor variables that had skew distribution were log transformed (Clarke and Warwick 2001). A search for any multi-collinearity between variables in the environmental data sets per habitat-locality was performed using Pearson correlation. Any variables that were collinear at 95% (r=0.95) or less than -95% (r=-0.95) were removed from the data set.

DistLM analyses without first removing environmental variables that do not (on average) significantly differ between comparison groups (i.e. between disturbed *vs.* undisturbed wetlands) leads to the inclusion of variables whose average value does not differentiate between groups. The removal of all environmental variables that individually do not contribute to the environmental difference between groups reduces the total variation that will be explained by the collective and linear combination of variables in a DistLM. It does, however, highlight those variables of greatest influence.

A distance-based Redundancy Analysis (dbRDA) ordination of different biotic samples (i.e. wetlands), is created with a vector overlay based on the multiple partial correlation of the linear combination (DistLM) of the environmental variables that contribute to the distribution pattern of the biotic assemblage (as displayed in Figure 2.9). This ordination of biotic samples is therefore constrained by the linear combination of environmental variables that best explain their distribution. The lengths of environmental vectors are based on Pearson Rank correlations (*r)* of each environmental parameter, reflecting their univariate importance in explaining the modelled vegetation assemblage pattern (Ter

Braak 1990). The interactive effects of parameters are not included in these vectors; however their multiple partial correlations do determine the position of biotic samples in the ordination and are reported in terms of their impact on this arrangement along the axes of the ordination. The percentage of total variation between biotic samples explained by the multiple partial correlations of environmental variables, is displayed on each axis of the dbRDA ordination. In an example based on the longitude, latitude and altitude of the Cape Flats wetlands is shown in Figure 2.9. Longitude, latitude and altitude are the environmental variables that describe the position the wetlands relative to one another. Based on multiple partial correlations: altitude (r=-0.97), longitude (r=-0.208) and latitude (r=-0.089) collectively explain 78.1% of total variation along the primary or x-axis (dbRDA 1). Along the y-axis, longitude (r=0.965) explains most of the variation, followed by altitude (r=0.19) then latitude (r=0.18); collectively explaining 21% of total variation. Having based this example on measurements with Euclidean distance, these two axes describe 99.1% of model fitted and of total observed variation. With the addition of a third axis (accounting in this case for altitude) 100% of variation between the positions of all wetlands is described by these variables.

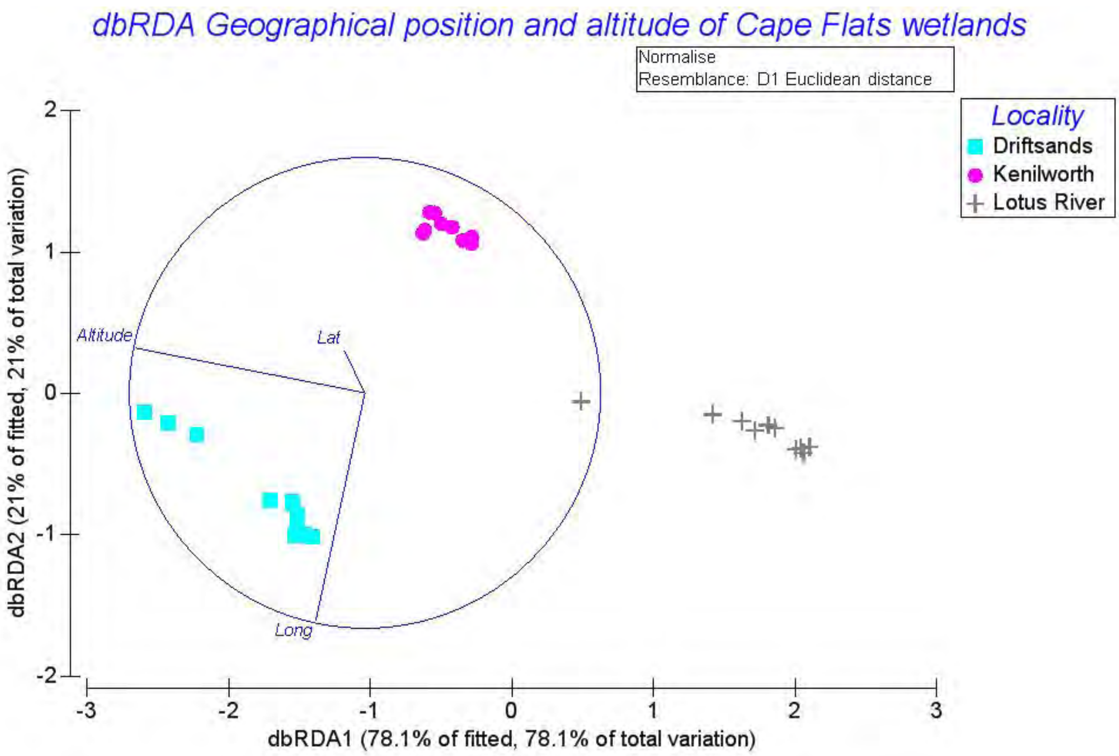


Figure 2.9: Example of a distance based Redundancy Analysis ordination of the wetlands of the Cape Flats as described by their longitude, latitude and altitude. Vectors are Pearson correlations (r>1).

2.10.6 Species diversity indices

An exploration of the species vegetation diversity variables wetlands that are disturbed relative to those that are undisturbed has the potential to reveal phyto-assessment metrics. A description of the following variables and their relationship to species diversity of vegetation is provided below:

- Total number of species as a concept of species richness (S);
- Species richness as determined by Margalef's index (d) (Margalef 1975);
- Species diversity: Shannon-Weiner (H') (Shannon and Weaver 1949) and/or Simpson's diversity index (Simpson 1949).

The area that a species covers, or the number of individuals of a given species, can be used interchangeably in the generation of diversity indices (Whittaker 1965). Although it is easier to sample vegetation using the Braun-Blanquet cover scale, rather than absolute measures of cover, t can produce a biased result if used (in place of abundance) in conjunction with diversity indices (Magurran 1992). In the present study, in which diversity measures are to be compared between samples in order to determine whether they can be used as a metric of difference between disturbed and undisturbed vegetation communities, the bias would impact all measures equally and is thus considered unimportant. That diversity should decrease as disturbance increases, has been shown to be dependent on the starting point of the natural environment of an area and be dependent on the intensity of disturbance influence (Tilman 1999). The use of diversity measures to compare between disturbance categories or environmental conditions, can only be done once causation and correlation of biotic association with said categories or conditions has been proven. The determination of the importance of human influence amongst the many environmental variables that caused difference between biotic assemblages using DistLM assists in the determination of causation.

2.10.6.1. Species richness

Richness is a measure of the number of different kinds of organisms present in a particular area. Species richness for a specific group of organisms is the number of different taxa of that type of organism present. Whilst species richness is often given simply as the number of taxa, this measure is obviously very dependent on sample size and therefore also on sampling effort. Margalef's index (d) determines species richness relative to the total median cover in a sample (N). Margalef's index is based on the number of different species within a sample standardized against the total cover each species occupies within that sample; therefore, species with little cover add proportionately less to the index. Per sample, Margalef's richness is calculated by dividing the total number of species less one, by the natural logarithm of their total cover or abundance:

$$\mathbf{d = (S\text{-}1)/Log_eN}$$

2.10.6.2. Species Diversity

Biological diversity can be quantified in many different ways. The two main aspects taken into account when measuring diversity are richness (as described above) and evenness. Evenness compares the abundance of each species present with that of every other species. If all species have similar cover/abundance in a specific area, then the evenness is higher. If there is one species that is highly dominant, it will reduce the evenness, and in many cases also the richness, since the dominant species will out-compete many others, including similar types of organisms. The *relative cover* or *relative abundance* of the different species making up the richness of the wetlands is thus a measurement of evenness (*sensu* Simpson 1949). The diversity indices employed in this project, which incorporate both evenness and richness, are those developed by (a) Shannon-Wiener and (b) Simpson.

2.10.6.2.1. Shannon-Wiener diversity index

The Shannon-Weaver index (H) measures overall biodiversity. H is calculated per sample (wetland) as the sum of the proportion of the total cover/abundance arising from the i^{th} species (p_i) multiplied by the natural logarithm of this proportion:

$$\mathbf{H' = -\textstyle\sum_i p_i \log_e(p_i)}$$

H is maximized when all species have the same number of individuals. For example, it is biggest when a wetland has 4 aquatic herbs, 4 graminoids, and 4 shrubs. H is smaller when a wetland has 1 aquatic herb, 2 graminoids, and 5 shrubs. Note that both wetlands have 8 inhabitants. The Shannon-Wiener index is however sensitive to the degree of sampling effort (Clarke and Warwick 2001).

2.10.6.2.2. Simpson's Diversity Index – Dominance or Evenness

Simpson's diversity index measures the richness and percentage cover of species in a sample or habitat. The index assumes that the proportional cover/abundance of species in an area indicates their importance to the species diversity of that ecosystem. Due to the importance of proportions, unlike the previous Shannon-Wiener index, Simpson's measure of species diversity is insensitive to the degree of sampling effort (Clarke and Warwick 2001). Simpson's diversity is a dominance index, in the sense that its largest values correspond to assemblages whose total cover/abundance is dominated by one, or very few, of the species present. Simpson's dominance as determined per sample (i.e. per wetland or per vegetation plot) is the sum of the square of proportion of the total cover/abundance arising from the i^{th} species (p_i):

$$\textbf{Dominance} = \lambda = \sum p_i^2$$

The range of this function is between 0 and 1. More biologically diverse samples score near 0 and more monotypic samples score near 1; where 1 represents no diversity, or that only one species is present (Clarke and Warwick 2001).

The inverse of this latter index is a measure of *evenness*, with the largest value when all species have the same cover/abundance:

$$\textbf{Evenness} = 1-\lambda = 1-(\sum p_i^2).$$

3. APPROACH AND METHODS

In this chapter, justification is provided for the approach adopted in developing phyto-assessment metrics in the coastal lowlands of the Western Cape and an explanation is given of the methods used. The hypotheses that will facilitate the development of metrics for a phyto-assessment index for palustrine wetlands of the Western Coastal Slopes are presented. These hypotheses do, however, require testing to confirm that the framework will provide sufficient material to enable the development of useful metrics. The proposed analyses for performing these tests are also presented. The methods that were implemented served to facilitate Objective 5 (Section 1.3) "the collection of vegetation sample data to test hypotheses" regarding Objective 6 (Section 1.3) "the relationship between macrophytes and the environmental condition of their habitats in order to develop metrics for phyto-assessment".

3.1. Approach adopted for phyto-assessment development

The intention of the present study is to identify (based on the analysis of vegetation and environmental data collected from field surveys), plant species and vegetation attributes that are consistently and characteristically associated with different environmental conditions. These species and attributes can each potentially be developed into a measure, or metric, of environmental condition. Metrics can be amalgamated into a multi-metric phyto-assessment index, thereby facilitating a quantitative determination of environmental condition. Several of these metrics may work in other wetland types and areas of South Africa but will need to be tested in the context of different wetland regions and wetland habitat types (Section 2.9). This project should be seen as an initial feasibility study into the development of a multi-metric phyto-assessment index; a process that has been in development in other parts of the world for the past 20 to 30 years.

The resources and time available for the present study limited the potential sample size that could be collated, and suggested that a single wetland region (*sensu* Cowan 1995 [Section 2.9.1]) and a single habitat type (*sensu* SANBI 2009 and Mucina *et al.* 2006a) should be used to determine the applicability of the phyto-assessment development framework. The wetland region that was chosen was the Western Coastal Slope (Cowan 1995) – a region in which the vegetation of the freshwater depressional wetlands is expected to be relatively uniform. Testing the development-protocol in this region would facilitate the development of ecologically valid metrics, even if they were somewhat limited geographically and in terms of ecosystem type. To focus on changes in plant community assemblage brought about by human-related stressors, rather than natural environmental differences between habitat units, an *a priori* classification of wetland habitat was carried out in the following manner:

1. The National Wetland Classification System (Ewart-Smith *et al.* 2006, SANBI 2009), the ecoregions of Kleynhans *et al.* (2005) (Section 2.9.3) and the wetland vegetation types of Mucina *et al.* (2006a) as incorporated within the wetland regions of Cowan (1995) (see Section 2.9.1), were used as the starting point to determine comparable habitat units of wetland vegetation. This study focused on comparable habitat units within:
   a) inland palustrine wetlands in the Coastal Foreland or lowlands of the Western Cape (Lambrechts 1979) that have a similar position in the landscape and a similar hydrogeomorphic setting (SANBI 2009);
   b) that are also within the mediterranean Western Coastal Slopes wetland region (SWm: Cowan 1995); encompassing:
   c) the south western and parts of the southern coastal belt ecoregions (Kleynhans *et al.* 2005); and
   d) wetlands containing Cape Lowland Freshwater (CLF) vegetation (Mucina *et al.* 2006a).
2. The study focused predominantly on isolated depressions (Ewart-Smith *et al.* 2006, SANBI 2009), within which the relationships between macrophytes and human stressors were investigated. Isolated depressions were chosen in order to reduce the potentially homogenizing influences of the flow of surface water between wetlands. Whilst the focus of the current research was largely on non-tidal depressional wetlands dominated by emergent plants, a number of other palustrine systems were sampled, namely: valley-bottom and floodplain wetlands, as well as wetland flats and seeps (*sensu* SANBI 2009). This was done with the intention of searching for metrics with the potential to be used in all of these wetlands types. The flats and seeps HGM types were sampled predominantly where they included habitat similar to the isolated depressions already sampled. The floodplain and valley-bottom wetlands assessed on the West Coast along the edge of Verlorevlei, are not isolated wetlands but were sampled because Verlorevlei was one of the initial joint study sites for the WHI Research Programme (Malan *et al.* 2006). An inventory of the abiotic parameters characterizing each wetland is presented in Appendix 7.

Subsets of wetland plant communities were sampled in three spatially disparate sub-regions of the Western Coastal Slopes, namely the West Coast, Cape Flats and Cape

Agulhas. Wetlands occur in close proximity in these three sub-regions, within which the intensity and extent of human land-use varies considerably (De Roeck 2007). Assessment effort was therefore concentrated in the three sub-regions with the dual intentions of:

- controlling for (minimizing) the potential changes brought about by natural environmental differences within each sub-region; and
- assessing the strength of gamma-diversity across the Western Coastal Slope region, as well as across each sub-region, and even, where necessary, within sub-regions.

The question needed to be asked whether, even within comparable (either reference or impacted) habitats, significant change (turnover) in plant diversity was apparent from one sub-region to the next. Significant differences in the percentage of unique (occurring exclusively in one sub-region) species in the vegetation plots and/or wetlands in the different sub-regions would indicate the existence of high gamma-diversity. If there is significant gamma-diversity across the chosen wetland region (i.e. SWm) then, in general, the number of wetlands that it is necessary to sample in each sub-region would be increased. Sixty wetlands were sampled in the present study with the intention of being able to develop metrics for the Western Coastal Slope region. This was with the assumption that wetlands in this region that contained Cape Lowland Freshwater vegetation should be considered a single unit of comparable wetland vegetation.

The sample size of the present study may prove to be insufficient to facilitate the development of robust metrics, if too great a variety of habitats and wetland vegetation types were inadvertently included. This all-inclusive approach could however, in theory, yield metrics that may be applicable over the full spatial and habitat-type range of the data set.

For wetland bioassessment in the Western Cape of South Africa this study represents a starting point to determine:

- Phytogeography of wetland taxa. The geographical area (i.e. wetland- or eco-regions or subsets thereof) within which ‘natural’ or reference condition wetlands have similar species assemblages. Or alternatively, whether there is significant gamma-diversity between different localities and sub-regions within the chosen region;
- Whether wetland plants, within this region or sub-regions thereof, are associated with different degrees of environmental condition as caused by human disturbance; and thus;

- The identification of potential indicator species or groups of species and other comparative measures of community assemblage such as evenness and diversity, which are representative of different categories of human disturbance.

3.2 Development of a Human Disturbance Score

An assessment of the variety, intensity and extent of land-use was proposed as an estimate of the degree of human disturbance impacting each wetland. This would therefore provide an *a priori* ranking of the amount of stress that a wetland had been exposed to as measured by 'human land-use affiliated disturbance', and could thus be used to assess the response of plant communities. An assessment method for integrating the different anthropogenic stressors, and thereby scoring the cumulative amount of disturbance impacting on each wetland, was formulated as part of this study and is presented in Section 3.5.4. This is referred to as the human disturbance score (HDS). Using this method, the environmental condition of the study wetlands was classified as reference (least-), moderately- or worst-impaired ecosystems. This method was intended to be quick, taking only a few hours per wetland to complete both the field assessment and calculate the final score.

This human disturbance assessment was derived from several sources:

- A mini-workshop with WHI team members using their expertise to collate information on the impacts of human land-use on aquatic ecosystems;
- The Western Cape Wetlands Inventory Datasheet (Dallas *et al.* 2006);
- The protocols stipulated by the Ohio Rapid Assessment Method, Version 5.0 (Mack 2001b);
- The Human Disturbance Score method of Gernes and Helgen (2002);
- The draft WET-Health protocol of Macfarlane *et al.* (2007 Draft, final version completed in 2008); as well as
- The draft Wetland Index of Habitat Integrity assessment tool (final version Rountree *et al.* 2007).

The tools and assessments that informed the final HDS method all followed the procedure of determining an overall level of impact for each wetland being assessed (Section 2.8.1.1). Whilst this procedure was used in this study, it should be noted that hydrological stressors impacting on the availability of water are not necessarily cumulative. Water can be added to, or subtracted from, a wetland depending on the nature of the anthropogenic activity. To determine an overall change to water availability, impacts causing water loss were scored negatively whilst those causing water gain were scored positively. Both states of change qualify as disturbance, however, and from a cumulative disturbance perspective both count to increase disturbance. Hence, for ranking purposes, both water gain and loss were scored positively so as to maximise the human disturbance score (see Section 3.5.4).

In summary:

- An integrated measure of human disturbance (the Human Disturbance Score; HDS) was used to determine the environmental condition of each wetland.
- Reference and disturbed conditions were defined and, based on this, the sampled wetlands were divided into three categories of disturbance intensity, namely least (Reference), moderately (Moderate) and most impaired (Worst) wetlands.

Nutrient concentrations in the soil and water were also measured and used as independent indicators of the influence of agriculture and urban land-uses. Each nutrient parameter that was measured (e.g. ammonium, nitrate) provided an independent trophic gradient against which to compare the patterns of the vegetation assemblage. Eutrophic concentrations of any of these nutrients were considered to indicate human disturbance and thereby used to corroborate the assignment of the categories of disturbance.

3.3. Hypotheses to be tested

The heterogeneity of wetlands in South Africa suggests that the assessment metrics developed in this project may not be applicable to all the biogeographical regions of the country. It is likely, however, that some overlap in metric applicability between wetland types and possibly even between biogeographical regions, is likely to emerge once a broader understanding of wetland ecology is achieved. From the literature review, a number of hypotheses were established concerning the relationship between wetland vegetation and its containing environment. These need to be resolved in order to facilitate the determination of phyto-assessment metrics. The hypotheses and the statistical procedures used to test them are presented below, and are separated into the chapters in which they are dealt:

3.3.1. Chapter 4

Hypothesis 1: Vegetation of similar wetland habitat (i.e. depressions (SANBI 2009) dominated by Cape Lowland Freshwater vegetation (Mucina *et al.* 2006a)) should represent a relatively uniform vegetation set with insignificant gamma-diversity between:

a) the sub-regions (West Coast, Cape Flats and Overberg) of the mediterranean South Western Coastal slope wetland Region (Cowan 1995); and similarly, between:
b) the West Coast and Cape Flats sub-regions contained within the South Western Coastal ecoregion (Kleynhans *et al.* 2005).

Test: Gamma-diversity differences will be determined by permutational analysis of dispersion between the Jaccard-based distance measure of percentage of unique species in each sub-region.

Hypothesis 2: Disjunctions in the distribution of vegetation within the Western Coastal Slope region are due to the spatial affinity of species for sub-regions (West Coast, Cape Flats, Overberg) or distinct locations within these sub-regions (e.g. the Cape Flats: Kenilworth, Lotus River or Kuils River).

Test: Disjunctions of spatial distribution of species/vegetation assemblages will be determined by ordination of the Jaccard, or Bray-Curtis based measures of similarity between wetlands of the different sub-regions. The significance and magnitude of the differences can be confirmed with PERMANOVA.

Hypothesis 3: Biogeographical differences between distinct groups of vegetation (*sensu* Hypothesis 2) are due to difference in environmental parameters, particularly macroclimatic and geological parameters.

Test: Spatial disjunctions in environmental conditions will be determined by ordination of the Euclidean distance-based measure of the multivariate environmental differences between sub-regions.

Hypothesis 4: In a given area (for example the entire Western Coastal Slope region, or sub-regions or locations) with uniform vegetation, the vegetation of different wetland HGM types is expected to be significantly different.

Test: Differences between the vegetation of different HGM types will be tested in ordinations of Bray-Curtis similarity. Significance and magnitude of differences will be confirmed with ANOSIM or Canonical Analysis of Principle Coordinates (CAP) (Anderson and Willis 2003).

Hypothesis 5: At the broad biogeographical scale (region or sub-regions) the impact of human disturbance on vegetation assemblages will have an homogenizing effect, reducing the difference between otherwise distinct spatial units of wetland vegetation.

Test: A reduction in difference between spatial units (i.e. essentially a more homogeneous species assemblage) will be sought in ordinations of the vegetation assemblages, which will reflect the anticipated reduction in the magnitude or significance of differences between spatial units. PERMANOVA or CAP will be used in this process.

3.3.2. Chapter 5

Hypothesis 6: Hyper-eutrophic or eutrophic soil and water conditions will show up as outliers to the range of nutrient concentration measurements for individual vegetation plots and or wetland average values.

Test: Outliers from a data set will be identified using ordination and dendrograms (Legendre and Legendre 2003).

Hypothesis 7: If the water quality component scores of the HDS accurately portray the effects of anthropogenic impacts on nutrient availability, then these scores should correlate with the trophic gradient of the nutrients assessed in the soil and water column of wetlands.

Test: Pearson correlation of the water quality gradient score to single nutrients (e.g. soil phosphorus, or orthophosphate) will be determined.

3.3.3. Chapter 6 – Testing the wetland weighted-average species data

Hypothesis 8: Wetlands from different categories of human impact (Reference, Moderate and Worst) are represented by different species assemblages within each sub-unit of vegetation identified in the analyses for Hypotheses 1 or 2. The wetlands from each category will have:

a) Characteristic and discriminatory species for each disturbance category. Such species will occur with consistently different weighted-average wetland cover/abundances per disturbance category; and
b) Different diversity measures (species richness, evenness or dominance) per disturbance category.

Test: a) Permutational multivariate analysis of variance (PERMANOVA) will be used to test the differences in species assemblages of different disturbance categories (Reference, Moderate and Worst) of spatial units (region/sub-regions or locations within sub-regions) which have been found to hold uniform wetland vegetation (in the analyses for Hypotheses 1 or 2).

b) Diversity differences between wetlands of different disturbance categories will be assessed in PRIMER-E with the tool DIVERSE.

3.3.4. Chapter 7 – Testing the plot-scale species data

Hypothesis 9: Significantly different species assemblages occur in the different hydrological zones (supralittoral and littoral) measured in each distinct spatial unit of wetlands identified in the analyses for Hypotheses 1 or 2.

Test: Analysis of similarity (ANOSIM) and/or PERMANOVA will be used to determine if plant assemblages of different hydrological zones are different.

Hypothesis 10: Where the analyses for Hypotheses 1 or 2 have identified spatial units with similar vegetation (i.e. locations, sub-regions or the whole Western Coastal Slopes), sample, or plot data will reveal a greater difference in vegetation between disturbance categories (Reference, Moderate and Worst) within each hydrological zone than was apparent when using the wetland average values (Chapter 6, Hypothesis 8).

Test: Permutational multivariate analysis of variance will be used to test the differences in species assemblages of hydrological zones of the different disturbance categories (Reference, Moderate and Worst). This will be done for all spatial units (region/sub-regions or locations within sub-regions) that have been identified as containing uniform wetland vegetation (as a result of analyses for Hypotheses 1 or 2).

Hypothesis 11: Human land-use and associated activities or stressors are predominantly responsible for the differences between vegetation assemblages categorised as being Reference, Moderate, or Worst.

Test: Distance Linear Modelling (DistLM) and distance based Redundancy Analysis (dbRDA) in PRIMER-E (Legendre and Anderson 1999, McArdle and Anderson 2001), will be used to determine which linear combination of variables are most responsible for the separation between the vegetation of different disturbance categories.

Hypothesis 12: The use of functional groups to define the species present in wetlands, increases the apparent similarity and thus comparability of wetlands from different sub-regions; and reduces the differences apparent in the analyses for Hypotheses 1, 2, 4, 5, 8, 9 and10.

Test: Substitution of Functional Groups for species in any of the analyses performed to test Hypotheses 1, 2, 4, 5, 8, 9 and 10.

3.4. Description of the region and sub-regions in the study

This study was focused south of 32^{0}S, west of 20^{0}E and below 200 mamsl on the coastal forelands (Lambrechts 1979) of the south-western corner of the Cape Floristic Region (CFR) (Goldblatt 1978, Goldblatt and Manning 2000) in the Cape Province of South Africa (Figure 3.1). The Cape Floristic Region has very high terrestrial plant species richness and endemism and it is thus recognized as one of the six phytogeographical Kingdoms of the world (*Capensis*) (Takhatajan 1986). The vegetation of The CFR is also very different from the rest of southern Africa (Werger 1978). Sixty-eight percent of the 9000 described plant species are endemic to the CFR region, along with 19% of the genera and six families (Goldblatt and Manning 2000). This project focused predominantly on wetlands within the Fynbos Biome of the CFR that contain Cape Lowland Freshwater (CLF) wetland vegetation (Mucina *et al.* 2006a) (see details in Section 2.9.1). The Fynbos Biome is comprised of three naturally distinct vegetation types (namely Fynbos, Renosterveld and Strandveld) and most of the endemism and biodiversity of the CFR is attributable to the Fynbos vegetation type of the Fynbos Biome (Rebelo *et al.* 2006).

The study area is characterised by a mediterranean-type climate, found nowhere else in sub-Saharan Africa, with cool wet winters and relatively dry warm summers that have been in place since the Late Pliocene (*circa* 3.2-2.5 Myr) (Deacon *et al.* 1992).

An areal loss of the Fynbos biome of between 51% and 65% is projected by 2050 (depending on the climate scenario used) as a result of anthropogenically driven climate change (Midgley *et al.* 2002). The majority of the loss is predicted to occur in the northerly (equatorward) latitudes, due to thermal and drought stress, but it is expected to be distributed more or less evenly with altitude. Increasing minimum temperatures along the western seaboard may also reduce the range of the Fynbos Biome in this area (Midgley *et al.* 2002). Other anthropogenic disturbances have already impacted the Fynbos Biome and are ongoing despite considerable conservation effort. These are mainly due to land transformation for agriculture and urban development, as well as the invasion of alien vegetation (Rouget *et al.* 2003).

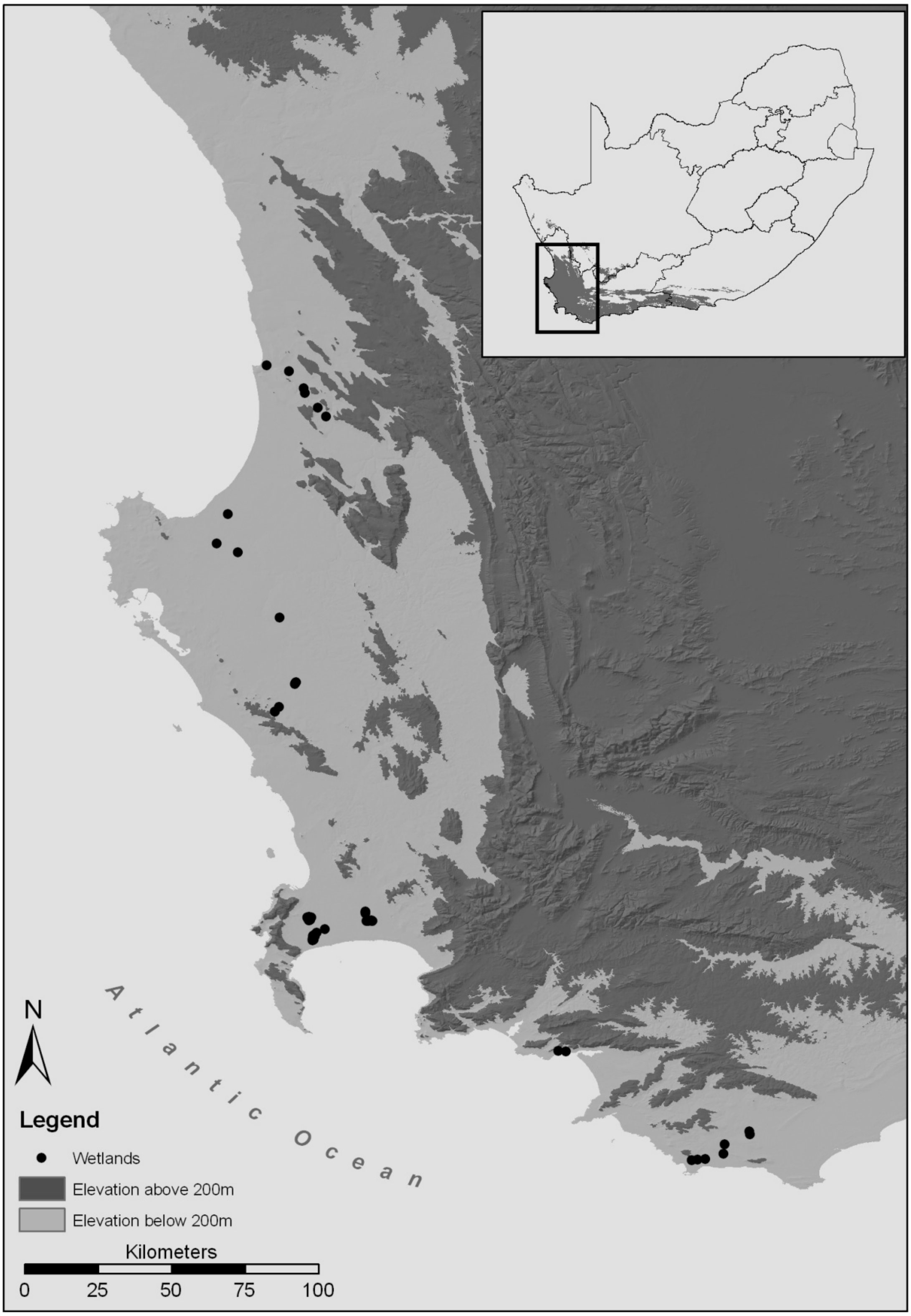


Figure 3.1: A map depicting the land below 200 mamsl in the south-western corner of the Fynbos Biome. This is the focus area for the present study and depicts the wetlands sampled in different areas of this region. Inset is a map of South Africa and its provincial boundaries, showing the location of the Fynbos Biome.

The wetlands assessed in this study are situated on the lowland plains of the Coastal Forelands, the physiographic zone between the Cape Fold Mountains and the coast in the Western Cape Province of South Africa (Lambrechts 1979). This geographical unit corresponds very closely with the Mediterranean climatic zone of the South Western Coastal Slope region (Cowan 1995) (see Figure 2.4), which is referred to in this study as the Western Coastal Slope (*sensu* Cowan 1995). Wetlands were sampled in three broad sub-regions within the Western Coastal Slope region (Cowan 1995) as shown in Figure 3.1. Within each sub-region there was a further concentration of sampling effort in distinct localities where, due to topography and relief, wetlands were most abundant.

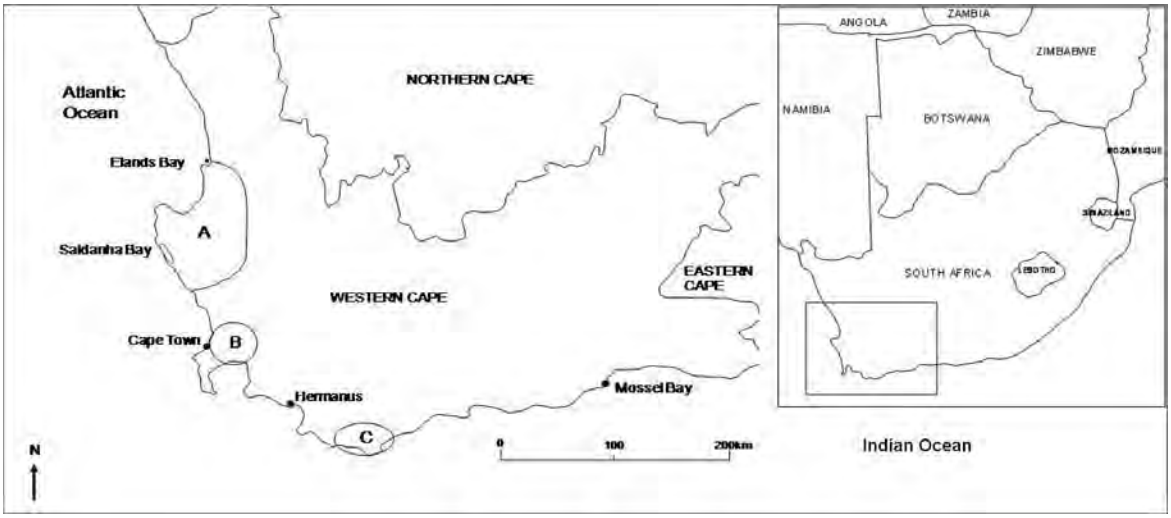


Figure 3.1: The sub-regions in which wetlands were sampled in the coastal lowland of the Western Cape: A = West Coast, B = Cape Flats, C = Agulhas Plain

The topography is predominantly flat with low to moderate relief (Lambrechts 1979, Kleynhans *et al.* 2005). For the wetlands that were assessed, altitude ranged from 5 to 120 metres above mean sea level (Google Earth, accessed 2007). Tertiary and Quaternary deposits of calcareous sands from the Cainozoic era form the geological basis of this area, within which limestone deposits are an occasional feature (Cowan 1995). Levels of soil nutrients, in particular nitrogen and phosphorus, are generally very low, and are known to act as determinants of the community assemblage of the dryland (as opposed to wetland) vegetation (Kruger *et al.* 1983, Rebelo *et al.* 2006). Geological and climatic differences within this region drive the expression of soil nutrient and salt concentrations, which result in different dryland vegetation types across the region (Rebelo *et al.* 2006 and see Appendix 7). Aridity increases from the Agulhas Plain in the south-east, to the Cape Flats and West Coast in the north-west (from C to A in Figure 3.1) (Rebelo *et al.* 2006). An increasingly seasonal concentration of rainfall on the West

Coast, relative to the Agulhas Plain (calculated from the duration and extent of mean winter rainfall as a percentage of mean annual rainfall), exacerbates this trend (Rebelo *et al.* 2006). A summary of this information is provided in Table 3.1.

Table 3.1: Environmental characteristics of the three sub-regions in which wetlands were investigated

Area	**West Coast (A)**	**Cape Flats (B)**	**Agulhas Plain + Hermanus (C)**
No. of wetlands investigated	17	33	9 + 3
Altitude range (m) [1]	2-120	5-40	10-65
Precipitation(annual average) ($mm.a^{-1}$) [2]	200-500	500-800	500-800
Evaporation (annual average) (mm^{-1}) [2]	2000-2500	2000-2500	<1500
Moisture balance (mm $month^{-1}$) [2]	Summer (Jan) -350 Winter (July) -25	Summer (Jan) -200 Winter (July) +50	Summer (Jan) -240 Winter (July) 0
Geology [3]	Cainozoic sandy and calcareous coastal deposit	Cainozoic sandy and calcareous coastal deposit	Cainozoic sandy deposits + Mio-Pliocene shallow water limestone and coastal sandy deposits
Surrounding dryland vegetation bioregions [3]	South West Fynbos, West Strandveld and West Coast Renosterveld.	South West Fynbos, West Strandveld	South West Fynbos, South Coast Fynbos and East Coast Renosterveld
Land-use [4]	Cultivated, grazed and fallow land and 'industrial urban edge'	Residential + 'industrial urban', agricultural and Conservation Area	Abandoned agricultural land and Conservation Area + Golf estate on urban edge

Citations: [1] Google Earth (2007); [2] Deacon *et al.* (1992); [3] Mucina *et al.* (2006a); [4] Field assessment.

3.4.1. The climate, geology and vegetation of the West Coast

In the area broadly stretching from Darling to Verlorevlei, the West Coast has a semi- to sub-arid climate, with less than 250 mm mean annual precipitation around Verlorevlei (Schulze 2006). Rainfall in the area is strongly concentrated in winter (80%). The geology is dominated by marine calcareous sands of high base ion (Mg^{2+}, Ca^{2+}) status. Generally the soils of the West Coast have developed from recent drift sands and near the coast the sands are highly calcareous, whilst inland the lime content gradually decreases through leaching (Rebelo *et al.* 2006). The dryland vegetation of the West Coast has large swathes of each of the three broad vegetation constituents of the Fynbos Biome, namely Fynbos, Strandveld and Renosterveld (Rebelo *et al.* 2006). Wetlands with Cape Lowland Freshwater vegetation, Vernal Pool vegetation and inland salt pans and associated 'Cape Inland Salt Pan' vegetation all occur in this area (Mucina *et al.* 2006a).

3.4.2. The climate, geology and vegetation of the Cape Flats

The Cape Flats is less arid than the West Coast, and is characterized by winter rains (Schulze 2006). The geology is dominated by sandy coastal deposits, with low base ion status in acid soils and higher base ion status in alkaline soils (Deacon *et al.* 1992). Cape Flats Sand Fynbos and Cape Flats Dune Strandveld dominate the dryland vegetation types, reflecting the spatial distribution of acidic and alkaline sandy soils respectively (Rebelo *et al.* 2006). CLF vegetation-dominated wetlands are mapped as distinct units of vegetation in this locality whilst Cape Vernal Pools, which are typically too small to be mapped, also occur in this area (Mucina *et al.* 2006a). During this project, wetlands from both the acidic and alkaline sands were assessed.

3.4.3. The climate, geology and vegetation of the Agulhas Plain

The Agulhas Plain is less arid than the West Coast but more so than the Cape Flats and receives predominantly winter rainfall (Schulze 2006). Along the coastal belt the geology of the Agulhas Plain consists of sandy deposits with shallow water limestone and ferricrete (Deacon *et al.* 1992). The dryland vegetation of the Agulhas Plain is dominated by vegetation of the broad Fynbos and Renosterveld type (Rebelo *et al.* 2006) Wetland vegetation units of Cape Inland Salt Pan and Cape Lowland Freshwater occur at a large enough scale to be mapped (Mucina *et al.* 2006a).

3.4.4. A summary of the three sub-regions

In summary, there are some broad climatic and geological similarities between the three sub-regions. The arid to semi-arid mediterranean climate and predominantly sandy geology result in conditions that commonly support ephemerally- to seasonally-inundated isolated depressional wetlands (Jones 2002) rather than the permanently inundated depressions common to less arid regions of the world. These similarities suggest the potential for each sub-region to contain similar wetland vegetation. The seasonality of the hydrological regime creates distinct hydrological zonation within wetlands of the Western Coastal Slopes, presenting different habitat for wetland plants within zones (see below).

3.5 Methods

The following are the methods used in the present study.

3.5.1. Timing of sampling

Sampling was conducted during the spring and early summer of 2007. This time period coincides with the flowering and seed set of graminoid species, and overlaps with end of the season for some geophytic taxa that flower in early spring.

3.5.2. Wetlands included in the study

a. Cape Lowland Freshwater vegetation (Mucina *et. al.* 2006a) was the chosen wetland vegetation unit as contained within the Western Coastal Slope wetland region (Cowan 1995). Where their vegetation appeared comparable to the Cape Lowland Freshwater vegetation units, a number of Vernal Pools, saline and alluvial vegetation units were included in the set of wetlands to be studied.
b. Following the National Wetland Classification System (SANBI 2009), the wetlands that were the focus of the present study were inland freshwater wetlands with a hydroregime of lentic conditions characterized by a range of ephemeral to permanent saturation. Inundation of the wetlands was predominantly seasonal, and the drainage was endorheic. These wetlands were in the South Western and Southern Coastal Belt ecoregions (Kleynhans *et al.* 2005), predominantly from a planal landform and with a depressional-HGM-type. A number of other HGM types (Floodplain-Flats and Valley-Bottoms) were also assessed where they were considered to contain similar habitat to the depressions.
c. Supralittoral, littoral and aquatic hydrological-habitat-zones were sampled in all of the wetlands where they were present.
d. The herbaceous structural vegetation unit was the predominant focus of the present study, but scrub-shrub vegetation was sampled in any hydrological-habitat-zone in which it was encountered

A reconnaissance was undertaken to identify wetlands that occur in close proximity to each other (in order to minimise factors such as climate that alter over geographical space), but that were differentially impacted by the intensity of human activities or land-use. Sampling was concentrated in three distinct areas where wetlands occurred with maximum abundance as described in the bullet points below and in Table 3.2.

A. On the West Coast: where the predominant impacts are due to agricultural land-uses, wetlands were sampled at Darling, along the Berg River, and at Verlorevlei in Elandsbaai. In these localities nine isolated depressions and a further eight non-isolated wetlands were sampled (six of which were separate areas within the Verlorevlei wetland itself);
B. On the Cape Flats: in the Kenilworth, Kuils River Floodplain (Mfuleni and Driftsands) and Lotus River areas, a total of 32 depressional wetlands and one wetland flat were sampled. These wetlands ranged from least impaired reference sites to severely impacted urban systems; and
C. In the Overberg on the south coast: seven depressions and two wetland flats were sampled on the Agulhas Plain (least impaired and agricultural-impacted wetlands); and three seeps with moderate to worst disturbance were sampled within the urban edge of Hermanus.

A table of the abiotic characteristics of each wetland and the dryland vegetation unit surrounding each wetland is presented in Appendix 7.

3.5.3. Delineation of wetland and hydrological zones

Where the wetland-dryland boundary was not easily apparent within a wetland, a rough delineation procedure was carried out by soil augering and by an examination of the vegetation and signs of past standing water using the method of DWAF (2003). The delineation process ensured:

- that all samples taken and observations recorded from each site were within the wetland rather than in dryland areas;
- that the full extent of the wetland area was assessed, including all of the ephemerally to seasonally saturated habitat of the supralittoral zone;
- that the approximate extents of the supralittoral, littoral and aquatic hydrological zones were determined; and
- that accurate descriptions could be made of the hydrological zone (aquatic, littoral or supralittoral) characterizing the habitat at each position where a vegetation sample was taken.

Table 3.2: Spatial hierarchy of wetlands with different vegetation and hydrogeomorphic habitat in localities from each sub-region of the Western Coastal Slope wetland region.

Sub-region	Locality	Types of Impacts	"Wetland vegetation-unit" + "HGM-type"			Dryland Vegetation Units
			depressions		Vegetation + HGM combinations	
			CLF-	Vernal-		
A=West Coast	Berg River	agricultural	1	1	1 Saline-floodplain	Saldanha Flats Strandveld, Hopefield Sand Fynbos
	Darling	agricultural and urban	3	2	2 Alluvial-floodplain	Swartland Granite Renosterveld, Swartland Alluvium Renosterveld, Hopefiled Sand Fynbos, Atlantis Sand Fynbos
	Verlorevlei	agricultural	-	-	3 CLF-Floodplain, 3 CLF-Valley-bottom	Leipoldtville Sand Fynbos, Lamberts Bay Strandveld
B= Cape Flats	Driftsands	urban	9	2	-	Cape Flats Dune Strandveld
	Kenilworth	urban	9	1	1 CLF-Flat	Cape Flats Sand Fynbos
	Lotus River	urban	11	-	-	Cape Flats Dune Strandveld, Cape Flats Sand Fynbos
C= Overberg	Hermanus	recreational	-	-	3 CLF-seeps	Overberg Sandstone Fynbos
	Agulhas	agricultural	4	1	1 Saline-floodplain, 1 Saline-flat,	Overberg Sandstone Fynbos, Elim Ferricrete Fynbos, Agulhas Limestone Fynbos, Central Ruens Shale Renosterveld

CLF = Cape Lowland Freshwater vegetation

3.5.4. Assessment of human disturbance

A targeted sampling approach (Section 2.10.3) was followed whereby suitable sites were chosen based on a rapid field-based assessment of the amount of anthropogenic disturbance as an *a priori* surrogate for overall environmental condition. Thereby, a relatively similar number of Reference, Moderate and Worst disturbed wetlands were assessed within the Western Coastal Slopes. The following is a description of the procedure used in the calculation of the HDS as recorded for each wetland. The field datasheet is shown in Appendix 1. The impact of human land-use activities were scored for the intensity and spatial extent of their impact on four major aspects of the wetland environment, namely:

- Water Quality Impacts;
- Hydrological Impacts;
- Disturbance to Physical Structure; and
- The presence and width of a buffer area around the wetland comprised of indigenous, or other, vegetation.

The likely impact of each anthropogenic stressor within the wetland, and within a radius of 500 meters from the wetland edge was assessed qualitatively, based on expert opinion, and scored as described in A-C below. These scores were collated into an overall rating of cumulative disturbance to water quality, hydrology and physical-structure. A measure of the buffer width of intact indigenous and/or altered vegetation surrounding the wetland was also estimated and included in the disturbance score as described in D and E below. The higher the resultant score, the greater the level of disturbance. Qualitative assessments were made within the wetland, within the first 100 metres from the wetlands edge and within the next 400 metres as three separate spatial units. These scores were then collated into a score per wetland.

The procedural steps in this qualitative assessment of human disturbance were as follows (a worked example of this procedure is shown in Table 3.3):

A. For water quality, hydrology and physical structure the spatial extent (0%, 1-25%, 25-50%, 50-90%, >90%: scored 0 to 4) and intensity (least to most: scored 0 to 5) of various land-use activities were combined into a qualitative score of “expected impact” on the environmental condition of a wetland. A range of expected activities and land-uses was provided as a guide on the datasheet, but other disturbances noted in the field were also included.

B. The intensity and extent scores for each land-use activity were multiplied together to give a rating (0-20) of the total impact on water quality, hydrology and physical-structure;

Table: 3.3: Extract from Human Disturbance Score sheet in Appendix 1, showing measurement of extent, intensity and resultant impact score of disturbance per land-use activity.

		Within Wetland***					
		Water Quality		Hydrology		Physical Structure	
Land-use/Activity	Extent	Intensity	**Impact**	Intensity	**Impact**	Intensity	**Impact**
Infilling	2	1	2	3	6	2	4
Sewage disposal	1	3	3	2	2	0	0
Solid waste	3	3	9	1	3	4	12
Water Abstraction	1	2.5	2.5	3	3	2	2
Sum of Impacts: "within wetland"			**16.5**		**14**		**18**

***The same exercise was repeated for the 100 m and the next 400 m spatial units of assessment around each wetland

C. The land-use activity impact ratings in B were then summed as three separate gradients of disturbance (as discussed in Section 3.2.5), namely: water quality, hydrology and physical-structure.

D. The width of buffer zone vegetation was scored in the following way (see Table 3.4):

i. On the four points of the compass (N, E, S, W) as four separate quarters, the width of natural indigenous vegetation and/or transformed vegetation was estimated according to seven categories (measured from broadest [least impacted] to narrowest [most impacted]: and scored from 0 to 6);

ii. The average of the four quarters was taken as the buffer width condition score for the wetland. "Narrowest" and "Worst state" reflects the highest score.

E. The score for buffer width was added to the scores generated per disturbance gradient (water quality, hydrology and landscape physical structure disturbance) in stage C, to give a human disturbance score for the entire wetland (Table 3.5).

Table 3.4: Extract from Human Disturbance Score sheet in Appendix 1, showing measurement of buffer width.

Width of dryland vegetation buffer	(0) natural state	(1) natural buffer > 50 meters	(2) natural buffer 25-50	(3) Transformed veg 25-50 meters	(4) Trf'd 10-25 meters	(5) Trf'd < 10 meters	(6) None
North side	**0**						
East side		**1**					
South side				**2**			
West side					**3**		
Score **6/4**	**0**	**1**		**2**	**3**		

The human disturbance scores per wetland facilitated the ranking and categorization of wetlands as being "least impaired" or in a relatively natural and "reference condition", relative to "moderately disturbed" conditions and wetlands with the "worst level of disturbance". Total scores were dependent on the number of land-use activities. Higher values thus represent greater disturbance on which no upper score limit was placed.

3.5.5. Assessment of water samples: physical and chemical properties

3.5.5.1. Physical variables

Oxidation-reduction (redox) potential and pH were measured using a Crison pH25 meter (accurate to 1 mV and 0.01pH). Dissolved oxygen was measured using a Crison OXI45 oxygen meter (accurate to 0.01 mg/L). Electrical conductivity was recorded using a Crison CM35 conductivity meter (accurate to 0.01µS/cm). Turbidity was measured from the water column immediately below the surface at two randomly selected points in each wetland using a Hach 2100P turbidimeter (accurate to 0.5 nephelometric turbidity units (NTU), the average of which was used for further analyses. Each of these meters was calibrated using appropriate standards. Measurement of turbidity using NTUs gives a value to the intensity of light scattered by the suspended solids in a sample. The greater the intensity of the scattered light, the greater the turbidity of the sample (McCarthy *et al.* 1974, US EPA 1979). Precipitation of dissolved constituents (for example, iron) causes measured turbidity values to be high. Coloured solutes can cause turbidity values to be low; and therefore the tannins in many Cape waters decrease NTU values. The Hach 2100P turbidity meter's optical system compensates for colour in the sample, light fluctuation and stray light, however, and hence, in theory, coloured solutes should not unduly lower NTU readings (Hach Website 2010).

Table 3.5: Extract from Human Disturbance Score (HDS) sheet in Appendix 1, showing addition of disturbance gradient scores across the spatial units and buffer width.

Gradient	Water Quality			Hydrology			Physical Structure			Buffer Width
	in wetland	100 m	500 m	in wetland	100 m	500 m	in wetland	100 m	500 m	-
Subtotal scores	16.5	X	Y	14	X	Y	18	X	Y	1.5
HDS =	(16.5	+ X	+ Y)	+ (14	+ X	+ Y)	+ (18	+ X	+ Y)	+ 1.5

*Spatial unit scores for 100 m and 500 m are marked X and Y respectively for each disturbance gradient.

3.5.5.2. Nutrients

A 2 litre surface water sample was collected from five locations within each wetland and pooled to form a bulk 10 L sample. This was then thoroughly mixed and sub-sampled to obtain a 200 mL sample for the analysis of nutrients levels in the laboratory. Water quality analyses were carried out at the Department of Oceanography at the University of Cape Town. Combined nitrate and nitrite – nitrogen (NO_3^{-2} and NO_2^- – N), ammonium – nitrogen (NH_4^+ – N) concentrations were estimated using a Lachat Flow Injection Analyser, as follows: ammonium was measured using Lachat's QuikChem® Method 31-107-06-1, based on the Berthelot reaction in which indophenol blue is generated. Nitrate and/or nitrite were estimated using Lachat's QuikChem® Method 31-107-04-1-E, in which nitrate is converted to nitrite and diazotized with sulfanilamide to form an azo dye. Approximate detection limits are 2.5µg.L^{-1} N for nitrate and nitrite and 5µg.L^{-1} N for ammonium. Details of the methods may be found at http://www.lachatinstruments.com. Total Inorganic Nitrogen (TIN) levels were determined from the addition of the concentrations of nitrate, nitrite and ammonium.

Inorganic phosphorus as ortho-phosphate (PO_4^{-3}–P) was measured manually by forming an antimony-phospho-molybdate complex using the method of Murphy and Riley (1962) adapted to a 5 ml sample size. The procedure is accurate for very low levels of phosphorus, having been specifically designed to measure concentrations from 1 to 160µg.L^{-1} and is accurate to a maximum concentration of 2000µg.L^{-1} (Murphy and Riley 1962).

Some of the assessed wetlands had no standing water, being seeps or saturated flats, or were assessed at a stage when no surface water was present. The number of wetlands for which physico-chemical parameters were measured is shown in Table 2 in Appendix 9.

3.5.6. Assessment of soil samples

At the first 16 wetlands sampled in the present study, only one or two soil samples were collected for analysis (n=20 samples). In the second phase of wetland sampling, soil samples were taken from almost every plot where vegetation was sampled (n = 242 samples).

A kilogram of soil was collected at each sample point to ensure that, after drying, sufficient mass (250 grams) would remain for analyses. All organic litter was removed from the soil surface and large pieces of organic matter were removed from each sample by hand. The soil was excavated and collected to a maximum depth of 25 cm. Soil samples were stored in individual plastic bags, until they could be spread out and air-dried before being delivered for laboratory analysis.

3.5.6.1. Soil Sample Analysis

Soil particulates and chemical composition were analyzed in accordance with the recommendations of the BAWWG (US EPA 2002c); other than the determination of concentrations of zinc, manganese, copper and boron which, were not performed. Soils were analyzed at BEMLAB (Pty) Ltd, Somerset West. Two blind duplicate samples were sent as a quality control to check the accuracy of analyses. Soils were air dried overnight at BEMLAB before analyses were performed.

The following soil variables were determined:

- **Soil particle size and silt/clay/sand distribution:** were determined by the mechanical hydrometer method (Van der Watt 1966).

- **Soil pH:** was determined by stirring 10 g soil in 25 mL 1M KCl at 180 r.p.m. for 5 seconds. After standing for 50 minutes the solution was re-stirred before measuring the supernatant pH with a calibrated meter.
- **Resistance** determination of electrical resistance was performed on a paste made by mixing the soil with de-ionized water and using US Bureau of Soil Standards electrodes and a resistance bridge.
- **Bulk density:** is the dry mass of the sample divided by the volume of the sample as determined by weighing a 50 mL volume of sieved soil (Hillel 1982, Jury *et al.* 1991).
- **Titratable acidity H^+ (For soils with pH ≤ 6.1):** 5 grams of soil were shaken together with the extractant solution (potassium sulphate / potassium acetate, phenolphthalein and potassium hydroxide 0.1M), before filtering, and titrating with sodium hydroxide 0.05M (Eksteen 1969).
- **Total nitrogen (%):** determined by digestion with a FP-528 Nitrogen Analyzer (Leco Corporation, St Joseph, USA). The percentage of nitrogen was multiplied by 10 000 to convert to mg $N.kg^{-1}$. The mg $N.kg^{-1}$ was divided by the bulk density to determine mg $N.m^{-3}$ and this was then divided by 1000 to convert to g $N.m^{-3}$. This value was further divided by four to determine the g $N.m^{-2}$ in the top 25 cm of the soil profile.
- **Phosphorus Bray No. 2 (for soils with pH < 6.9):** Plant available phosphorus was determined in soils with pH less than 6.9 with the Bray No. 2 reagent. The soil was prepared for Bray No. 2 P analysis by shaking 6.6 grams of soil in Bray 2 solution (150 mL ammonium fluoride in 4 L of water with 50 mL of HCl) (Bray and Kurtz 1945) before filtering and analyzing using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Varian Vista MPX, Melbourne, Australia).
- **Phosphorus Olsen (for soils with pH ≥ 6.9):** For soils with a pH greater than or equal to 6.9, the determination of plant available phosphorus was carried out using the Olsen reagent. These soils were prepared for analysis by shaking 5 grams of soil with sodium bicarbonate solution 0.5M (Olsen and Sommers 1982) before filtering and analyzing using an ICP-OES.
- **Percentage organic matter (Walkley-Black) (For soils with pH ≥ 6.5):** determined by the Walkley Black method (1934).
- **Percentage organic matter (LECO C/N-Analyzer) (For soils with pH < 6.5):** determined with a LECO CN-Analyser (Leco Corporation, St Joseph, USA).
- **Cation Exchange Capacity:(** determined by following the procedure of Chapman (1965) in which 10 grams of soil are washed three times with 30 mL of 0.2M ammonium acetate;

then washed three times with a 1:1 water and methylated spirits mixture after which soil is eluted with 0.2M potassium sulphate and ammonium was measured using an auto-analyzer.

- **Exchangeable cations (contained in calcium, magnesium, potassium and sodium):** were displaced from 10 g soil with 25 mL of 0.2M ammonium acetate. The samples were filtered through Reeve Angel Grade 307 filter paper, made up to 200 mL and thereafter exchangeable cations of potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg) were measured using ICP-OES analysis.
- **Water-soluble cations (contained in calcium, magnesium, potassium and sodium):** a saturated paste was prepared with de-ionised water, the soil and solution were separated by centrifugation and soluble cation concentration of the supernatant was determined by ICP-OES using appropriate standards.

Detection limits and calibration ranges of these analyses (Appendix 9) are sufficient for the intents and purposes of the present study.

3.5.7. Other environmental variables

3.5.7.1. Climatic variables

Climatic variables for each wetland were extracted from the 2002 (*a*) data base of the South African Atlas of Climatology and Agrohydrology (Schulze 2006). The variables extracted were:

- Mean daily minimum Temperature (ºC);
- Mean daily maximum Temperature (ºC);
- Mean annual precipitation (mm);
- Mean annual potential evaporation (mm); and
- Mean annual potential evapotranspiration (mm).

3.5.7.2. Quantitative variables measured at plot scale

The following quantitative and semi-quantitative variables were recorded and collated with the soil variables that were measured per quadrat (see field sheet in Appendix 4):

- Slope and aspect were estimated by eye;

- Soil depth in ordinal classes (<0.2 m, 0.2-1.5 m, >1.5 m) was estimated by hand augering to a depth of 1.5 metres, below which the depth was recorded as 2 meters;
- pH and redox potential were measured in the field in each vegetation plot using a Crison pH25 meter. In order to take these measurements with probes designed for use with solutions, a fixed quantity of soil was mixed with a fixed quantity of water with neutral pH (pH 7) and shaken vigorously. This solution was left to settle for a minute before taking both pH and redox readings. For soils in which an analyses of pH was determined in the laboratory, however, these values were used rather than the above field measurement

3.5.7.3. Qualitative or descriptive variables

In the vegetation sampling process that will be described in section 3.5.8, a number of environmental parameters were described for each vegetation sample plot (see the field sheet in Appendix Four). These variables were:

- **Habitat description:** e.g. slope, flat, hypersaline flat, channel, microdepression, aquatic;
- **Hydrogeomorphology:** e.g.
    - landform (basin, flat, channel, slope),
    - HGM type (Ewart-Smith *et al.* 2006),
    - water flow velocity (none, slow, fast).
- **substrate and soil types:** e.g. aeolian or alluvial sands and texture;
- **sedimentation/erosion:** e.g. erosion, stasis, deposition (chemical / mineral / organic)
    - Deposition and burial by mineral (clastic) deposits: sand / clay;
- **dominant vegetation structure:** e.g. graminoid, herbaceous or shrub;
- **roughness** or density of vegetative cover; and
- **vegetation utilization:** e.g. none, mowed / grazed / harvested, overgrazed / excessively harvested.

For the description of plot habitats, the six examples provided in the first bullet point above were augmented by appropriate descriptions as devised during sampling work. All descriptions were summarised into eight categories of increasing wetness to assist in the development of a concept of hydroregime (See below).

3.5.7.4. Hydrological variables at plot and wetland scales

For each wetland the following hydrological variables were recorded (see field sheet in Appendix 2):

- Wetland size in hectares (ha) was judged according to seven pre-determined categories (<0.5, 0.5-1, 1-5, 5-10, 10-20, 20-50, >50);
- Maximum annual water depth in meters (m) was estimated according to four categories (<0.5, 0.5-1, 1-2, >2).

The above categories were each assigned a score (1-7) and (1-4) respectively. An ordinal concept of water **volume per wetland** was thereby developed by multiplying size by depth scores (i.e. wetland size: 3 x depth class: 2 = volume category 6). This facilitated a ranking of the relative volume of water each in wetland and allowed the comparison of wetlands.

For every vegetation sample plot the following hydrological variables were recorded (see field sheet in Appendix 4):

- **Current (present) water depth** (millimetres) above (inundation) or below the ground surface (water table depth);
- Estimation of **potential maximum depth** of annual inundation (millimetres);
- **Current hydrological condition,** e.g. dry, moist, saturated or inundated; and
- **Hydrological regime,** e.g. temporary, seasonal or permanent.

The combination of current depth, estimated potential depth, current hydrological condition and hydrological regime were used along with the habitat description to determine whether a sample was aquatic, littoral or supralittoral as defined in Section 2.9.3.1.

The habitat description for each plot (flat, channel, micro-depression, aquatic, etc.) was assigned a score from 1 to 8 in terms of least, to most wet (e.g. rapidly draining sandy flats to permanently-inundated-clay-aquatic habitat). This habitat description score was used to augment the determination of the abovementioned hydrological regime categories (temporary, seasonal or permanent). The habitat description score was multiplied by a weighting based on the current hydrological condition (dry, moist, saturated, inundated, and scored 1 to 4) of the sampled plot. This weighting brings a temporal element to the hydrological regime (hydroregime). When a plot is wet even in the drier seasons of the index period, such a weighting raises the hydroregime score – thus emphasizing greater wetness.

When measured in the midst of the wet-season, a higher score suggests a greater period of wetness. The weighted hydroregime parameter was used in later analyses, rather than any of the three components of its construction.

3.5.8. Vegetation sampling

Within each wetland the vegetation was examined to obtain a holistic overview and to determine homogeneous and 'representative' stands of vegetation (Section 2.10.1). During this process, all species encountered in the wetland were recorded (inventoried) on a separate data sheet for each wetland (see Inventory field sheet in Appendix 3). **A full species list for all wetlands is listed in Appendix 12.**

3.5.8.1 Specimen collection and identification

In the field all species were recorded by their Latin binomial nomenclature when identification was certain. Unidentifiable species were collected for later identification and assigned a field code following a specific protocol to maintain accurate records (see description of field tag code in Appendix 4). For all specimens collected, floral structure and colour, rooting medium (water/soil) and a habitat description were recorded on the field tag. When possible, specimens were pressed in the field to ensure quality of preservation for identification and voucher specimen purposes (vouchers were used to facilitate later field identifications).

In cases where species were considered to be rare (fewer than 20 individuals of an unidentifiable species in existence at a site) specimens were photographed rather than collected to aid identification efforts.

Collected specimens were identified in the Bolus Herbarium at the University of Cape Town. A number of difficult specimens were identified by experts at, or affiliated with, the Bolus Herbarium or the Compton Herbarium at Kirstenbosch. Non-vascular taxa were identified only to the lowest commonly recognizable taxonomic level. Nomenclature followed Goldblatt and Manning (2000), Germishuizen and Meyer (2003) and Govaerts *et al.* (2010).

3.5.8.2. Selection of vegetation sample plots

Homogeneous stands of vegetation were chosen that best characterized the various hydrological zones for the entire wetland and the different vegetation habitat units (sand versus clay soils or impacted and un-impacted) within these zones. Within each chosen homogeneous stand, a single sampling plot, considered to be a characteristic representation of the vegetation, was assessed. This resulted in a relevé (or list) or species representative of that stand. The number of relevés per wetland was thus dependent on the number of different stands of homogeneous and representative vegetation. Sample plots were laid out so that any variation within the plot was minimized – in other words if the unit being assessed was very narrow a 1 x 1 m or 0.5 x 0.5 m plot would be more appropriate than a 2 x 2 m plot. The 2 x 2 m plot size, however, was most commonly used (99% of all quadrats). The standardized plot sampling sheet (Appendix 4) was based on that of Sieben (2003).

For each sample plot the cover and abundance of each species were recorded in the Barkman *et al.* (1964) adjusted scale of Braun-Blanquet (1928) and may be seen in Table 3.6. These values were adjusted to a representative median percentage to assist in their statistical analysis.

Table 3.6: Cover and abundance values, representative codes, and median percentage values (after Barkman, Doing and Segal (1964))

Cover	Abundance	Braun Blanquet code	Median % cover
<5%	1	R	1
<5%	2-10	+	2
<5%	11-100	1	3
<5%	>100	2m	4
5-12.5%	-	2a	8
12.5-25%	-	2b	18
25-50%	-	3	38
50-75%	-	4	68
75-100%	-	5	88

3.5.8.3. Wetland weighted-average species values

A cover or abundance value was calculated for each species per hydrological zone (supralittoral/littoral/aquatic), thus providing an estimate of the representative cover or abundance of each species per wetland. The percentage area that each hydrological zone occupies in the wetland was used to weight the average value of each species per hydrological zone. As a theoretical example: species F's average cover value from four different samples for the supralittoral zone in Wetland Dar01 = 48%. The supralittoral zone occupies 35% of wetland Dar01. Hence 0.35 x 48 suggests the supralittoral extent of species *F* has a cover value of 16.8% for wetland Dar01. Should species F be found in other hydrological zones, the sum of the weighted cover values for each hydrological zone (i.e. $16.8\%_{supralittoral} + x\%_{littoral}$) would equal the total cover value for species F for that wetland.

3.6. Data Analysis

The data analysis in this study followed the procedures detailed in Section 2.10.5. The statistical analysis package Plymouth Routines in Multivariate Ecological Research (PRIMER-E: Clarke and Warwick 2001, Clarke and Gorley 2006) and its add-on Permutational Multivariate Analysis of Variance (PERMANOVA: Anderson *et al.* 2008) were used for all analyses.

4. BIOGEOGRAPHY OF WETLAND PLANT ASSEMBLAGES

4.1. Introduction

This chapter examines Hypotheses 1 to 5 as listed in Section 3.3 of this volume. Essentially these hypotheses seek to determine what geographical regions (spatial units) have naturally homogenous communities of wetland vegetation. In order to minimize the number of phyto-geographical spatial units, within which separate metrics for phyto-assessment must be developed, the aim is to determine the largest spatial units within which vegetation is relatively uniform. Both *a priori*-determined impacted and un-impacted wetlands were included in the analyses in the current chapter to determine the impact of human disturbance on the homogeneity of vegetation communities. Within a region with naturally similar vegetation communities in similar habitat, it is hypothesized that the impacts of human disturbance on vegetation will be discernable as anomalous species or vegetation assemblages. For a given habitat type, considerable change in the natural species composition between areas within a region (i.e. gamma diversity *sensu* Cody 1975 and 1983, Cowling *et al.* 1992) would make it difficult to determine the impacts of human disturbances on vegetation communities for the whole region. In a similar manner, but from a different biological perspective, natural and consistent differences in phyto-sociological pattern (referred to hereafter as “vegetation assemblage”) between wetlands from different areas within a given region could potentially mask the impacts of human disturbance. The identification of geographical regions with naturally homogeneous wetland flora enhances the potential to detect anomalous vegetation assemblages or unnatural species compositions resulting from anthropogenic disturbance. Delineation of regions with homogenous wetland flora would therefore also enhance our ability to develop metrics for phyto-assessment for any of these regions (Section 2.10.1.3).

Habitat of the same type and with naturally similar environmental parameters, by virtue of relative proximity, can be expected to hold a greater percentage of similar species than habitat units that are a considerable distance apart (e.g. Whittaker 1962, MacArthur and Wilson 1967). If this theory of decreasing floristic similarity with decreasing proximity holds true, then the wetlands of the different sub-regions (West Coast, Cape Flats and Overberg) of the present data set can be expected to hold different plant communities. Superimposed on natural differences, anthropogenic impacts are expected to result in anomalous wetland plant assemblages. The data for the three sub-regions allowed us to determine the strength of gamma diversity across the Western Coastal Slopes region and thereby to decide whether it is possible to develop phyto-assessment tools for the region as a whole.

For an inventory of the physical parameters differentiating the sub-regions and localities within them see Table 4.2. Appendix 7 contains an inventory of this data per individual wetland. Depressions dominated by Cape Lowland Freshwater vegetation (CLF depressions), represented 37 of the 60 wetlands sampled and so only these 37 wetlands, representing a single habitat unit, were included in the determination of gamma diversity differences between sub-regions (Section 4.2). For all other analyses in this chapter, all wetland data were used.

In this chapter, the patterns of gamma diversity, and the differences and discontinuity in vegetation assemblage patterns between and within sub-regions are described. The influence of environmental parameters on species distribution across the Western Coastal Slopes between sub-regions and localities is examined. The question of whether different HGM types hold different vegetation is also briefly explored. A determination of the impact of human disturbance on reducing the heterogeneity of wetland assemblages is performed. Finally, the implications of these analyses for the development of phyto-assessment indices for the Cape coastal lowlands are discussed.

4.2. The data used for biogeographical analyses

Two levels of assessment were performed in the vegetation survey of each wetland (see Section 3.3.6.2):

1. An inventory was made of all species encountered within the boundary of every wetland; and
2. Characteristic stands of wetland vegetation were intensively surveyed to determine:
   i. species cover and/or abundance values for each vegetation plot surveyed; from which,
   ii. (a) a weighted wetland-average cover value per species per wetland; and (b) an average per species per hydrological zone, was calculated.

The cover/abundance values per individual vegetation plot and the wetland-average cover values provide two different scales of data for each wetland. The former is a measure for each characteristic stand of vegetation, and the latter an average for the whole wetland. **(See Appendix 12 in the accompanying CD for both cover vlaues per plot and for wetland-average cover.)**

4.2.1. Wetland inventory: species data

A total of 549 species were recorded from the study wetlands assessed in the Western Coastal Slopes region of the Cape coastal lowlands. **(See Appendix 12 in the accompanying CD)** A summary of the geographical association of the number of species with different floristic characteristics is presented in Table 4.1. The species inventory incorporated many terrestrial and atypical species, namely singletons, those that occurred in only a single wetland. Such singletons are not useful in the process of development of metrics for phyto-assessment, providing no potential for statistical rigour. They are also not useful in the determination of geographical units of land (ecoregions) with homogenous species composition. Singletons do potentially have biogeographical value, particularly if they are known to be rare species with limited distributional range. Such biogeographical detail is however predominantly unknown in this context (Mucina *et al.* 2006a). A total of 336 species were common to more than one wetland. In general, however, the presence/absence inventory data provided less useful information for potential phyto-assessment metric development than the cover/abundance data recorded in the intensively-sampled plots from characteristic vegetation stands. The inventory data were therefore not used for comparative analyses between wetlands.

4.2.2. Wetland vegetation samples: species data

A total of 374 species were recorded within the vegetation plots sampled from characteristic stands of vegetation in the 60 wetlands of the study. **(See Appendix 12 in the accompanying CD)** Removal of singletons left only 196 species that were recorded in plots from more than one wetland. Only 28 species were shared between all three sub-regions. Four of these were alien, or non-indigenous species more typically associated with dryland or terrestrial conditions. A further three were indigenous dryland/terrestrial species, and two were non-indigenous wetland species. A considerable number of the species in the Cape Flats and Overberg wetlands were unique, in the sense that they occurred exclusively in one sub-region; whilst very few species were unique to the West Coast. A summary of the comparative floristic character of each of the sub-regions is provided in Table 4.1 and Figure 4.1. In Table 4.1, the term “terrestrial” refers to species that were recorded in the wetlands but that are more typically associated with dryland conditions. Differentiation between terrestrial and wetland taxa was determined according to the US Fish and Wildlife Service indicator categories (Reed 1988: Section 2.9 and Table 3) as used by Glen to assign such categories based on herbarium data (unpublished, see Section 2.9 and Appendix 5 of this volume).

Table 4.1: Wetland floristic character of sub-regions in the Cape coastal lowlands

Geographical area	No. of species	***No. sp.>1 wetland	††Terrestrial / Wetland	Alien / Indigenous	Annual / Perennial	Unique species per sub-region and (no. of these that are alien)	
	374	**196**	**104 *vs.* 270**	**74 *vs.* 300**	**96 *vs.* 278**	Wetland	Terrestrial
West Coast	123	82 (55)	34 *vs.* 89	37 *vs.* 86	45 *vs.* 77	9 (3)	6 (3)
Cape Flats	201	141 (50)	53 *vs.* 148	44 *vs.* 158	48 *vs.* 153	65 (7)	30 (7)
Overberg	192	113 (59)	43 *vs.* 148	24 *vs.* 167	35 *vs.* 156	65 (3)	20 (2)

***Species common to more than one wetland in the SWm study region (and in each sub-region).
††Terrestrial / Wetland as per frequency of occurrence in dryland/wetland habitat (Reed 1988) See Appendix 5.
More than 100% cover occurs as a result of the sum of different layers of cover

The larger proportion of terrestrial, alien and annual species in the West Coast wetlands is generally indicative of greater levels of perturbation (natural or unnatural). This is possibly a consequence of the greater natural seasonal variation in hydrology in these wetlands relative to those of the other two sub-regions (see Table 2.4 for justification).

Figure 4.1 summarizes the comparative floristic cover of each sub-region based on growth form. The growth forms that were used were developed by (Mucina *et al.* (2006b). They are based on several major features of the structural and functional life history of plants, such as longevity, architecture, height, woodiness, succulence, parasitism, carnivory, etc.

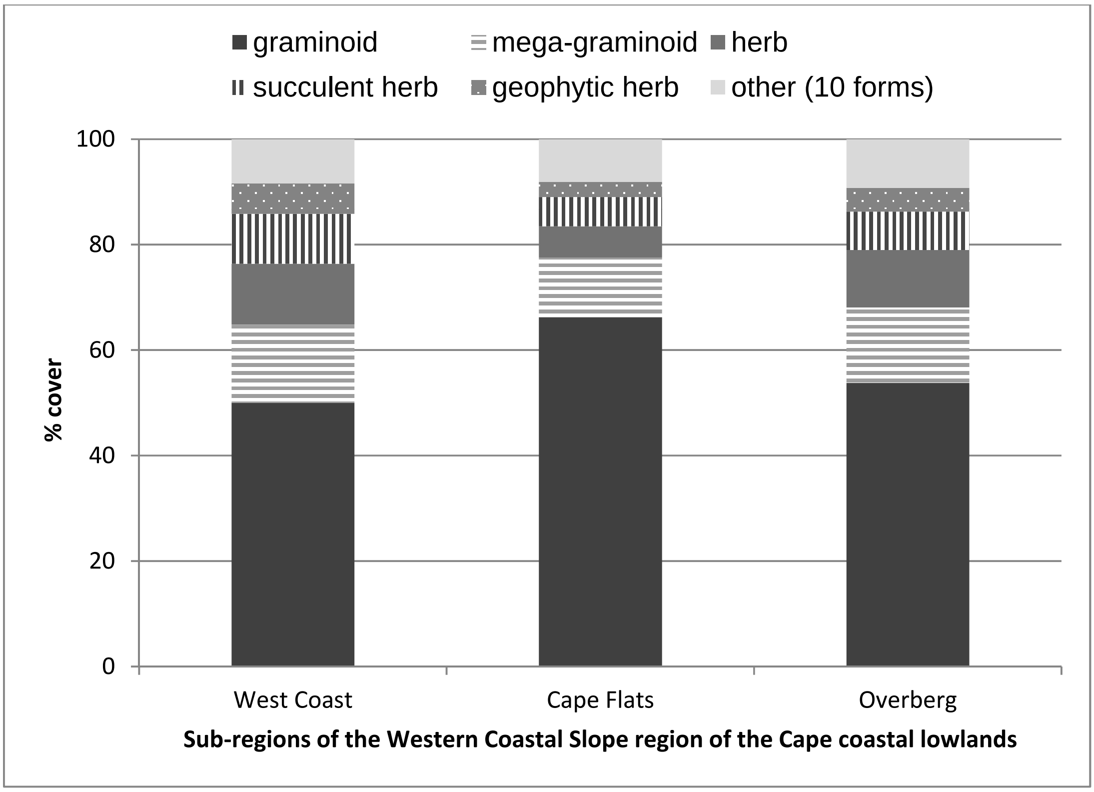


Figure 4.1: Percentage cover of the 5 dominant growth forms (*sensu* Mucina *et al.* 2006b) and a compilation of 10 'other' less dominant forms in the wetlands of the different sub-regions in the Cape coastal lowlands.

In Figure 4.1, the 'other' component of the vegetation cover consisted of standing dead litter and the following growth forms: aquatic herbs, macro-algae, carnivorous herbs, herbaceous climbers, mosses, succulent shrubs, low shrubs, tall shrubs, and small trees. Spatial cover in wetlands of these sub-regions is dominated by graminoid taxa ranging from short, mat-forming lawn grasses to tall reed beds composed of mega-graminoids.

A summary of the habitat combinations as sampled in the present study within localities and sub-regions of the study area is presented in Table 4.2:

Table 4.2: Spatial hierarchy of wetlands with different vegetation and hydrogeomorphic habitat in localities from each sub-region of the Cape coastal lowlands.

Sub-region	Locality	Wetland code***	Disturbance categories represented**	"Wetland vegetation-type" + "HGM-type"		
				CLF depressions	**Vernal-depressions**	**Other vegetation + HGM combinations**
West Coast	Berg River	Ber/Vel	1 M, 2 W	1	1	1 Saline floodplain
	Darling	Dar	1 R, 4 M, 2 W	3	2	2 Alluvial-floodplain
	Verlorevlei	Ver	4 M, 2 W	-	-	3 CLF-Floodplain, 3 CLF-valley bottom
Cape Flats	Driftsands	Dri	2 R, 6 M, 3 W	9	2	-
	Kenilworth	Ken	2 R, 5 M, 4 W	9	1	1 CLF-Flat
	Lotus River	Lot	5 R, 1 M, 5 W	11	-	-
Overberg	Hermanus	Her	2 M, 1 W	-	-	3 CLF-seeps
	Agulhas	Agu	5 R, 1 M, 1 W	4	1	1 Saline-floodplain, 1 Saline floodplain

***Wetland codes relate to the first three letters of the locality names as depicted in Table 3.2

**Disturbance categories R = Reference, M = Moderate, W = Worst

4.3. Gamma diversity – testing Hypothesis 1

Hypothesis 1: Vegetation of wetland depressions (*sensu* SANBI 2009) dominated by Cape Lowland Freshwater vegetation (Mucina *et al.* 2006a) is relatively uniform with insignificant gamma diversity between:

a. the sub-regions (West Coast, Cape Flats and Overberg) of the mediterranean South Western Coastal Slope wetland Region (Cowan 1995); and similarly, between

b. the West Coast and Cape Flats sub-regions contained within the South Western Coastal ecoregion (Kleynhans *et al.* 2005).

Sufficient CLF depressions were sampled in each of the sub-regions to determine whether gamma diversity is significant across the Western Coastal Slopes region of the Cape coastal lowlands for this wetland vegetation and HGM type ("habitat combination"). A significant proportion of unique species between CLF depressions from different sub-regions across the

Western Coastal Slopes would indicate a biogeographical gradient with concomitant high species turnover (gamma diversity) between sub-regions (e.g. Cody 1975 and 1983, Cowling *et al.* 1992).

Using Jaccard resemblance, a presence/absence measure of the existence of unique species (variability of the species composition) in the samples from different sub-regions can be determined. This test assesses the range, or variability, in species composition between samples (wetlands or vegetation plots) from different sub-regions (homogeneity of dispersion: Anderson *et al.* 2008). When a comparison is made of the species from a habitat combination of a similar vegetation unit and HGM-type across a broad area, such as the Western Coastal Slope, this analysis effectively determines the difference in species composition between the incorporated wetlands of different sub-regions and is thereby a determination of gamma diversity (*sensu* Cody 1975 and 1983, see Section 2.10.1.3).

Species composition data from both individual vegetation plot and wetland data sets were used to examine differences in gamma diversity between the different sub-regions. The vegetation plots provide a greater number of samples per sub-region, thus increasing the reliability of the dispersion test (Anderson *et al.* 2008).

4.3.1. Gamma diversity across the Cape coastal lowlands

For the CLF depressions sampled in different sub-regions, using the species listed per wetland, strong and significant differences in the variability of species composition per sub-region (significant heterogeneity of multivariate dispersion) was evident from a test for homogeneity of dispersion between sub-regions ($pseudo\text{-}F_{2,34}$=23.4, $p<0.01$). A list of these results may be seen in Table 4.3.

A posteriori pair-wise tests revealed that significant gamma diversity exists only between the CLF depressions of the Cape Flats and the West Coast sub-regions (t=6.7, $p<0.01$: Table 4.3). No significant gamma diversity apparently exists between the Cape Flats and Overberg or between the Overberg and West Coast as determined from these lists of species occurring in each wetland. A similarly significant result was apparent when using only those species occurring in more than one wetland (196 vs. 374 species), thereby reducing the influence of rare, and thus potentially uncommon species. The low number

Table 4.3: Jaccard distance-based test for homogeneity of multivariate dispersion between the species composition of CLF depressions from each sub-region. Significance at p<0.05 is marked * and is indicative of gamma-diversity.

pseudo-F $_{2,\,34}$ = 23.417, p (permutations) = 0.003*			
a posteriori PAIR-WISE COMPARISONS		t	p (permutational)
West Coast vs. Cape Flats		**6.67**	**0.003***
West Coast vs. Overberg***		2.44	0.14
Cape Flats vs. Overberg		1.7	0.94
MEANS AND STANDARD ERRORS			
Sub-region	**No. of Wetlands**	**Average Distance**	**Standard Error**
West Coast	4	42.73	5.56
Cape Flats	29	60.32	0.69
Overberg	4	56.94	1.75

***In the Overberg, CLF depressions were sampled only in the vicinity of Agulhas Plain.

of West Coast and Overberg CLF depressions in these analyses (n = 4 in each sub-region, see Table 4.2) limits the potential for comparison between sub-regions. A minimum of 5 samples is recommended for accurate determination of dispersion (Anderson *et al.* 2008). Comparison were therefore made between sub-regions using vegetation plots rather than whole-wetland data.

4.3.2. Gamma diversity across the Cape coastal lowlands using vegetation plots

Considerable heterogeneity of dispersion or gamma diversity (*pseudo-F*$_{2,261}$=48.3, p<0.001) and with a higher confidence level (p-value), was apparent when using the lists of species from the independent vegetation plots per depression.

Using the greater number of samples represented by vegetation plots rather than wetlands, *a posteriori* pair-wise tests revealed that significantly different dispersion and therefore gamma diversity also exists between West Coast and Overberg depressions (t=5.5, p<0.001) as well as between West Coast and Cape Flats depressions (t=9.98, p<0.001), as already revealed above (Table 4.3). Unconstrained multidimensional scaling (MDS) ordination of this dataset, as seen in Figure 4.2, shows the variability in dispersion between sub-regions that is indicative of different species compositions and hence of significant turnover of species or gamma-diversity difference between sub-regions.

Table 4.4: Jaccard distance-based test for homogeneity of multivariate dispersion between the species composition of individual vegetation samples from Cape Lowland Freshwater vegetation dominated depressions in different sub-regions. Significance is marked *

pseudo-*F*: 48.34 df1: 2 df2: 261 P(permutational): 0.001*			
PAIR-WISE COMPARISONS			
Sub-regions compared	**t**	**P(perm)**	
(West Coast vs. Cape Flats)	9.9769	0.001*	
(West Coast vs. Overberg)	5.4669	0.001*	
(Cape Flats vs. Overberg)	1.154	0.523	
MEANS AND STANDARD ERRORS			
Sub-regions	**No. of Samples**	**Average**	**Standard Error**
West Coast	19	58.13	1.5356
Cape Flats	210	66.84	0.224
Overberg	35	66.1	0.6822

The significant difference between West Coast and Cape Flats samples reveals significant gamma diversity difference or species turnover across the south-western coastal belt ecoregion (Kleynhans *et al.* 2005). These results suggest the West Coast and Cape Flats and the West Coast and Overberg sub-regions need to be tested independently for the impact of human disturbances on vegetation and subsequent creation of phyto-assessment metrics. No significant gamma diversity was apparent between the Cape Flats and Overberg wetlands, suggesting potential similarities and the possibility for treatment as a single sub-region for developing phyto-assessment tools for CLF depressions.

It is important to ascertain whether significant gamma diversity exists between the different localities, both within and between each sub-region, i.e. within and between the following localities: West Coast (Darling, Berg River and Verlorevlei), Cape Flats (Kenilworth, Lotus River and Driftsands) and Overberg (Ratels River and Waskraalvlei).

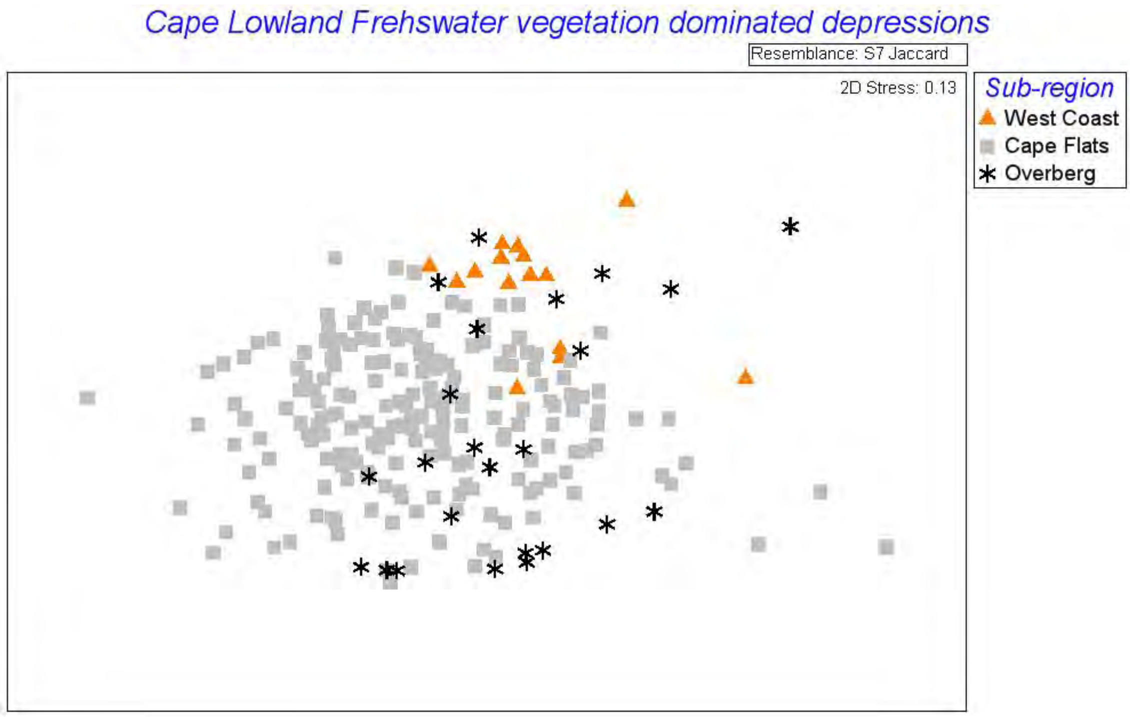


Figure 4.2: Multidimensional scaling ordination of the Jaccard resemblance (dissimilarities based on percentage of unshared species) of species compositions in individual vegetation samples from CLF dominated depressions in the Cape coastal lowlands.

4.3.3. Gamma diversity between localities and sub-regions

The species listed per vegetation plot in CLF depressions (n=264) provided sufficient samples to facilitate a comparison of the turnover of unique species (gamma diversity) between the localities as shown in Figure 4.2. Significant gamma diversity does exist between a number of the localities in different sub-regions and between two localities within the Cape Flats sub-region ($pseudo\text{-}F_{5,\ 258}$=22.5, p<0.001). The results for these tests of gamma diversity are presented in Table 4.5.

4.3.3.1 West Coast

Within the West Coast, no significant differences in gamma diversity (no heterogeneity of dispersion) were apparent between the vegetation samples of different localities (Darling [n=15] and Berg River [n=4]) where CLF depressions were sampled.

4.3.3.2. Cape Flats

Within the sub-region of the Cape Flats, significant differences in gamma diversity (heterogeneity of dispersion) occur between the species listed from the vegetation plots of the Kenilworth (n=54) and Lotus River (n=91) localities (t=2.8, p=0.01). The dryland vegetation types of these localities are known to be associated with acidic soils at Kenilworth and alkaline soils at Lotus River (Rebelo *et al.* 2006).

4.3.3.3. Overberg

In the Overberg, CLF depressions were sampled only on the Agulhas Plain. There were no gamma diversity differences (there was homogeneity of vegetation community dispersion) between the samples from CLF depressions in the Waskraalvlei (n=18) and Ratels River (n=17) localities on the Agulhas Plain.

4.3.3.4. Cape Flats versus Overberg

In the first two analyses above (Section 4.2.1 and 4.2.2) the vegetation from the CLF depressions of the Cape Flats and Overberg sub-regions were investigated and reflected insignificant gamma diversity. *A posteriori* analyses between vegetation plots of the different localities within these sub-regions (presented in Table 4.5), however, revealed significant gamma diversity difference between the Kenilworth (n=54) and Agulhas Plain (n=35) localities (t=2.7, p=0.01).

4.3.3.5. West Coast versus Overberg and West Coast versus Cape Flats

Comparison of the list of species for the vegetation plots of the different localities on the West Coast *vs*. Overberg and West Coast *vs*. Cape Flats suggests significant differences in gamma diversity (dispersion) between many of the locations (Table 4.5). These results were expected, given the significant gamma diversity differences shown in the first two analyses between the broader sub-region sample sets (Table 4.3 in Section 4.2.1 and Table 4.4 in Section 4.2.2).

4.3.4. Conclusions to Hypothesis 1

From the previous analyses it is apparent that there is significant gamma diversity between wetlands of the West Coast and the other two sub-regions. Whilst comparing between sub-regions only suggests no gamma diversity difference between Cape Flats and the Overberg, it is apparent when *posterior* pair-wise tests (based on data from vegetation plots) are used, that significant gamma diversity exists between the Kenilworth (Cape Flats) and Agulhas Plain (Overberg) wetlands. Generally, within the West Coast, Cape Flats and Overberg sub-regions there is an insignificant proportion of unique species (gamma diversity) amongst the different localities at which depressional wetlands were assessed. On the Cape Flats however, between the acidic and alkaline wetlands at Kenilworth and Lotus River specifically, there is a difference in dispersion along a pH gradient that suggests a high gamma diversity difference between these localities.

Table 4.5: Jaccard distance-based test for homogeneity of multivariate dispersion between the species composition of the individual vegetation plots sampled per CLF depression from each locality and within each sub-region. Significance at p<0.05 is marked *

pseudo-F $_{5, 258}$=22.5, p=0.001			
PAIR-WISE COMPARISONS			
Cape Flats vs.	**West Coast**	**t**	**p (permutational)**
Kuils River (Driftsands)	Darling	6.9	0.001*
Kenilworth	Darling	4.2	0.001*
Lotus River	Darling	7.9	0.001*
Kenilworth	Berg River	4.8	0.02*
Lotus River	Berg River	7.8	0.003*
Kuils River (Driftsands)	Berg River	6.9	0.003*
Overberg vs.	**Cape Flats**	**t**	**p(permutational)**
Agulhas	Kenilworth	2.7	0.01*
Agulhas	Driftsands	1.3	0.2
Agulhas	Lotus	0.9	0.48
Overberg vs.	**West Coast**	**t**	**p(permutational)**
Agulhas	Darling	7.8	0.001*
Agulhas	Berg River	7.3	0.004*

Within West Coast		t	p(permutational)
Darling	Berg	2.2	0.2
Within Cape Flats		**t**	**p(permutational)**
Driftsands	Kenilworth	2.2	0.2
Driftsands	Lotus	0.6	0.55
Kenilworth	Lotus	2.8	0.01*
Within Agulhas Plain of Overberg		**t**	**p(permutational)**
Ratels	Waskraal	0.9	0.9

MEANS AND STANDARD ERRORS

Group	No of samples	Average	SE
Berg River	4	45.1	6.78
Darling	15	54.8	1.57
Driftsands	65	64.8	0.6
Kenilworth	54	62.7	0.89
Lotus River	91	65.3	0.48
Agulhas	35	66.1	0.68
Agulhas subset: Ratels	18	62.4	1.62
Agulhas subset: Waskraal	14	62.1	2.02

Hypothesis 1, (i.e. that CLF depressions represent a uniform set of vegetation with insignificant gamma diversity across the Western Coastal Slope region), must be rejected. The heterogeneity of multivariate dispersion based on the Jaccard measure highlighted the magnitude of the collective difference in species composition for each sub-region and suggested that significant gamma diversity does exist across the CLF depressional wetlands of this Western Coastal Slope wetland region. These results suggest that the Western Coastal Slope wetland region of Cowan (1995) and the intrazonal Cape Lowland Freshwater vegetation unit (Mucina *et al.* 2006a) incorporate too great a diversity of wetland vegetation within depressions to facilitate the accurate development of metrics for phyto-assessment development for the entire region.

The West Coast, Cape Flats and Overberg do, however, provide three sub-regions within which the amount of statistical noise caused by unique species is limited. Within each of these sub-regions it may be feasible to search for suitable phyto-assessment metrics for CLF depressions. The differences between the Kenilworth and Lotus wetlands on the Cape Flats emphasises the diversity differences along a known soil pH gradient. It is possible, however, that other environmental parameters exist that also influence the turnover of species between these locations.

In order to determine whether sub-regions or locations (distinction made in Table 4.2) are the best spatial units for the development of phyto-assessment techniques (see Section 4.4), it is necessary to determine whether vegetation assemblage patterns are consistently different. In other words, confirmation is required as to whether phyto-sociological differences exist between sub-regions and also between locations within sub-regions.

4.4. Disjunctions in vegetation composition – testing Hypothesis 2.

> Hypothesis 2: Disjunctions in the distribution of vegetation within the Western Coastal Slope region are due to the spatial affinity of species for sub-regions (West Coast, Cape Flats, Overberg) or distinct locations within these sub-regions (e.g.: the Cape Flats: Kenilworth, Lotus River or Kuils River).

In order to demonstrate that disjunctions in the distribution of wetland plants show affinity for the spatial units of the different sub-regions, ordinations were produced to examine the distributions; firstly of the vegetation of CLF depressions, and secondly of all of the vegetation unit – HGM habitat combinations.

4.4.1. Sub-regional disjunction in species distribution of CLF depressions

Disjunctions in the distribution of wetland plants of the CLF depressions among the three sub-regions were demonstrated by an MDS plot on the basis of the Jaccard-measure of species presence/absence (Figure 4.3). Difference between the sub-regions was more apparent in the 3-dimensional ordination. The proximity of the single Cape Flats wetland (Lot06) to the West Coast wetlands (at right of Figure 4.3) can possibly be explained by the extreme levels of eutrophication at this site. The naturally greater availability of nutrients in Renosterveld soils of wetlands Dar01, Dar01b and Dar02 of the West Coast (Rebelo *et al.* 2006) may explain the proximity of these wetlands to the eutrophic Cape Flats wetland

(Lot06). The other West Coast depression in this group (Vel02 – from the Berg River floodplain) was from a Fynbos area but had eutrophic soil phosphorus concentrations and was highly impacted by agricultural activities. The Cape Flats wetland Lot14, located at the top of Figure 4.3 is an extremely disturbed artificial wetland and its species composition is unnatural, reflecting high levels of disturbance.

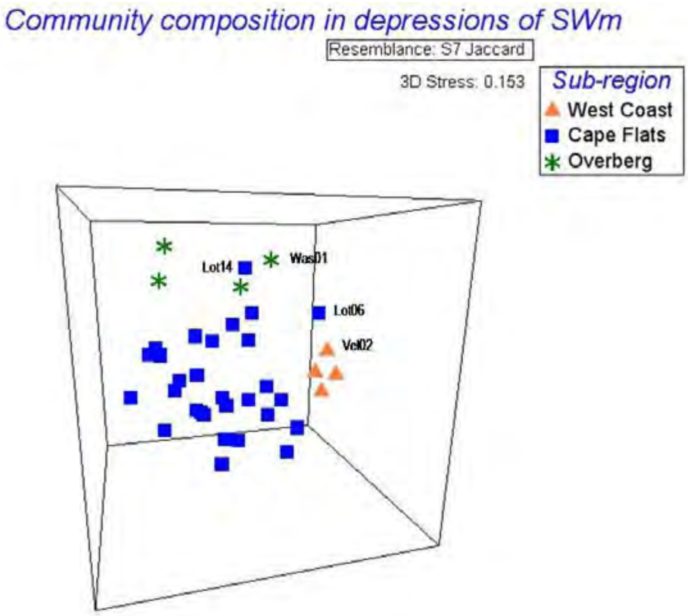


Figure 4.3: Multi-Dimensional Scaling ordination of the plant species composition in CLF depressions (n=37) within the Western Coastal Slopes (SWm), as based on the Jaccard-measure of species presence/absence.

4.4.2. Multiple vegetation- and HGM-type habitat combinations

As stated in the introduction to this chapter, numerous wetland vegetation habitats were assessed in the present study by virtue of the combination of multiple HGM types and vegetation units. The analyses described so far have examined only the CLF depression habitat combination. Examination of the aggregate of all habitat combinations will show whether different vegetation units and HGM types hold species that would reduce the differences shown above to exist between the sub-regions.

Ordination using the Jaccard-resemblance measure of the species listed per wetland (presence/absence data) for all HGM and vegetation types revealed considerable differences between the sub-regions, particularly between the West Coast and Cape Flats (Figure 4.4). Even greater difference was apparent in three dimensions, however, interpretation of the three-dimensional image in a two-dimensional format is not feasible, hence this three-dimensional ordination is not shown. The three-dimensional ordination makes it apparent that within the two-dimensional ordination, the Overberg wetlands were orthogonally overlaid on Cape Flats and West Coast wetlands. In the three-dimensional ordination, the Overberg-Agulhas and Overberg-Hermanus wetlands each lump together, but in two separate planes. Within the Overberg sub-region, the assemblages of the Hermanus and Agulhas wetlands are very different and this is apparent in these ordinations. Within the ordinations, the separate and predominantly clumped distribution of wetlands from each sub-region signifies generally different sets of species in each of the sub-regions.

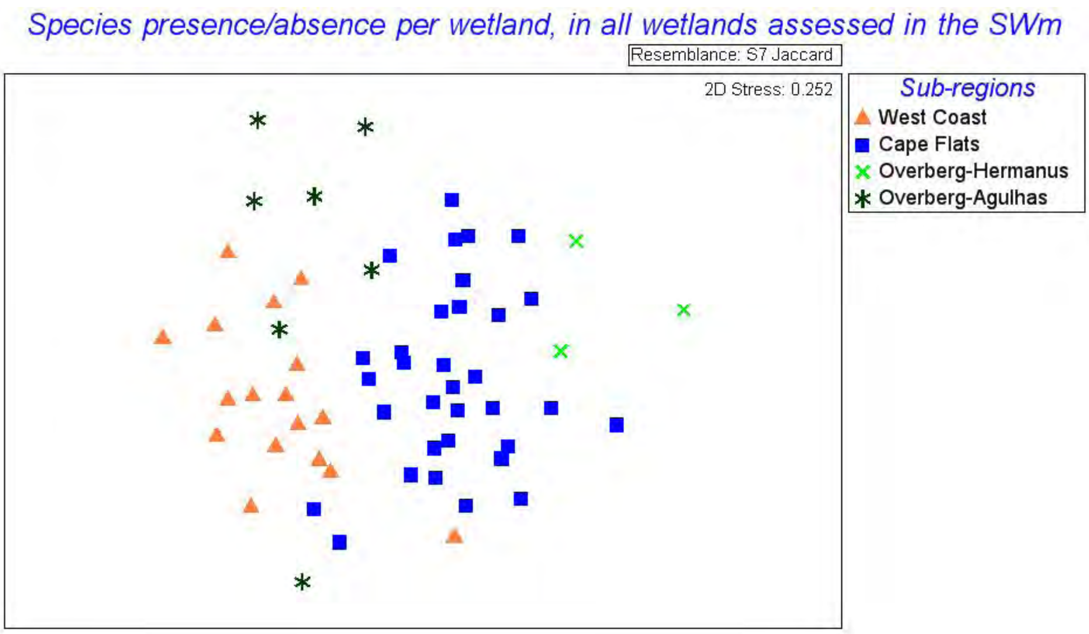


Figure 4.4: Multi-Dimensional Scaling ordination of the vegetation community in the aggregate of all wetland habitat combinations assessed in the Western Coastal Slope (SWm) wetland region as based on the Jaccard-measure of species presence/absence.

Using the intensively sampled vegetation plots, it is possible to determine whether the inclusion of all habitat combinations decreases the confidence (p-value) obtained using the

CLF depressions and with which we can say sub-regions have different species composition. Comparing the vegetation plot samples from the aggregate of depressions, flats, seeps and valley bottoms (*pseudo-*$F_{2,397}$=4.2, p=0.04: Table 4.6) suggests at 95% confidence level that the sub-regions hold different communities. When using only the CLF depressions, the confidence level is 99% (*pseudo-*$F_{2,261}$=48.3, p<0.001: Table 4.4), suggesting that the inclusion of more habitats lowers the confidence with which we can differentiate between sub-regions using dispersion tests of diversity differences between sub-regions.

Determining the dispersion in the aggregate of all habitat combinations is no longer a test of gamma diversity as it incorporates multiple habitats (numerous HGM-types and wetland vegetation units.) across spatial and also environmental gradients. *A posterior* pair-wise tests suggest significant differences in species composition of vegetation plots sampled in the West Coast and Overberg (t=2.78, p=0.01) and between Cape Flats and Overberg wetlands (t=2.7, p=0.05) but not between West Coast and Cape Flats wetlands Table 4.6). These tests confirm differences between the sub-regions and show that the inclusion of samples from all habitat combinations reduces the confidence (p-value) with which we can say the floristic communities of sub-regions differ.

Table 4.6: Jaccard distance-based test for homogeneity of multivariate dispersion between the species composition of individual vegetation samples of all wetlands sampled within the West Coast, Cape Flats and Overberg sub-regions. Significance is marked *

pseudo-*F*: 4.26 df1: 2 df2: 397 P(permutational): 0.05			
PAIR-WISE COMPARISONS			
Localities compared	**t**	**P(perm)**	
(West Coast vs. Cape Flats)	0.684	0.5	
(West Coast vs. Overberg)	2.78	0.01*	
(Cape Flats vs. Overberg)	2.72	0.05*	
MEANS AND STANDARD ERRORS			
Sub-region	**No. of Samples**	**Average**	**Standard Error**
West Coast	92	66.69	0.394
Cape Flats)	232	66.97	0.21
Overberg	76	68.02	0.24

4.4.3. Conclusions to Hypothesis 2

The spatial disjunctions apparent in ordinations of species occurring in CLF depressions (Figure 4.3: Section 4.3.1) and in the aggregate of all habitat combinations (Figure 4.4: Section 4.3.2) indicate sub-regional differences in species distribution. Further separation is apparent within the sub-regions between the wetlands of different locations (, as shown by gamma diversity difference between localities Kenilworth and Lotus on the Cape Flats (Section 4.2.3); and by differences between the species compositions of Hermanus and Agulhas wetlands in Figure 4.4. Further differences in species composition between localities can be shown by ordination. Rather than an examination of difference in species composition based on *presence/absence* data, in the following section (Section 4.4) the difference between plant species *cover/abundance* assemblage patterns will be used. Wetlands from different localities will be investigated in order to confirm vegetation differences between phytogeographical units. This is another way of testing hypothesis 2.

4.5. Vegetation assemblage pattern – re-testing Hypothesis 2

> Hypothesis 2: Disjunctions in the distribution of vegetation within the Western Coastal Slope region are due to spatial affinity of species for sub-regions (West Coast, Cape Flats, Overberg) or distinct locations within these sub-regions (e.g.: on the Cape Flats: Kenilworth, Lotus River or Kuils River).

In the previous analyses, a significant proportion of unique species was shown to occur in the CLF depressions of each sub-region across the Western Coastal Slope of the Cape coastal lowlands (Section 4.2). The aggregate of all HGM and vegetation – habitat combinations also showed significant numbers of unique species between the West Coast and Overberg and between the Cape Flats and Overberg but not between Cape Flats and West Coast (Section 4.3.2). Ordination of the aggregate of habitat combinations did, however, suggest a difference in the spatial distribution of wetland plant species between West Coast and Cape Flats, sub-regions (Fig 4.3). Rather than focusing on numbers of unique species, another approach is to examine the phyto-sociological cover/abundance pattern or *vegetation assemblage pattern.* Vegetation assemblage patterns were used, as explained in the following section, to search for phyto-geographical units with distinct biotas (or ‘biotic assemblages’). Comparison of the assemblages between sub-regions using individual *HGM* and *vegetation* – combinations, rather than the aggregate of all habitats, is only feasible with the CLF depressions, as insufficient replicates of any other combinations were recorded. An analysis of all habitat combinations was considered to be more informative than using only the CLF depressions.

To determine whether patterns of vegetation assemblages differ between wetlands of all habitat combinations assessed in different areas of the Western Coastal Slope region, the main structure in the data was sought using MDS ordination. Analyses using the aggregate of all habitats were performed both at the scale of the entire wetland assemblage and also independently for the supralittoral and littoral hydrological zones to determine if, in any of these data sets, significant similarity exists across the Western Coastal Slope wetland region (Section 2.9.1).

The vegetated aquatic zone was minimal in all wetlands, accounting for only 7%, or 27 of 400, vegetation sample plots. The aquatic habitat type was found in only 11 wetlands and hence does not provide sufficient samples to determine meaningful difference between location or disturbance groups. The aquatic-zone samples were therefore incorporated into the littoral zone, following the recommendations of the BAWWG (Section 2.9.3). The supra-littoral zone accounted for 55% (221/400) of sample plots and the combination of aquatic (27/400) and littoral (152/400) zones accounted for the other 45% of sample plots. Species occurring in only one wetland were removed from these data sets as they do not provide any comparative potential except in the Jaccard-resemblances of percentage of unique species as performed in Sections 4.2 and 4.3.

For the cover and abundance data per wetland, values used were based on a weighted-average (*sensu* Cochran 1977, Krebs 2003) percent cover calculated per taxon in each hydrological zone (Section 3.5.8.2). The cover/abundance of species were recorded in every wetland using the Braun Blanquet scale (Braun Blanquet 1928) which is, effectively, a log scale transformation of the data (Van der Maarel 1979). The weighted-average as determined per species per wetland was the mean value of species coverage across all of what were often an irregular number of vegetation samples in each hydrological zone. Thus, this weighted wetland average no longer represents a log-scale. The number of samples in each hydrological zone was dependent on the number of different homogenous stands of vegetation. The supralittoral and littoral zones were also often of different size, thus represented different weights (See Section 3.5.8.2). For the purposes of comparing vegetation assemblages, the weighted-average values were fourth-root transformed to down-weight the excessive contributions of quantitatively dominant species in the assemblage patterns (Field *et al.* 1982, Clarke and Gorley 2006). The frequency distribution of a numerically dominant species in the dataset (*Stenotaphrum secundatum*) is presented below showing that both square root and fourth root transformations effectively normalized the distribution (Figure 4.5). These transformations were similarly successful at normalizing the distribution of infrequently encountered species with low weighted-average cover values.

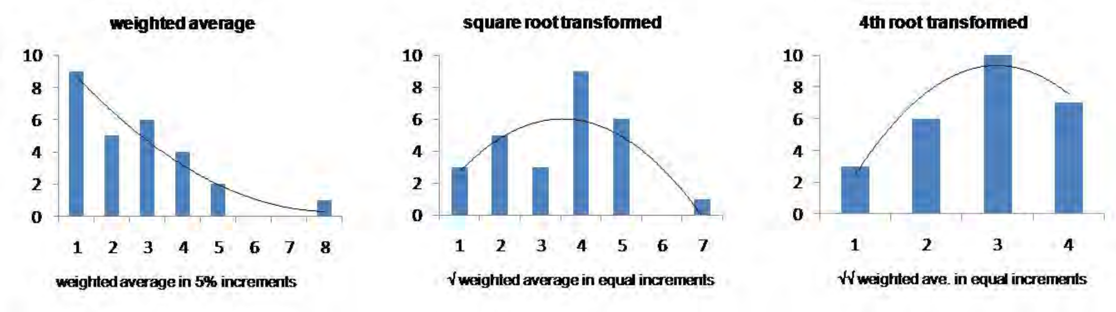


Figure 4.5: Frequency distribution under different transformations of *Stenotaphrum secundatum*, a numerically dominant species in the data set, having high weighted-average cover and occurring in approximately 50% of the wetlands sampled.

The Bray-Curtis resemblance measure of (dis)similarity was used to compare samples (wetlands) on the basis of vegetation assemblage patterns (as determined from 4^{th} root transformed species cover) as this has been shown to be an ecologically meaningful resemblance measure (Field *et al.* 1982).

4.5.1. Comparisons of vegetation assemblages

Wetlands with similar patterns of vegetation assemblage were expected to exhibit grouped distribution in dendrograms and ordinations. Furthermore it was expected that spatial correlation of wetland vegetation assemblages with the environmental conditions prevalent in the different sub-regions would produce sub-region-related groupings and clusters in ordinations and dendrograms.

4.5.1.1. Unconstrained ordination of vegetation assemblages

In unconstrained ordination (MDS) both as depicted in 2- or 3-d (not shown) there was relatively clear distinction between species assemblages of wetlands from the West Coast and Cape Flats sub-regions. Overberg wetlands were split into Hermanus and Agulhas contingents that were orthogonally aligned to the axis that best explained West Coast and Cape Flats vegetation assemblage distribution (Figure 4.6). The MDS stress is high (0.251 and 0.177 in 3-d) and a Principle Coordinate Ordination (PCO; not shown) accounted for only 31% of multivariate distribution of the vegetation data when graphed in three-dimensions.

The high stress, low percentage of explained multivariate distribution and yet spatial correlation of wetlands from each sub-region, also apparent in a cluster dendrogram depicted in Figure 4.7, suggested that there are more underlying geo-spatial relationships between wetlands than can be explained in the two or three dimensions of the unconstrained ordinations.

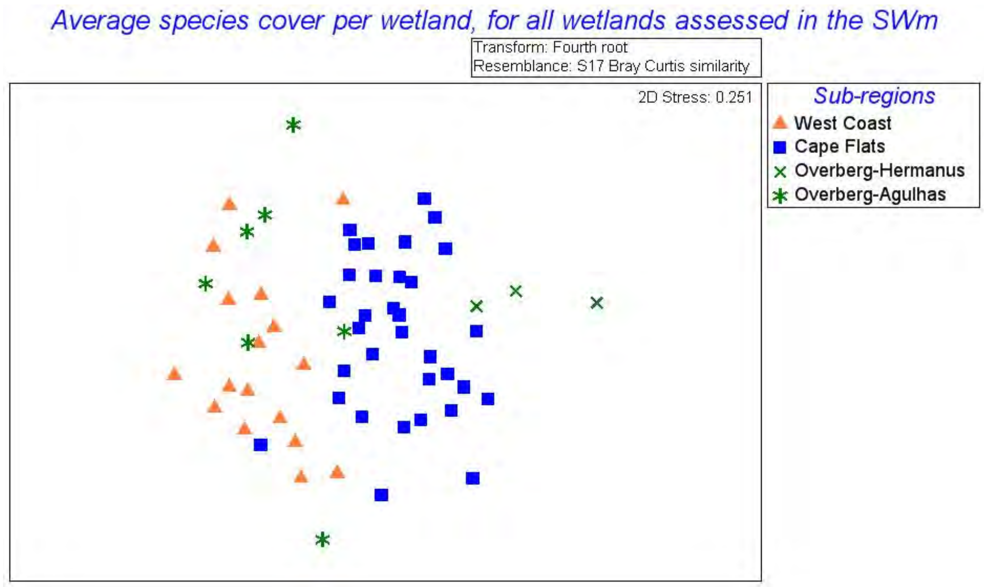


Figure 4.6: Multi-Dimensional Scaling ordination of (Bray-Curtis) vegetation assemblage patterns per wetland, derived from 4th root transformed weighted-average cover of typical plant taxa per wetland (n=60) in the conglomerate of all habitat combinations sampled in all sub-regions of the Western Coastal Slope (SWm) region of the Cape coastal lowlands.

It is apparent from these ordinations and dendrograms that there is some spatial disjunction in the distribution of species assemblages among all three of the sub-regions. Thus wetlands of each sub-region do have greater spatial affinity/correlation with others from the same sub-region, than with those from other sub-regions. The difference between the Overberg wetlands and those of the other two sub-regions is, however, not made clear by these analyses.

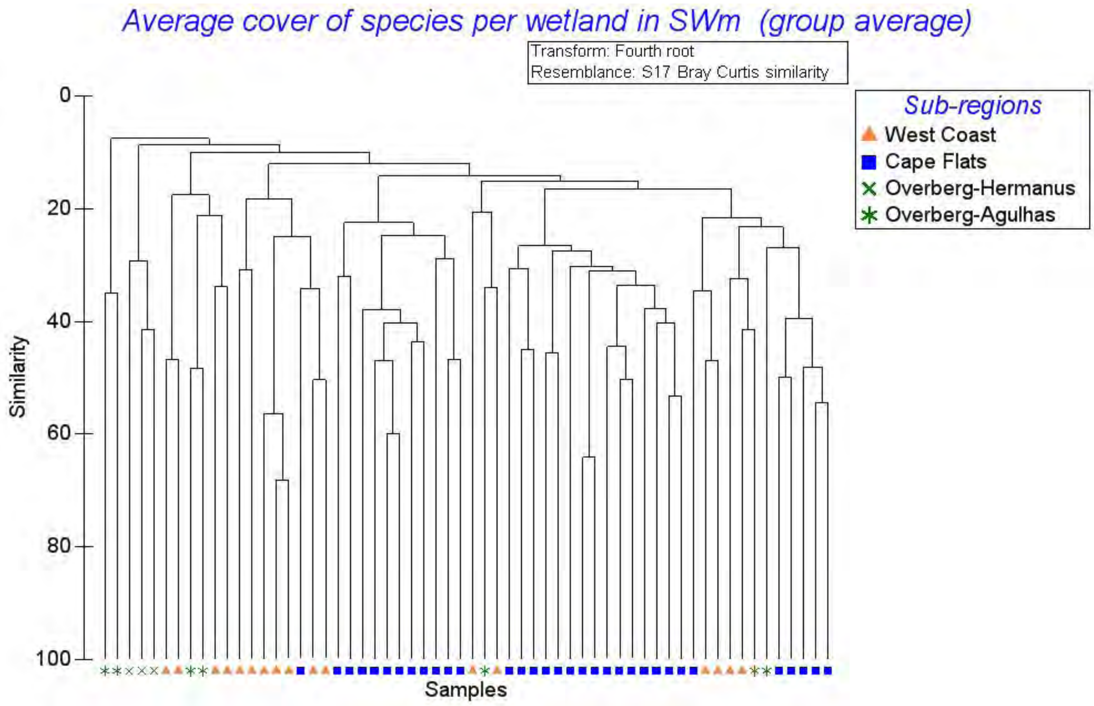


Figure 4.7: Cluster dendrogram, using group average clustering, of Bray-Curtis resemblance of the vegetation assemblage pattern per wetland as derived from 4^{th} root transformed weighted-average cover of species for all of the 60 wetlands sampled in the Western Coastal Slope (SWm) region of the Cape coastal lowlands. Note sub-regions dominate major clusters.

4.5.1.2. Analysis of similarity of vegetation assemblages

Analysis of the similarity (ANOSIM) of vegetation assemblages was performed using two approaches. An initial comparison (a) of the *wetland-average* species cover data was made, followed by (b) separate comparisons of the average cover data of species in the assemblages of each of the hydrological zones.

a) Significant difference in vegetation assemblage patterns of wetlands from each sub-region was revealed by ANOSIM (global-R=0.465, p<0.001; $R_{\text{all pairs}}$>0.345, p<0.003) (Table 4.7 "All habitats combined"). Using only the CLF depressions a similarly significant difference (R-value) between assemblages was apparent between all sub-regions (Table 4.8). It is apparent from the large R-values that the wetlands of each sub-region have significantly different species assemblages. In other words, the assemblage patterns of wetlands from each of the sub-regions have greater affinity for other wetlands from the same sub-region than to those of any other sub-region.

Table 4.7: Analysis of similarity of the Bray-Curtis resemblance of the weighted average vegetation assemblages of all habitats for all wetlands after 4^{th} root transformation. Group comparisons that are significant are marked *.

All habitats combined: vegetation assemblages for each wetland					
Global R = 0.465, p < 0.001*, 999 permutations, # permutations ≥ Global R = 0					
Pair-wise Tests	**R Statistic**	***P*-value**	**Possible Permutations**	**Actual Permutations**	**No. ≥ Observed**
West Coast vs. Cape Flats	0.465	< 0.001*	Very large	999	0
West Coast vs. Overberg	0.347	< 0.003*	8436285	999	2
Cape Flats vs. Overberg	0.519	< 0.001*	Very large	999	0
Supralittoral habitat: vegetation assemblages of all wetlands.					
Global R = 0.54, p < 0.001*, 999 permutations, # permutations ≥ Global R = 0					
West Coast vs. Cape Flats	0.51	< 0.001*	4.73E+08	999	0
West Coast vs. Overberg	0.41	< 0.001*	92378	999	0
Cape Flats vs. Overberg	0.59	< 0.001*	1.24E+08	999	0
Littoral habitat with emergent and aquatic vegetation.					
Global R = 0.36, p < 0.001*, 999 permutations, # permutations ≥ Global R = 0					
West Coast vs. Cape Flats	0.35	< 0.001*	Very large	999	0
West Coast vs. Overberg	0.337	< 0.007*	50388	999	6
Cape Flats vs. Overberg	0.4	< 0.002*	8347680	999	0
Aquatic assemblages of all wetlands in which this habitat exists.					
Global R = 0.46, p < 0.02*, 999 permutations, # permutations ≥ Global R = 7					
West Coast vs. Cape Flats	0.6	< 0.06	35	35	2
West Coast vs. Overberg	0.4	< 0.09	35	35	6
Cape Flats vs. Overberg	0.3	< 0.1	35	35	4

b) Within the data sets of the supralittoral and littoral hydrological zones, significant difference in the vegetation assemblages of these two habitats was apparent between sub-regions as revealed by ANOSIM (Table 4.7). This difference between sub-regions, is evident whether based on vegetation assemblages of the different hydrological zones or, based upon their overall combination per wetland ("all habitats combined"). Thus despite the fact that each of the supralittoral or littoral hydrological zones provide similar habitat, with similar drainage and hydroregime, in wetlands of different HGM types, samples from these zones do not present homogenous assemblages of vegetation between the different sub-regions of the Western Coastal Slope region. Susceptibility to seasonal dehydration means that the supralittoral zone is more influenced by macroclimatic factors than the wetter littoral zone, thus having more affinity with surrounding dryland vegetation. Greater difference was therefore apparent between the supralittoral vegetation of different sub-regions than was apparent between littoral vegetation (compare R-values in Table 4.7: Supralittoral *vs.* Littoral vegetation).

Table 4.8. Analysis of similarity of the Bray-Curtis resemblance of the weighted average vegetation assemblages of CLF depressions after 4^{th} root transformation. Significant group comparisons are marked *.

Global R = 0.509, p < 0.001*, 999 permutations, # permutations ≥ Global R = 0

Pair-wise Tests	**R Statistic**	***P*-value**	**Possible Permutations**	**Actual Permutations**	**No. ≥ Observed**
West Coast vs. Cape Flats	0.49	< 0.001*	40920	999	0
West Coast vs. Overberg	0.56	< 0.29	35	35	1
Cape Flats vs. Overberg	0.52	< 0.002*	40920	999	1

4.5.1.3. Constrained ordination of vegetation assemblages

Canonical Analysis of Principal coordinates (CAP: Anderson and Willis 2003) is a constrained ordination technique. CAP can be used to define a linear combination of the Bray-Curtis resemblances of the vegetation assemblage of the aggregate of different wetlands. In this way, the differences between distinct spatial units of wetlands with similar vegetation assemblage become more apparent (See Section 2.10.5.1). Constrained ordination was used (a) to compare the species assemblages of the aggregate of all habitat combinations between sub-regions; and (b) to compare the species assemblages of the CLF depressions between sub-regions.

4.5.1.3.1. Aggregate of all habitat combinations

With constrained ordination, comparison of wetland assemblages in all habitat combinations revealed difference between the West Coast and Overberg relative to the Cape Flats wetland assemblages as separated along the primary or x-axis: CAP1 (Figure 4.8). The Overberg and West Coast wetlands are also relatively distinct from one another, as shown by separation along the y-axis: CAP2 (Figure 4.7). The most distinct sub-region, which had a 96.97% successful allocation rate, was the Cape Flats (in which the greatest number of wetlands (n=33) were sampled). Allocation success in the West Coast

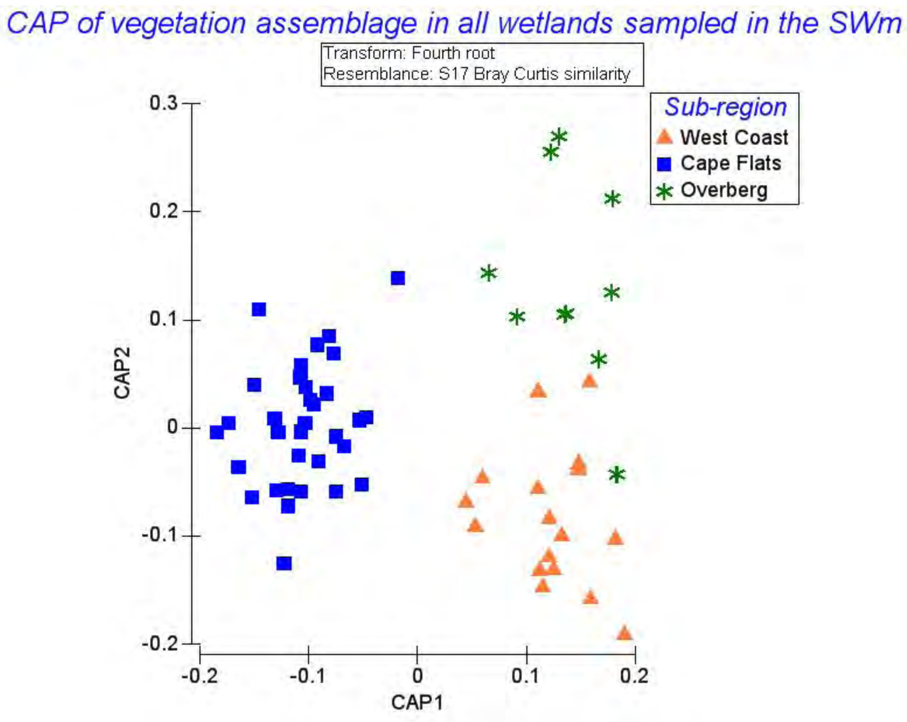


Figure 4.8: Canonical Analysis of Principal coordinates of the fourth-root-transformed weighted-average species cover per wetland in the different sub-regions of the Western Coastal Slope wetland region. The squared canonical correlation to the first axis (CAP1) = (δ_1^2) = 0.90. For CAP2, δ_2^2= 0.55. Both Hermanus and Agulhas localities are included in the Overberg wetlands.

(n=17) was still high at 88.24%, dropping to 60% for the Overberg (n=10). This suggests that the CAP was accurate for making predictions of which species assemblages represent sub-regions and whether the sub-regions are different. With a "misallocation" error of 11.6% (as based on wetlands that were allocated to another sub-region due to their assemblage pattern) there was a significant difference found between the vegetation assemblages of the sub-regions (trQ_m'HQm=1.45, p=0.0001; 9999 permutations: Table 4.9).

This analysis included *all* of the wetlands sampled in the Western Coastal Slopes and thus incorporated an aggregate of vegetation- and HGM-type habitat combinations, as well as natural and anthropogenic environmental differences. Numerous reasons can thus be suggested as to why some of the wetlands were "misallocated" in the CAP

Table 4.9: Diagnostics for discriminant analysis between species assemblages of different sub-regions using Canonical Analysis of Principal coordinates.

tr(Q_m'HQ_m)=1.456262, p=0.0001; No. of permutations used: 9999

Total correct: 53/60 (88.33%): Misclassification error: 11.6%

Original group	Classified as: West Coast	Cape Flats	Overberg	Total n***	%correct
West Coast	15	0	2	17	88.2
Cape Flats	1	32	0	33	96.97
Overberg	3	1	6	10	60

***n = number of wetlands

Individual samples that were misclassified/misallocated:

Sample	Original group	Classified as	Disturbance	HGM (SANBI 2009)	Wetland Vegetation Unit (Mucina *et al.* 2006a)
Ber01	West Coast	Overberg	Worst	Depression	Vernal Pool
Ver1B tidal	West Coast	Overberg	Moderate	Floodplain	Cape Lowland Freshwater
Lot06	Cape Flats	West Coast	Worst	Depression	Cape Lowland Freshwater
Rat03	Overberg	Cape Flats	Reference	Depression	Cape Lowland Freshwater
Rat04	Overberg	West Coast	Reference	Depression	Cape Lowland Freshwater
Was01	Overberg	West Coast	Reference	Depression	Cape Lowland Freshwater
Was02	Overberg	West Coast	Worst	Floodplain	Cape Inland Salt Pan

CLF = Cape Lowlands Freshwater Vegetation analysis; there is always some level of misallocation by multivariate techniques and the low percentage of misallocation in the present example does not meant that the ordination is inaccurate, only that the data suggested the wetland was more similar to another sub-region. Despite the inclusion of numerous HGM, soil and vegetation types, and different degrees of human impact (Table 4.9), the constrained ordination accurately separates species assemblages between sub-regions. This therefore suggests that natural environmental differences between each sub-region are potentially important parameters determining the species assemblage differences shown to exist between each sub-region. This supposition is explored in Section 4.5 of this volume.

It is apparent that the Western Coastal Slope wetland region does not support a homogenous set of wetland vegetation within the aggregate of habitat combinations that were included in the present study. This wetland region can be said to support multiple wetland vegetation communities that represent phyto-sociologically different units of freshwater wetland vegetation.

4.5.1.3.2. CLF depressions

Similar results suggesting even greater magnitude of difference in the vegetation assemblages between each sub-region were obtained using only the CLF depressions (tr(Q_m'HQ_m)=1.8, p=0.001, misallocation error 5.4%). For this single vegetation-HGM-habitat combination the differences in assemblage between the sub-regions (tr(Q_m'HQ_m value) was more marked than the aggregate of habitat combinations. The greater accuracy of classification to each sub-region, and greater magnitude of difference between sub-regions obtained for CLF depressions rather than using all habitat combinations, serves only to emphasize the natural differences between sub-regions.

4.5.1.4. Summary of vegetation assemblage comparisons

The results from all of the above analyses (Section 4.4), suggest considerable difference between vegetation assemblages of each sub-region. These marked sub-regional differences suggest that the search for the spatial units that will facilitate development of metrics for phyto-assessment purposes would best be conducted within each of the sub-regions of the Western Coastal Slope wetland region. The Western Coastal Slope cannot be considered to be a geographical unit of land that holds a homogenous, or relatively uniform, set of wetland vegetation.

4.5.2. Difference between localities within each sub-region

The differences among the vegetation assemblages of all habitat combinations, including both impacted and un-impacted wetlands, suggests considerable and significant difference between **all of the localities of the sub-regions** when simultaneously examined in a CAP analysis (tr(Q_m'HQ_m)=5.013, p=0.001, misallocation error of 20.3%). This finding is explained below, for each vegetation type.

4.5.2.1 Spatial units of homogenous wetland vegetation on the West Coast

As is apparent from the spatial hierarchy of wetlands sampled on the West Coast (Table 4.2), sampling focused on three localities, namely the Berg River, Verlorevlei and Darling. Using wetland average data, a CAP analysis revealed significant differences between the wetland

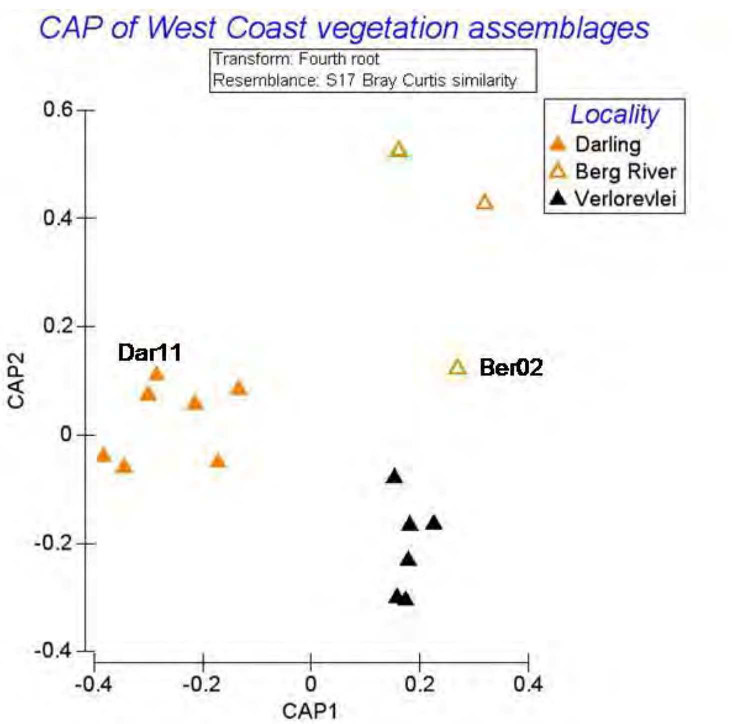


Figure 4.9: Canonical Analysis of Principal coordinates of the fourth root transformed weighted-average species cover per wetland on the West Coast. $\delta_1^2 = 0.928$, $\delta_2^2 = 0.804$.

assemblages of each locality (tr(Q_m'HQ_m)=1.7, p=0.035, misallocation of 25%), (Figure 4.9). Similar differences were apparent with the CAP analysis based on the vegetation plot samples, rather than wetland averages ((tr(Q_m'HQ_m)=1.14, p=0.001, misclassification of 26%).

The limited graphical spread of the wetlands of each locality in the ordination suggests affinity to the soil types and climatic conditions in each locality. The dryland vegetation types in these three localities (Veldrift + Berg River, Verlorevlei and Darling) have been classified according to soil type and affinity into broad classes of Fynbos, Strandveld or Renosterveld vegetation (Rebelo *et al.* 2006). The dryland vegetation classifications of all of these localities were presented in Table 3.2 whilst that surrounding each wetland is shown in Appendix 7.

Wetland Ber02, is located midway between the Berg and Verlorevlei wetlands in Fig 4.9. It potentially experiences some tidal influence, being a backwater depression in the Berg River floodplain. The soils of Ber02 were saline-sodic as revealed by average conductivity (416 $mS.m^{-1}$) being higher than 400 $mS.m^{-1}$. This is the level above which soils are considered to be saline. The sodium adsorption ratio of 81 is considerably higher than the level of 15, above which soils are considered sodic (Ellis and Mellor 1995). One other wetland on the West Coast (Dar11) also has saline-sodic soils (500 mS.m-1, and SAR 37), yet the vegetation assemblage of this wetland was not an outlier from the rest of the wetlands from the same locality such as Ber02 appears to be in Figure 4.9.

4.5.2.2. Spatial units of homogenous wetland vegetation on the Cape Flats

On the Cape Flats, the variability in vegetation assemblage pattern (range of dispersion) was not significantly different (i.e. not heterogeneous) when comparing the three intensively sampled localities of Kenilworth, the Lotus River and Driftsands. The wetlands of the Kenilworth area fall within the Cape Flats Sand Fynbos matrix of dryland vegetation, as do those south-western wetlands in the Lotus River area that occur within the Rondevlei Nature Reserve. The remainder of the Lotus River and the Driftsands (Kuils River Floodplain) wetlands fell within the Cape Flats Dune Strandveld vegetation (See Table 3.2 and Appendix 7). Analysis of similarity revealed significantly different vegetation assemblages between the Fynbos-associated wetlands of Kenilworth and the Strandveld wetlands of the Lotus River and Driftsands (Global-*R*=0.53, p<0.001) as shown by the results displayed in Table 4.10.

The lower magnitude of difference apparent between the Lotus and Driftsands wetlands (*R*=0.34, p<0.001) is important as it suggests closer affinity between these two predominantly Strandveld-associated sets of wetlands than between these and the Fynbos-associated (Kenilworth) wetlands. Furthermore the existence of some Fynbos elements in the land surrounding the south-western wetlands of the Lotus locality perhaps explains the smaller difference exhibited between Kenilworth and Lotus wetlands (*R*=0.51, p<0.001) relative to that between Kenilworth and Driftsands wetlands (*R*=0.74, p<0.001). A difference

Table 4.10: Analysis of similarity of the wetland vegetation assemblages in the different localities on the Cape Flats. Significance marked *.

Global-R=0.531, p<0.001*, 999 permutations, # permuted R ≥ global-R = 0

Pairwise Tests	R Statistic	*P*-value	Possible Permutations	Actual Permutations	Number ≥ Observed
Driftsands, Kenilworth	0.738	0.001*	352716	999	0
Driftsands, Lotus	0.342	0.001*	352716	999	0
Kenilworth, Lotus	0.509	0.001*	352716	999	0

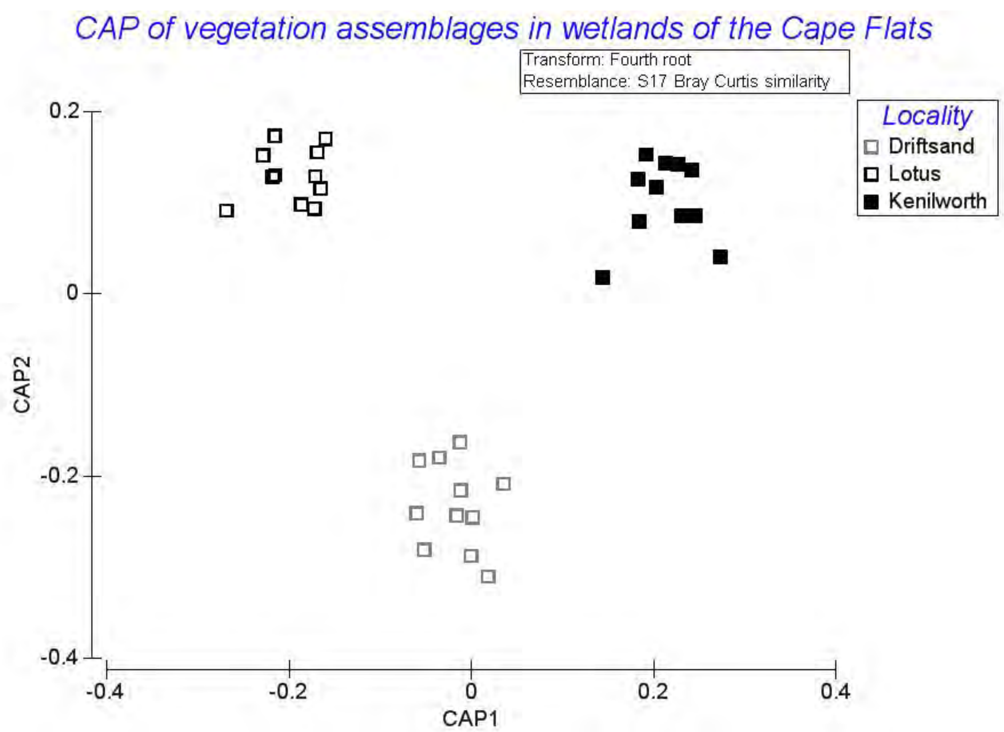


Figure 4.10: Canonical Analysis of Principal coordinates of the fourth root transformed weighted-average species cover per wetland on the Cape Flats. δ_1^2 = 0.96, δ_2^2= 0.95.

was also apparent between assemblages of the different localities using CAP (tr(Q_m'HQ_m)=1.9, p=0.001, with misallocation error of 3%), with clear distinction between the localities visible in the constrained ordination plot (Figure 4.10).

4.5.2.3. Spatial units of homogenous wetland vegetation within the Overberg

In the Overberg, a CAP analysis showed significant difference between the vegetation assemblages of the different localities (tr(Q_m'HQ_m)=0.97, p=0.018, with no misallocation), (Figure 4.11). The wetlands of the Hermanus locality were hillslope seeps within a dryland or zonal vegetation matrix of Overberg Sandstone Fynbos. The Agulhas Plain wetlands (predominantly depressions) were variously associated with Overberg Sandstone Fynbos, Elim Ferricrete Fynbos, Agulhas Limestone Fynbos and Central Ruens Shale Renosterveld (See Table 3.2 and Appendix 7).

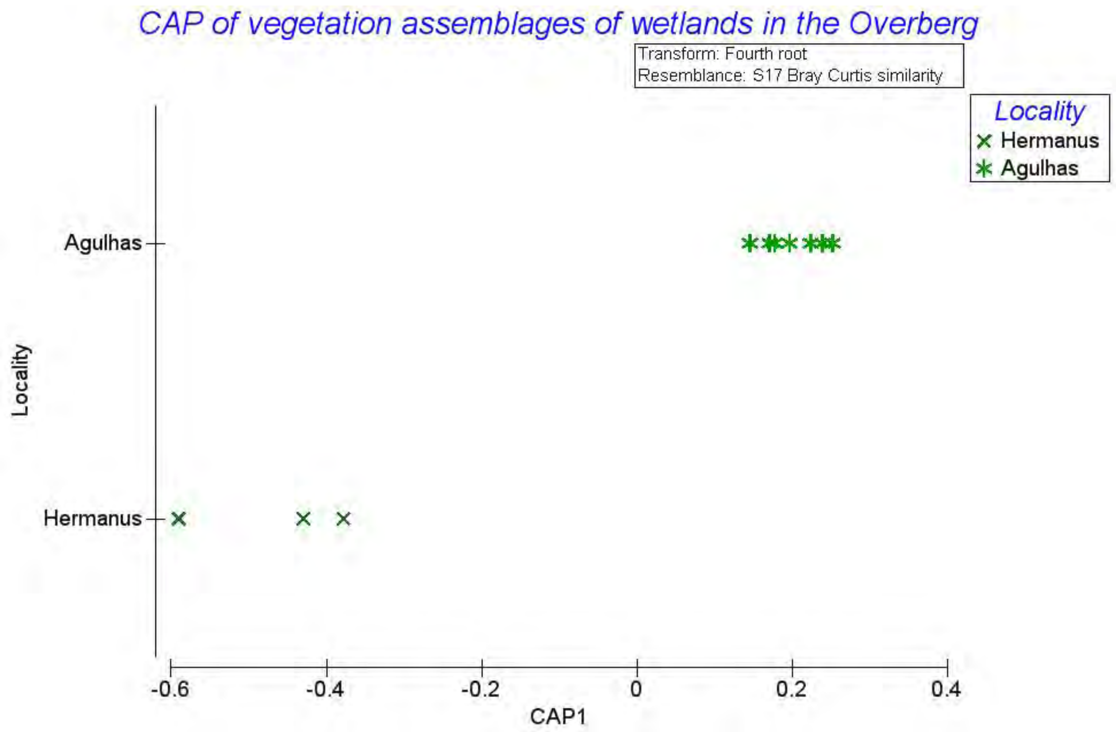


Figure 4.11: Canonical Analysis of Principal coordinates of the fourth root transformed weighted-average species cover per wetland in the Overberg. Correlation with primary axis of separation is 98% (δ_1 = 0.98 and δ_1^2 = 0.97).

4.5.3. Conclusions regarding the sub-region and location differences

The analyses in Section 4.4 show that the wetlands at each locality and within each of the three sub-regions, hold different vegetation assemblages. The impact of anthropogenic disturbance in many of these wetlands does not reduce the affinity of their vegetation assemblages for the localities within which they occur. It was anticipated that even if the Western Coastal Slope region did not hold homogenous wetland vegetation, then at least within each sub-region, sufficient wetlands would have been sampled to facilitate comparisons between disturbance categories. The differences in vegetation assemblages between the localities within sub-regions (Section 4.4.2) potentially reduces the ability to discern consistent and typical species response to disturbance within sub-regions. For instance, in the Overberg, only Moderate and Worst wetlands were sampled at Hermanus, whilst only Reference and Moderate wetlands were assessed on the Agulhas Plain (See Table 4.2). The Hermanus and Agulhas wetlands are significantly different (Section 4.4.2.iii), but as there was only wetland disturbance category that was found in both – Moderate wetlands – they cannot be legitimately compared. The development of metrics for phyto-assessment on the Overberg would only be feasible between Reference and Moderate wetlands of the Agulhas Plain and/or between Moderate and Worst wetlands of the Hermanus locality. Similarly on the West Coast, predominantly only wetlands with Moderate and Worst levels of disturbance were found and could be assessed. A greater range of disturbance was sampled amongst the locations on the Cape Flats. Even in this sub-region, however, the magnitude of the differences between disturbance categories (Reference, Moderate and Worst) in the Kenilworth and Driftsands (Kuils River) areas was limited.

4.6. Environmental difference between localities – Testing hypothesis 3

> Hypothesis 3: Biogeographical differences between distinct groups of vegetation (*sensu* Hypothesis 2) are due to differences in environmental parameters, particularly macroclimatic and geological parameters.

The difference in vegetation assemblages between different areas of the Western Coastal Slope wetland region may be due to environmental differences between localities and not simply to the element of distance *per se* (*sensu* Whittaker 1962: Section 2.6.1). Across a region where environmental conditions are variable, the affinity of plants for specific conditions creates a mosaic of characteristic plant assemblages (Whittaker 1962, Walter 1973). Geological and climatic differences driving the expression of soil nutrient and salt concentrations are likely to result in differing sets of wetland plant communities, as they do in dryland vegetation types (Cowan 1995, Mucina *et al.* 2006a, Rebelo *et al.* 2006). Constrained ordination was used to search for environmental difference between localities. A total of 34 non-collinear environmental parameters (as determined using multiple Pearson correlations in PRIMER-E) were employed, including variables pertaining to climate, wetland water-volume, anthropogenic disturbance, buffer width, land-aspect, slope, altitude and physico-chemical soil properties (see Table 4.11). There was a large magnitude (high trace value) of environmental difference evident between all sub-regions and localities for the wetlands assessed in the Western Coastal Slope region (tr(Q_m'HQ_m)=7.1, p=0.001; misclassification error 8.3%). This demonstrates a significant multivariate difference in these 34 environmental parameters between all sub-regions and localities.

4.6.1. Spatial units of homogenous environment on the West Coast

On the West Coast, significant differences were apparent between the environmental parameters of each locality (Darling, Verlorevlei and Berg River) (Figure 4.12: tr(Q_m'HQ_m)=1.8, p=0.002; with 18.7% misallocation). The misallocated wetlands were from the Berg River locality which, with only three wetlands, was essentially under-represented in the analysis. The constrained ordination does, nevertheless, separate each location as having distinctly different environmental conditions. Comparison of Figure 4.9 displaying the vegetation association with locations and Figure 4.12 reveals a very similar pattern of separation between localities, suggesting strong affinity of plants for certain environmental conditions. In these West Coast wetlands, the differences between distinct groups of wetlands with similar vegetation assemblage patterns in each location can be seen to be due to difference in environmental parameters at each location.

Wetland Ber02, which was argued earlier (Section 4.4.2.i) to have a different vegetation assemblage possibly as a consequence of saline-sodic soils, did not appear to be an outlier

Table 4.11: The 34 environmental variables used in the differentiation between localities of the Western Coastal Slope. **(See Appendix 12 – last worksheet)**

Parameter	**Variable Type**
Altitude	Geomorphology
Aspect	Geomorphology
Slope	Geomorphology
Soil depth	Geomorphology
potential depth	Hydrology
Wetland Volume	Hydrology
Weighted Hydroregime	Hydrology
%_Silt	Soil physical
%_Sand****	Soil physical
Bulk Density	Soil physical
pH(KCl)	Soil chemical
Resistance	Soil chemical
Exchangeable cations of hydrogen$^+$	Soil chemical
Plant available phosphorus	Soil chemical
Plant available potassium ***	Soil chemical
Exchangeable cations of sodium	Soil chemical
Exchangeable cations of calcium	Soil chemical
Exchangeable cations of magnesium	Soil chemical
Total nitrogen %	Soil chemical
Total carbon %	Soil chemical
Cation exchange capacity	Soil chemical
Water soluble sodium	Soil chemical
Water soluble potassium	Soil chemical
Water soluble calcium	Soil chemical
Water soluble magnesium	Soil chemical
Buffer Width	Anthropogenic disturbance
Cumulative disturbance score	Anthropogenic disturbance
Vegetation Utilization	Anthropogenic disturbance
Mean Temperature	Climate
Maximum Temperature	Climate
Minimum Temperature	Climate
Evaporation**	Climate
Rainfall	Climate
Roughness of wetland vegetation	Vegetation

****Percentage of sand in the sediments is 97%inversely collinear with the percentage of clay

***Plant available potassium is 97% collinear with exchangeable cations of potassium

**Evaporation is 98% collinear with evapotranspiration

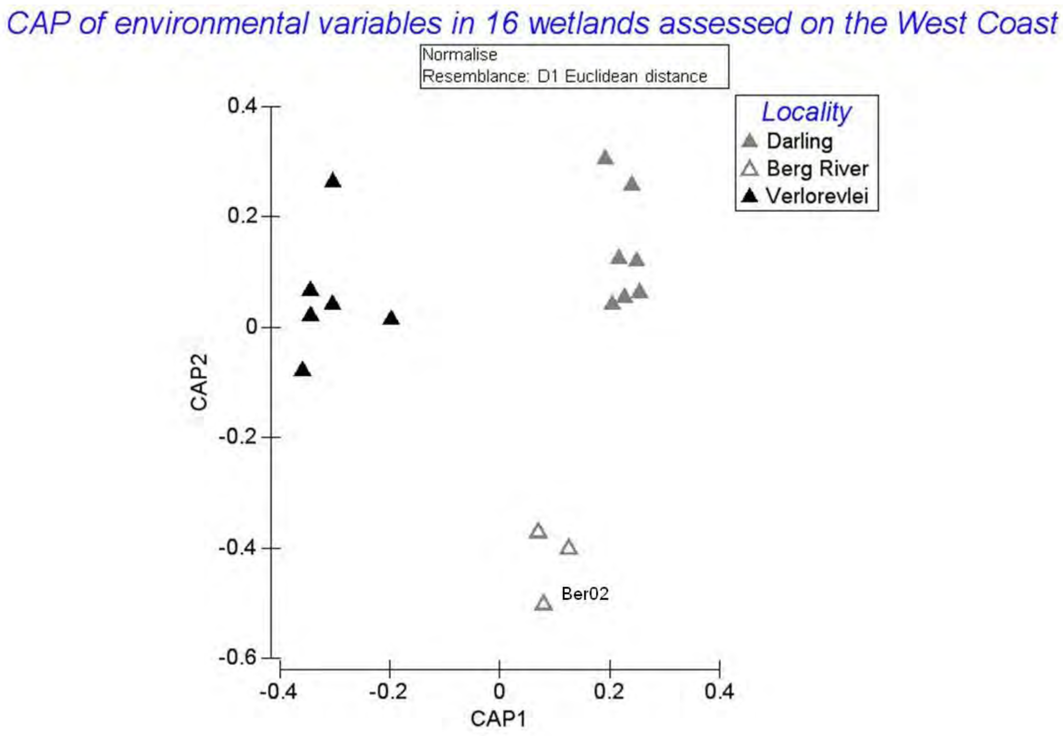


Figure 4.12: Canonical Analysis of Principal coordinates of the environmental parameters per wetland on the West Coast. Correlation with primary axis of separation is 98.8% and 91% with the secondary axis ($\delta_1 = 0.988$, $\delta_2 = 0.91$ and $\delta_1^2 = 0.98$, $\delta_2^2 = 0.83$).

from the other two Berg River wetlands when the analysis included more environmental parameters than just salinity. It is possible that other environmental variables/determinants that were not measured in this project may help explain the differences in vegetation assemblage portrayed in Figure 4.9.

4.6.2. Spatial units of homogenous environment on the Cape Flats

On the Cape Flats, significant difference was apparent between the environmental parameters of the different localities (Kenilworth, Lotus and Driftsands: tr(Q_m'HQ_m)=1.89, p=0.001; with no misallocation: Figure 4.13). Comparison between the ordination of vegetation assemblages of Cape Flats wetlands in Figure 4.10 and their environmental parameters in Figure 4.13 reveals similar distribution pattern. Thus, the vegetation assemblages have strong affinity for environmental parameters, suggesting these parameters have a controlling influence on species distribution and assemblage.

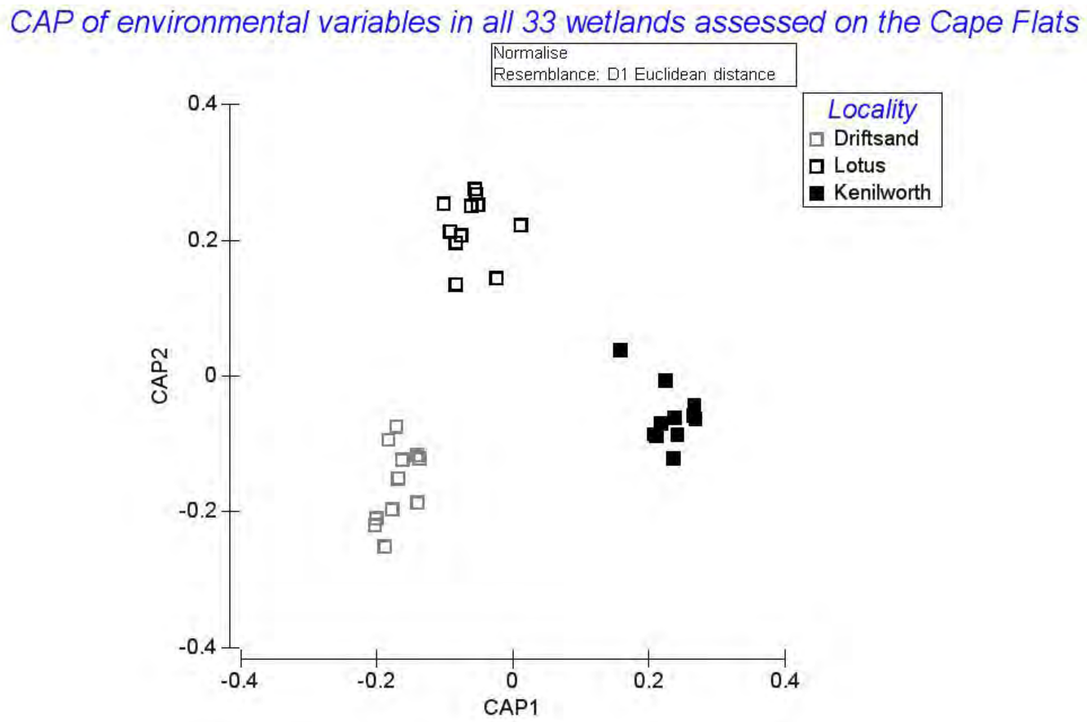


Figure 4.13: Canonical Analysis of Principal coordinates of the environmental parameters per wetland on the Cape Flats. Correlation with primary axis of separation is 98.6% and 95.9% with the secondary axis (δ_1 = 0.986, δ_2= 0.959 and δ_1^2 = 0.97, δ_2^2= 0.92).

4.6.3. Spatial units of homogenous environment in the Overberg

In the lowlands of the Overberg, significant differences were apparent between the environmental parameters of the Hermanus and Agulhas wetlands (tr(Q_m'HQ_m)=0.98, p<0.01, with no misclassified wetlands: Figure 4.14). Comparison of the ordination of wetland vegetation assemblages in Figure 4.11 with the wetland environmental parameters in Figure 4.14 reveals a similar pattern, suggesting affinity of the vegetation for environmental parameters.

4.6.4. Conclusions as to the affinity between vegetation assemblages and environmental parameters

Biogeographical differences between the vegetation assemblages of the wetlands in each location of the Western Coastal Slopes region of the Cape coastal lowlands mirror the environmental differences between locations. Whittaker's (1962) premise, that vegetation

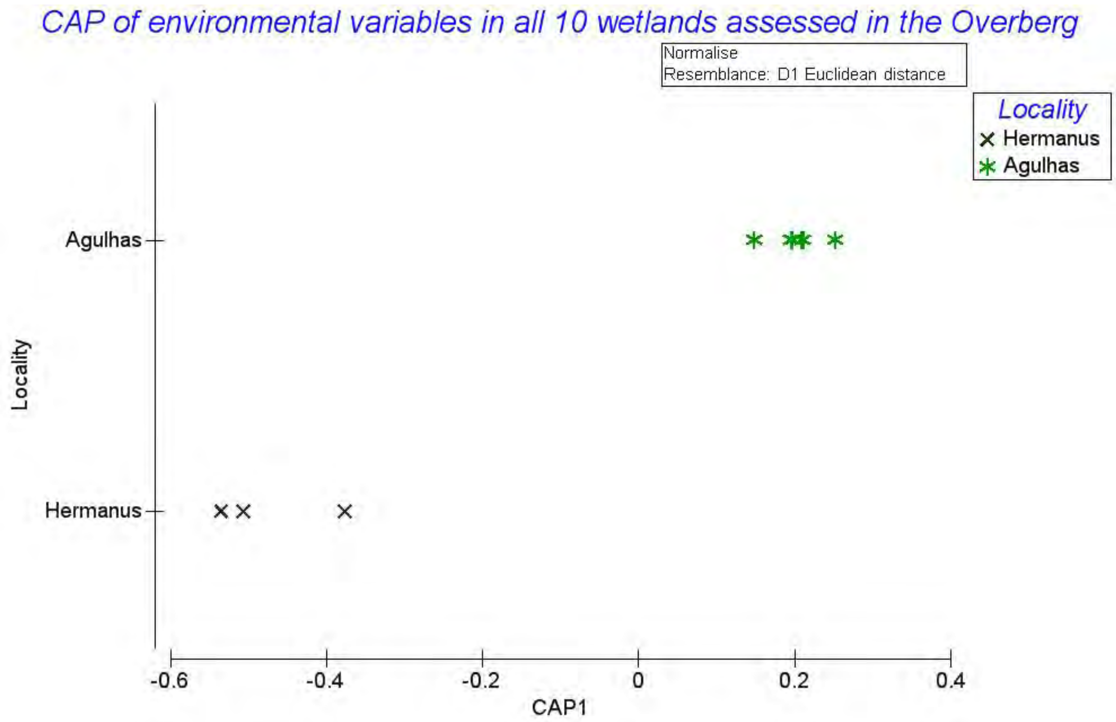


Figure 4.14: Canonical Analysis of Principal coordinates of the environmental parameters per wetland in the Overberg. Correlation with primary axis of separation is 98.98% (δ_1 = 0.988 and δ_1^2 = 0.98).

has affinity to particular environmental conditions, holds true for the vegetation of palustrine wetlands assessed in these Cape coastal lowlands. Wetland vegetation is considered by Mucina *et al.* (2006a) to be azonal, being controlled by the hydrological regime and/or the related concentrations of salts created by levels of waterlogging. These factors result in vegetation assemblages that deviate from the typical surrounding zonal or dryland vegetation in which macroclimatic factors, particularly rainfall, influence salt (base) concentration by leaching (See Section 2.10.1). The influence of the hydrological regime was assumed by (Mucina *et al.* 2006a). to "*exert an overriding influence on floristic composition, structure and dynamics over macroclimate*" leading to the classification of all Cape Lowland Freshwater (AZf1) wetlands as a single vegetation unit (AZf1) with a (sub)cosmopolitan character. It is apparent from the present study, however, that the lowland freshwater wetlands in the Cape coastal lowlands **do not** form a homogenous unit of vegetation. The differences that the present study has shown to exist between wetland habitat and vegetation assemblages of different localities within the Cape coastal lowlands is an important finding that should influence how wetlands in this area are managed and conserved. For the purposes of developing phyto-assessment tools, therefore, within the Cape Lowland Freshwater vegetation unit, different wetland vegetation habitats must be determined by a framework based on HGM types, soil types and macroclimatic parameters (i.e. rainfall and temperatures).

4.7. Vegetation of different HGM types – Testing hypothesis 4

> Hypothesis 4: Within an area (Western Coastal Slope wetland region (SWm), sub-regions or locations) with uniform vegetation, different wetland HGM types are expected to be significantly different in terms of vegetation.

Hydrogeomorphic (HGM) units differ in terms of their water-holding capacity, which depends on geomorphology (Brinson 1993). Wetlands of different HGMs can therefore be expected to provide different habitats for plants. The differences in vegetation assemblages between localities, as evidenced by the above analyses, suggest that every locality has different vegetation. Whilst the wetlands sampled in the localities of the Cape Flats were dominated by depressions (32/33), other HGM types were sampled in the Overberg and West Coast. There were, however, insufficient sample replicates in all of the sub-regions and at all of the localities, to determine whether different HGM types can contain different vegetation within one location. No formal testing of hypothesis four, that the vegetation of different HGM types is significantly different, was therefore possible.

Although no stringent statistical testing is feasible, some general conclusions can be made. From the data available in the present study, based upon the “important taxa” recorded to occur in each of the wetland vegetation units listed by Mucina *et al.* (2006a), it is apparent that HGM types sampled in the present study support distinct vegetation units (*sensu* Mucina *et al.* 2006a) (Appendix 7). Important or dominant and characteristic species of the Cape Lowland Freshwater vegetation were associated with depressions. Important Vernal pool species were also associated with the edge (supralittoral zone) of many depressions mostly with seasonal and shallow depressions or micro-depressions that do not exceed 10 cm in maximum inundation depth. Floodplain HGMs frequently support important Cape Lowland Freshwater taxa, but more often support important taxa associated with the Cape Lowland

Alluvial vegetation type. Important taxa of the Cape Inland Salt Pan vegetation and Cape Estuarine Salt Marsh were also associated with floodplain HGM's in the present data set. The three hill-slope seeps sampled in Hermanus held important taxa of the Cape Lowland Freshwater vegetation unit as well as endemic Fynbos Freshwater Wetland species, typically associated with shrub dominated ericaceous vegetation units (Section 2.9.1.1). Overall, there is some broad overlap of habitat among HGM types. In particular the supra-littoral zones of wetlands present similar vegetation habitat. Being infrequently inundated for any length of time and, in the Mediterranean climate of the Cape, being more often susceptible to seasonal dehydration, these supralittoral zones represent similar habitats.

4.8. Human disturbance and assemblage composition – testing Hypothesis 5.

> Hypothesis 5: At the broad biogeographic scale (region or sub-regions), the impact of human disturbance on vegetation assemblages will have a homogenizing effect, reducing the differences between naturally distinct spatial units of wetland vegetation.

Human disturbance has been shown to have a homogenizing effect on wetland species diversity (US EPA 2002c). Decreased species diversity is brought about as disturbance-tolerant plants come to dominate disturbed sites, outcompeting intolerant taxa. The proximity of the eutrophic Cape Flats depression Lot06 to West Coast depressions in the ordination in Figure 4.2 is a good example of this effect. No CLF depressions sampled on the West Coast were considered to be in a Reference or Moderate environmental condition (See hierarchical structure of wetlands in Table 4.2) and none in the Overberg were considered to be in a Worst condition. It was therefore not possible to test whether there was greater gamma diversity between un-impacted, relative to impacted, depressions across all of the different sub-regions. Significant gamma diversity is apparent between the relatively un-impacted CLF depressions of the Cape Flats and Overberg (*pseudo*-$F_{3,174}=6.7$, p=0.038). *A posteriori* pair-wise tests between these un-impacted depressions suggest significant species turnover or gamma diversity between the Lotus and Kenilworth localities, both on the Cape Flats, and between Kenilworth and the Agulhas Plain locality of the Overberg (Table 4.12).

Table 4.12: Jaccard distance-based test for homogeneity of multivariate dispersion between the species composition of individual vegetation samples of Reference and Moderately disturbed CLF depressions in wetlands of the localities within the Cape Flats and Overberg sub-regions. Significance is marked *

pseudo-*F*: 7.73 df1: 3 df2: 174 P (permutational): 0.002*			
PAIR-WISE COMPARISONS			
Localities compared	**t**	**P(perm)**	
(Kenilworth vs. Agulhas)	3. 57	0.001*	
(Lotus vs. Kenilworth)	3.398	0.02*	
MEANS AND STANDARD ERRORS			
Localities (Sub-region)	**No. of Samples**	**Average**	**Standard Error**
Kenilworth (Cape Flats)	39	60.5	1.35
Lotus (Cape Flats)	57	64.9	0.555
Agulhas (Overberg)	35	66.1	0.682

Between the impacted (Worst) CLF depressions of the Cape Flats and West Coast, considerable gamma diversity is apparent between locations (*pseudo*-$F_{4,81}$=4.3, p=0.05) (Table 4.13).

Table 4.13: Jaccard distance-based test for homogeneity of multivariate dispersion between the species composition of individual vegetation samples of Worst disturbed CLF depressions of the localities within the Cape Flats and West Coast sub-regions. Significance is marked *

pseudo-*F*: 4.34 df1: 4 df2: 81 P(permutational): 0.05			
PAIR-WISE COMPARISONS			
Localities compared	**t**	**P(perm)**	
(Kenilworth vs. Darling)	2.28	0.05*	
(Lotus vs. Darling)	2.34	0.05*	
(Lotus vs. Kenilworth)	0.37	0.705	
MEANS AND STANDARD ERRORS			
Localities (Sub-region)	**No. of Samples**	**Average**	**Standard Error**
Kenilworth (Cape Flats)	15	59.69	1.48
Lotus (Cape Flats)	34	60.6	1.497
Darling (West Coast)	15	54.78	1.57

On the Cape Flats, impacted (Worst) and un-impacted (Reference and Moderate) depressions were sampled in both the Kenilworth and Lotus CLF depressions. Between CLF depression samples from these two localities, there is considerable gamma diversity between un-impacted (Reference and Moderate) sites (t=3.39, p=0.002) but none between impacted (Worst) sites (t=0.37, p=0.705) (See both Tables 4.11 and 4.12). Thus, at least for these Cape Flats wetlands, the homogenizing effect of anthropogenic disturbance is apparent as it reduces the diversity or number of species unique to each of these localities. This has important implications for the conservation of wetlands, suggesting that human disturbances will decrease biodiversity differences between localities or sub-regions that have been shown above to naturally hold different vegetation (Section 4.5).

4.9. Discussion

The analyses in this chapter sought to determine what phyto-geographical regions in the Cape coastal lowlands have naturally similar wetland floras. Significant gamma diversity differences were shown between the sub-regions in terms of the Cape lowland Freshwater vegetation within depressional wetlands (Section 4.2). Consequently, the Western Coastal Slope wetland region (Cowan 1995) does **not** represent a spatial unit with homogenous wetland vegetation for CLF depressions (Section 4.2). Using either CLF depressions or the aggregate of habitat combinations and including all impacted wetlands and un-impacted wetlands, the Western Coastal Slope region and the Cape Lowland Freshwater vegetation unit (AZf1: Mucina *et al.* 2006a) cannot be considered to hold a homogenous set of wetland vegetation, and cannot be considered to be azonal.

It is possible that with a greater number of representative CLF depression wetland/vegetation samples from the Overberg and the West Coast, less overall difference would be apparent between these sub-regions. The analysis techniques that were employed, however, are not invalidated by unequal or small sample size (Clarke and Warwick 2001, Anderson *et al.* 2008).

Despite disturbance to some of the wetlands, analyses revealed considerable differences in vegetation assemblages in the aggregate of HGM-vegetation habitats, both between sub-regions and within them (i.e. between localities) (Section 4.4). This finding suggests that it would be useful to deal separately with each sub-region and locality of the Cape coastal lowlands when ascertaining whether plant assemblages of different disturbance categories have different vegetation assemblages. The considerable differences between the vegetation assemblages, as associated with different environmental parameters at each locality and in each sub-region, emphasizes the environmental heterogeneity within the Cape coastal lowlands. Such environmental differences as were shown to exist in Section 4.5 are also apparent in the classification of numerous dryland vegetation types, in and around each of the localities assessed in this study (Rebelo *et al.* 2006 and Appendix 7). Geological and climatic differences within this region, driving the expression of soil nutrient and salt (base) concentrations, have thus been shown to result in different sets of wetland plant communities, as they do in dry lands. This has important implications for the conservation and management of wetlands in this area and also suggests that these differences need to be taken into account when developing phyto-assessment tools. The homogenizing affect of human disturbances on vegetation distribution, briefly examined in Section 4.7, reduces the magnitude of difference in vegetation composition between sub-regions and therefore can be considered to have a negative impact on freshwater wetland vegetation diversity Anthropogenic impacts did not, however, make a marked difference to the association of species for localities or sub-regions as evidenced by the fact that wetlands of all categories of disturbance are included in all of the ordinations that show difference between sub-regions or localities (Figures 4.3, 4.4, 4.8, 4.9, 4.10 and 4.11). The spatial cohesion of vegetation assemblages to localities and associated environmental parameters that were apparent in the CAP analyses within sub-regions (Figures 4.8 to 4.13) suggests that there is potential for further phyto-sociological classification of wetland vegetation units within the Cape Lowland Freshwater wetland vegetation unit (AZf1) of Mucina *et al.* (2006a). Such phyto-sociological classification is, however, beyond the scope of the present study.

The South Western Mediterranean coastal slopes region (SWm: Cowan 1995) has been shown to hold at least three distinct sub-regions of wetland flora: on the West Coast, on the Cape Flats and in the Overberg. The south-western coastal belt ecoregion (Kleynhans *et al.* 2005) incorporates the West Coast and Cape Flats sub-regions. The southern coastal belt ecoregion (Kleynhans *et al.* 2005) incorporates the Agulhas and Hermanus wetland contingents of the Overberg sub-region which have also been shown to hold different vegetation albeit in different HGM types. These two areas are only the western edge of this latter ecoregion and the likelihood is that considerable variation (heterogeneity) will be apparent between more distant localities of the ecoregion. Development of a single phyto-assessment index of environmental condition for palustrine wetlands of these ecoregions, or for the Cape coastal lowlands is unlikely to be feasible. It will be necessary to search within each of the sub-regions and potentially at an even smaller spatial scale within the localities in order to determine ecologically sound assessment metrics.

The spatial cohesion of particular species and vegetation assemblages for locations was shown to be determined by the natural environmental parameters determining species distribution in each locality (Section 4.5). Within South Africa, regions with greater spatial homogeneity of environmental parameters that drive species distribution, than apparent in the present study, will require less subdivision for the purposes of phyto-assessment development. For instance, the environmental parameters and resultant vegetation diversity across the western Free State are considered to be more homogenous than is known to be typical across the Capensis and Drakensbergensis areas of high vegetation endemism (Van Wyk and Smith 2001).

5. HUMAN DISTURBANCE AND TROPHIC STATE

Before comparison of the vegetation assemblages of wetlands from different disturbance categories can be performed, it is first necessary to determine whether the categorization of wetlands in terms of different levels of human disturbance is an accurate reflection of environmental condition. In the present chapter, an examination of the nutrient concentrations of the soil and water column of the wetland is undertaken in order to determine whether they corroborate the Human Disturbance Scores.

This chapter fulfils the objective (4.ii in Section 1.3) of facilitating an *a priori* categorization of wetlands within the study set as Reference, Moderate or Worst in terms of their environmental condition. This *a priori* categorization was based on:

1. An assessment of the cumulative impact of human activities and land-uses within, and surrounding wetlands, as calculated by a Human Disturbance Score (HDS), which was used to rank and categorize wetlands; and
2. Measurement of nutrient concentrations in the soil and water-column in order to assign trophic state, which in turn was used to corroborate the HDS-determined categories of environmental condition.

It is hypothesized that measurements of the nutrient levels in each wetland may reveal eutrophic and therefore impacted states in wetlands that might otherwise (based on land-use alone) be allocated to a Reference or Moderate HDS category. There is extensive evidence that increased human disturbance decreases the environmental condition of wetlands (for a review see Adamus *et al.* 2001). Increased nutrient load in wetlands is one component of the full complement of anthropogenic stressors known to impact wetland environmental condition (Section 2.4.2). Of course, elevated levels of nutrients do not always accompany other forms of human disturbance as some anthropogenic stressors do not necessarily result in raised nutrient concentrations. Elevated water depth, prolonged saturation periods and increased vegetation harvesting/utilization are examples of stressors that may in fact reduce nutrient concentrations in wetlands.

Nutrient concentrations and related trophic states provide independent and quantitative measures of anthropogenic impacts to wetland environmental condition. Trophic state (oligo- meso- and eutrophic) relates to the concentrations of nutrients, in both the soil and water-column, which are known to influence biological productivity (e.g. Keddy 2000, Cronk and Fennessy 2001, Stevens *et al.* 2010) and to be affected by anthropogenic influence (for reviews see for example Coetzee 1995, Chorus and Bartram 1999, US EPA 2002a, Batchelor *et al.* 2002, Malan and Day 2005b). In the international context, nutrient-scarce environments result in wetlands that are nutrient limited under natural, un-impacted conditions (e.g. Keddy 2000). The Cape coastal lowlands (SWm) of South Africa are typified, under natural conditions, by nutrient scarcity in terrestrial and riverine environments (Milewski 1982, Stock and Allsop 1992, Brown *et al.* 1996). The natural nutrient status of wetlands in the Cape coastal lowlands is less well documented (Malan and Day 2005b). In the South African context, anthropogenic disturbance has been shown to result in elevated levels of nutrients in the water-column (Coetzee 1995; Malan and Day 2005b; Sections 2.4 and 2.8.5 of this volume).

Nutrient concentrations allow the allocation of wetlands to trophic state categories that are assumed to reflect the impacts of human disturbance (See Section 5.1.2). Ranking the level of human disturbance and nutrient concentrations thus facilitates a two-pronged approach to the categorization of wetlands based on environmental condition. The categorization of trophic states assists in improving the accuracy of categorization of environmental condition for each wetland before comparison is made between the vegetation assemblages. In this chapter, trophic states were thus compared to the HDS categories for each wetland to determine if there were any obvious discrepancies

5.1. Human disturbance score and environmental condition

Within the present study, the degree of human disturbance in each wetland was assessed, as described in Section 3.5.4, by generation of HDS scores, which were then used to rank wetlands from least to worst disturbed. In keeping with the recommendation of Malan and Day (2005b), that in the absence of sufficient ecological understanding the graduation of ecological condition between relatively natural and highly impacted wetlands is difficult to determine, only three broad ecological categories of environmental condition were recognized:

1. Largely natural or **Reference** conditions;
2. Moderately impacted or **Moderate**; and
3. Highly modified or **Worst** environmental condition.

5.1.1. Human Disturbance Score

A bar graph of the HDS for the sampled wetlands revealed no obvious separations that suggested category boundaries between Reference, Moderate and Worst disturbed wetlands (Figure 5.1). The boundaries between groups were decided upon by slight breaks in numerical sequence of scores (i.e. using expert judgement). The data set was divided into three categories, consisting of:

- seventeen **Reference** wetlands with HDS scores ≤75 and ranging from 17 to 73;
- twenty four wetlands with a **Moderate** degree of human impact and with scores ranging from 77 to 109;
- the nineteen **Worst** disturbed wetlands with HDS scores ≥110 and ranging from 112 to 251.

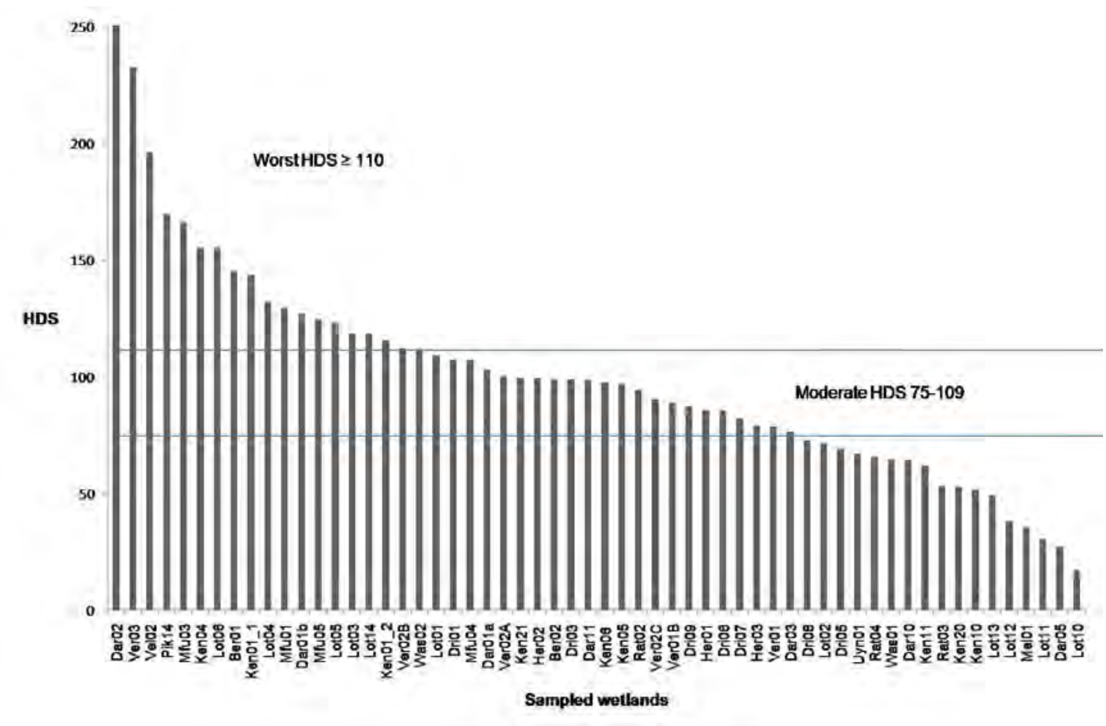


Figure 5.1: Human Disturbance Scores (HDS) for each of the study wetlands

Best professional judgement of the relative degree of human impact on environmental condition was used to categorize those wetlands that fell close to the upper and lower limits of the Moderate category.

5.1.2. Trophic State

A table of nutrient concentrations in the soil and the water-column, HDS categories and other site characteristics of each wetland is presented in Appendix 9.

It is recognised that typically more nutrients are bound up in wetland sediment than in most terrestrial systems (Mitsch and Gosselink 2007). Wetlands are frequently coupled to adjacent ecosystems through chemical exchange, facilitated by ground- and surface-water movements, which significantly affect both systems. Endorheic wetlands, due to the influx of nutrients and pollutants in sediments, and due to their primary means of water loss being evaporation, are susceptible to concentration of nutrients. In rivers, the lotic hydroregime results in the constant removal of suspended and dissolved nutrients. Flood events in rivers flush nutrient-bearing sediment from upper reaches, depositing it in downstream reaches with lower energy. River waters can therefore generally be expected to hold lower nutrient concentrations than wetlands (particularly endorheic and floodplain wetlands), that accumulate sediments. Norms for nutrients of importance in the water-column of wetlands are presented below (Table 5.1a). These values are presented as a baseline from which it will be necessary to develop norms that are more applicable to South African wetlands of different regions.

Table 5.1a: Norms for water-column nutrient concentrations in wetlands with a range of environmental conditions as measured in South Africa (µg $P.L^{-1}$ and µg $N.L^{-1}$).

Nutrient	South African Wetlands (n=99)[1)]	WHI wetlands(n=97) [2)]
P	85	169
N	681	674

1) Malan and Day 2005b

2) Bird 2010

Studies of soil nutrient concentrations in the Fynbos Biome, predominantly focusing on dryland vegetation but also including winter saturated soils, have revealed considerable variations, depending on underlying geology and soil type (Witkowski and Mitchell 1987). Plant-available phosphorus concentrations and total nitrogen content have been reported in a number of studies (Stock and Lewis 1986a and Witkowski and Mitchell 1987) focused on different locations in the Fynbos Biome (Table 5.1b).

Table 5.1b: Plant-available (resin extractable) phosphorus concentrations and total nitrogen levels for soils of the Fynbos Biome.

Nutrient	Strandveld Calcareous sand	Renosterveld Shale	Lowland Fynbos non-calcareous sands
mg $P.kg^{-1}$	300	160	31
Organic matter (%)	2.4	7.8	1.8
pH	7.6	5.8	4.8
g $N.m^{-2}$	-	-	27

Witkowski and Mitchell 1987

Stock and Lewis 1986a

The South African Target Water Quality Range (TWQR see Table 2.2 and 2.6) values for water-column nutrient concentrations in aquatic ecosystems were derived primarily from river, not wetland, data (DWAF 1996 and 2002). A review (Malan and Day 2005b) found that these TWQR values are not appropriate for wetlands.

In the absence of accurate TWQRs appropriate for inland wetlands and in the absence of soil nutrient norms, cluster analyses and ordination were used to in the present study to estimate potentially appropriate boundaries between trophic levels for each nutrient assessed. For each nutrient, outliers with extremely high concentrations were sought that would suggest nutrient enrichment (eutrophic or hyper-eutrophic states) and thus impacted environmental states. Breaks in numerical sequence of concentrations are very apparent in ordination and dendrograms. For a given nutrient, such breaks result in separate clusters (in this case of wetlands), each with a distinct concentration range. Obvious outliers, with high (or low) concentrations relative to those of all the other wetlands within the sample sets, were thus identified. These separate clusters and outliers were taken as suggestive of different trophic states. High- *vs.* low- concentration outliers were, respectively, considered indicative of impacted or eutrophic, *vs.* natural or oligotrophic, states. Wetlands with high nutrient concentrations were used as an indication of un-natural and therefore anthropogenic impact that suggested a Moderate to Worst category of environmental condition.

5.2. Results of water-column nutrient concentrations in wetlands

Comparison of the HDS scores with water-column phosphorus (SRP) and nitrogen (TIN) concentrations did not reveal any significant levels of correlation for the 33 wetlands in which these nutrients were measured (Pearson's *r* (31) = [HDS *vs.* SRP = 0.165] and [HDS *vs.* TIN = 0.239], p > 0.1 and Figure 5.2).

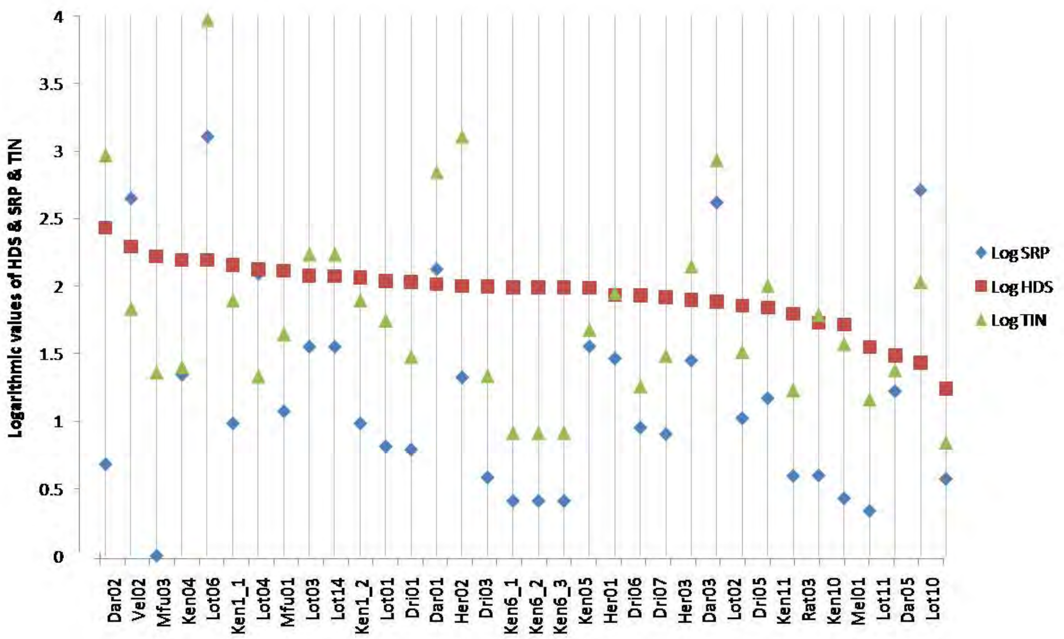


Figure 5.2: The variability of concentrations of total inorganic nitrogen (TIN) and soluble reactive phosphorus (SRP) relative to the cumulative amount of human disturbance (HDS) is evident. Log HDS ranked from Worst to Least disturbed *vs.* log[TIN] and log[SRP]

5.2.1. Soluble Reactive Phosphorus

Soluble Reactive Phosphorus (SRP) will be used in this document only to refer to water-column phosphorus concentrations. The present macrophyte study and the invertebrate assessment (Bird 2010) of the Wetland Health and Integrity Programme (WHI) provide data on SRP for a considerable number of palustrine and endorheic wetlands (n=98). A dendrogram (not shown) of the SRP values of these 98 palustrine wetlands revealed three apparently hyper-eutrophic outliers: Lot06 (1277 µg.L^{-1}), Lot07 (1407 µg.L^{-1}) and Dar04 (2827 µg.L^{-1}). The value of 2827 µg.L^{-1} SRP is above the maximum concentration for which the analytical method antimony-phospho-molybdate is known to be accurate. Nevertheless, the value is probably close to the actual concentration (H. Waldron, Oceanography Dept, University of Cape Town, pers. comm. 2010). The SRP data were not normally distributed and a log-transformation was therefore performed on the data before clustering them (Figure 5.3). A significant split is apparent, with 61 of the 98 wetlands assessed having concentrations of less than 25 µg.L^{-1} (as represented by 'A' in Figure 5.3). Further significant divisions [1] are depicted in Figure 5.3, with SRP concentrations from 25 to 250 µg.L^{-1}, and greater than 250 µg.L^{-1}. Ordination of the same data (not shown) also reveals a significant split or break in the number sequence, this time between wetlands with more or less than 250 µg.L^{-1} of SRP. The three groups of SRP concentrations were designated "oligotrophic," "mesotrophic" and "eutrophic" as shown in Fig. 5.3.

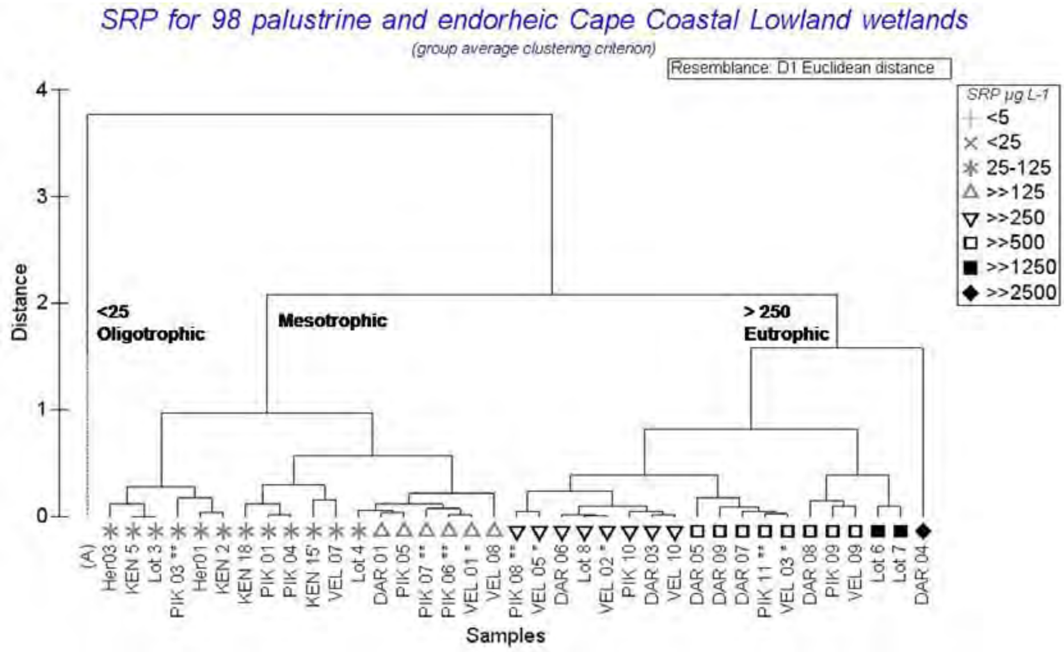


Figure 5.3: Dendrogram, using group average clustering criterion, of the log-transformed SRP concentration in 98 palustrine and endorheic wetlands in the Cape Coastal Lowlands. Three distinct clusters are apparent. "(A)" represents 61 wetlands with the lowest SRP concentrations, considered to be oligotrophic. Mesotrophic and eutrophic clusters and a potentially hyper-eutrophic outlier (Dar04) are indicated.

Nutrient concentrations of inland wetlands of South Africa are known to be the result of many factors including the influence of hydrology and geology (HGM type) as well as climate, topography and season of measurement (Malan and Day 2005b). Sub-regional differences in water-column nutrient concentration were therefore examined in the WHI data set (Bird 2010). Soluble reactive phosphorus levels on the West Coast were on average the highest and those in the Overberg were the lowest (Table 5.2). The SRP value for each individual wetland is presented in Table 2 in Appendix 9.

Table 5.2: Soluble reactive phosphorus: mean, median and maximum concentration (µg P L^{-1}) for wetlands from the different sub-regions in the macrophyte (present study) and invertebrate (Bird 2010) WHI studies.

	West Coast		Cape Flats		Overberg	
No. of wetlands	32		38		27	
Median	247		7		4	
Average	391		98		9	
Maximum including outliers (excluding outliers)	2827	(999)	1407	(440)	44	(44)
Standard Error	93		50		4	
No. eutrophic wetlands:>125 µg.L^{-1} (according to DWAF 2002 values)	22		3		0	
No. eutrophic wetlands:>250 µg.L^{-1} (according to DWAF 1996 values)	16		3		0	

If the three hyper-eutrophic outliers (Lot 06, Lot07 and Dar04) noted previously are omitted from the analysis, there were still significantly different ranges of SRP in different sub-regions ($F_{2,92}$=58.6, p<0.001) as identified by permutational analysis of dispersion (PERMDISP: Anderson 2006). Most of the difference was between the range in West Coast relative to Overberg (t=8.3, p<0.001) and West Coast versus Cape Flats (t=7.8, p<0.001) as is evident from Table 5.2 above. The Overberg and Cape Flats had relatively similar ranges. With uncertainty about the nutrient load of SRP that represents different trophic states for inland wetlands it makes sense to compare the wetland SRP concentrations within the context of the range of values obtained within each sub-region, rather than comparing values between sub-regions. This also facilitates a search for outliers (suggestive of eutrophication) from the range of values measured within each sub-region.

5.2.1.1. West Coast

SRP concentrations suggest that wetlands on the West Coast are eutrophic or in “poor condition” relative to the riverine TWQR (DWAF 1996 and DWAF 2002: Table 5.3). Less than a third of all sampled West Coast wetlands were considered to be in a Reference condition as determined by HDS, with considerable agricultural impact widely evident. A dendrogram (Figure 5.4) of the West Coast wetland SRP concentrations (Bird 2010) revealed wetland Dar04 (2827 µg.L^{-1}) to be a hyper-eutrophic outlier. Eight other wetlands had SRP concentrations greater than 500 µg.L^{-1}, suggestive of eutrophic conditions. There was, however, a lack of obvious anthropogenic impacts likely to cause elevated SRP concentration in some of these eutrophic wetlands, all of which were within 300 meters distance of each other (Dar05=511 µg.L^{-1}, Dar07=615 µg.L^{-1} and Dar08=816 µg.L^{-1}). The only visible impact to these wetlands was a sparse (less than 15% total area cover) infestation of alien legumes (*Acacia saligna*), which had recently been cut down. Phosphorus content of the litter layer beneath an infestation of another alien acacia, *A. cyclops* was shown to be greater (1 050 µg.kg^{-1}) than that of litter under indigenous Fynbos vegetation (360 µg.kg^{-1}) (Witkowski and Mitchell 1987). Wetland Dar05, which was the only one of these West Coast wetlands to be assessed in the macrophyte survey, had a soil phosphorus (Bray No.2) concentration of 1050 µg.kg^{-1}. Wetlands Dar07 and Dar08, had soil phosphorus concentrations of 31 000 µg.kg^{-1} and 1000 µg.kg^{-1} respectively. The elevated water-column SRP concentrations may therefore have been a result of the alien vegetation; although the extremely high soil P concentration at Dar07 is perhaps more difficult to explain. column SRP and soil P concentrations (Pearson correlation: *r* (31) = 0.093, p > 0.1). Different soil types and the associated mineral and soil colloids associated with these soils

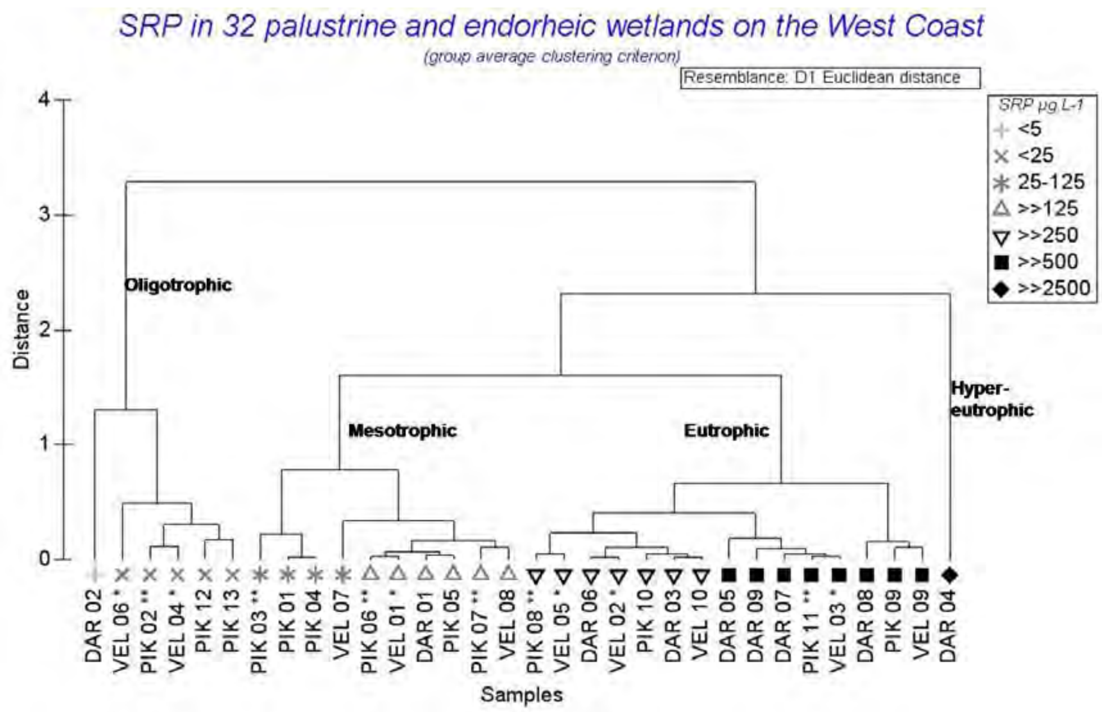


Figure 5.4: Dendrogram, using group average clustering criterion, of the resemblance of Soluble Reactive Phosphorus concentrations for 32 palustrine wetlands from the West Coast (After Bird 2010).

Wetlands Dar01, 02 and 03 all had higher levels of Bray No.2 P than Dar05 but lower levels of water-column SRP. In this data set there is no consistent correlation between water may explain some of the discrepancies between soil P and water-column SRP in different localities. The three Darling wetlands (Dar01, 2 and 3) noted above, are situated in Swartland Granite Renosterveld vegetation whereas, Dar04, 5, 7 and 8 are in Hopefield Sand Fynbos (*sensu* Rebelo *et al.* 2006). As the nutrient concentrations of Renosterveld soils are generally held to be higher than those of Fynbos soils (e.g. Stock and Allsopp 1992, Rebelo *et al.* 2006), the higher levels of soil P in Dar01, Dar02 and Dar03 are understandable.

SRP values for wetlands in the Darling locality (n=8) ranged from 4 to 2827 $\mu g.L^{-1}$. Wetland Dar04 (2827 $\mu g.L^{-1}$) is a hyper-eutrophic outlier, while Dar02, with only 4 $\mu g.L^{-1}$, represents a nutrient-scarce outlier. Despite the very low SRP concentration, Dar02 was categorised as being Worst disturbed by the HDS, being a shallow (±1.5 meters) road gravel quarry, and thus characterized by extreme levels of geophysical disturbance and vegetation removal.

When compared to the 31 other wetlands assessed on the West Coast, the SRP concentration of the endorheic wetland Dar05 (511 $\mu g.L^{-1}$) is more than two standard errors higher than the median value, suggesting a eutrophic condition relative to other wetlands in the West Coast set (Appendix 9). In the macrophyte study, Dar05 was categorized as having a Reference environmental condition but, SRP concentration was 30 times higher than that of the next wetland in the Reference group. This SRP concentration is high relative to the median of previously assessed “un-impacted” endorheic (20$\mu g.L^{-1}$) and palustrine (160$\mu g.L^{-1}$) wetlands in South Africa (Malan and Day 2005b). For the purposes of the present macrophyte study, wetland Dar05 was downgraded from a Reference to a Moderately impaired environmental condition (HDS) due to the high SRP concentration.

In the group categorized as having Moderate human disturbance for the wetlands assessed for macrophytes, the two wetlands with highest SRP concentration were also both from Darling (Dar03=413 $\mu g.L^{-1}$ and Dar01=132 $\mu g.L^{-1}$). The dendrograms of all 98 wetlands (Figure 5.3), and of West Coast wetlands (Figure 5.4), suggest that Dar01 is best categorized as mesotrophic and Dar03 as eutrophic. As the concentration of SRP in Dar03 is not particularly high, downgrading it to the “Worst” category seemed unjustifiable and it was retained in the “Moderate” category.

5.2.1.2. Cape Flats

On the Cape Flats, wetlands Lot06 (1277 µg.L^{-1}), Lot07 (1407 µg.L^{-1}) and Lot08 (440 µg.L^{-1}) represent outliers in terms of SRP concentrations. Thus excluding these sites, the maximum SRP is below 125 µg.L^{-1} (Figure 5.5). Of these outliers only wetland Lot06 was surveyed in the present macrophyte study and was accorded the Worst category of disturbance by the HDS. No further discrepancies from the assigned HDS categories were apparent in the Cape Flats wetlands based on SRP concentrations.

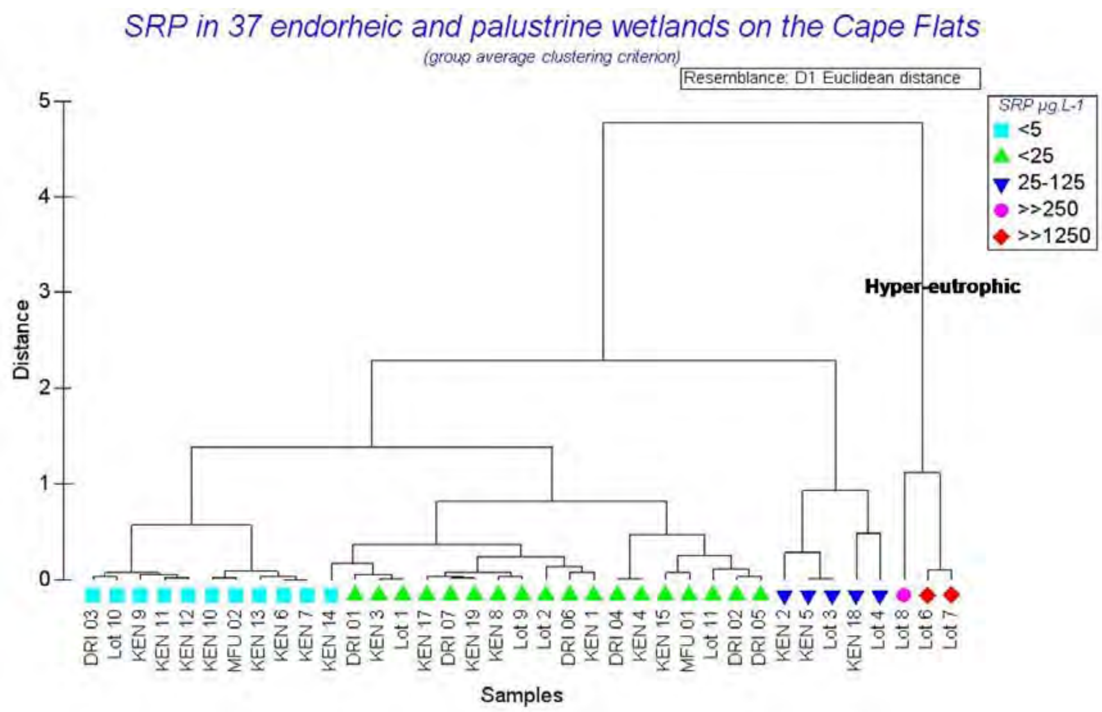


Figure 5.5: Dendrogram, using group average clustering, of the resemblance of Soluble Reactive Phosphorus concentrations for 37 endorheic and palustrine wetlands from the Cape Flats (After Bird 2010).

5.2.1.3. Overberg

In the Overberg, no wetlands had SRP levels that were considered to be outliers from the general range of measurements. The three Hermanus wetlands, Her01 (44 µg.L^{-1}), Her02 (24 µg.L^{-1}) and Her03 (39 µg.L^{-1}), had the highest SRP concentration. As hillslope seeps, these wetlands have a continual, but slow flow of groundwater. They are therefore unlikely to accumulate nutrients in the manner of endorheic wetlands. Unlike rivers, however, hillslope seeps are not flushed of sediment or nutrients by seasonal flood events or even by regular surface water flow. A natural build-up of SRP is therefore possible with the concentration dependent on local geology. The acidic soils of this Hermanus locality support fynbos vegetation which is recognised as being nutrient scarce. The SRP concentration in these Hermanus wetlands is thus perhaps suggestive of Moderate levels of impact and Mesotrophic conditions. No discrepancies from the HDS categories of disturbance were apparent.

5.2.1.4. Summary of findings for Soluble Reactive Phosphorus

Higher SRP concentrations were apparent in the West Coast relative to Cape Flats and Overberg wetlands. Ordination of the SRP data, for the 98 wetlands in which SRP was sampled revealed a considerable break between wetlands with more, or less than 250 µg.L^{-1}. For palustrine and endorheic wetlands within these two sub-regions of the Western Cape, SRP of 250 µg.L^{-1} represents a concentration level above which wetlands could potentially be considered impacted and to have eutrophic concentration. A concentration boundary of less than 25 µg.L^{-1} was also apparent from the dendrogram (Fig 5.3). These boundaries suggested by the WHI data, as well as those previously proposed for aquatic ecosystems, are presented below (Table 5.3).

Table 5.3: Benchmark soluble reactive phosphorus concentrations as trophic state category boundaries for inland aquatic systems of South Africa, compared to boundaries suggested by the present WHI study as appropriate for palustrine and endorheic wetlands of the West Coast and Cape Flats sub-regions of the Cape Coastal Lowlands** (values in µg P.L^{-1}).

DWAF 1996	**DWAF 2002**	**Malan and Day 2005b*** **	**WHI – this study**
Derived for "aquatic ecosystems" (mainly rivers)	**Derived for rivers**	**Derived for wetlands**	**Derived for wetlands**
SRP (ortho-phosphate or PO_4^{-3})			
Oligo**-** ≤ 5	Natural ≤ 5	80	Oligo**-** < 25
	Good 5-25		
Meso**-** 5-25	Fair 25-125		Meso**-** 25-250
Eutro**-** 25-250	Poor > 125		Eutro**-** >250

**The Overberg represents a sub-region in which the DWAF 1996 boundaries are perhaps most accurate.

***'Best Guess' default water quality objective for wetlands

The generally higher SRP concentrations observed in wetlands from the West Coast, relative to the other sub-regions, are perhaps explained by the fact that there were few un-impacted wetlands measured in this sub-region. The HDS-determined Reference West Coast wetlands discussed above (Dar05, 07 and 08) did have impacts that may have resulted in anthropogenic elevation of SRP concentrations. Other than the apparently hyper-eutrophic Cape Flats wetlands Lot06 and Lot07, and the eutrophic Lot08, the levels of SRP concentration attained in "Worst disturbed wetlands" in other sub-regions were predominantly far lower than those attained on the West Coast. These results suggest that the SRP concentrations that represent trophic boundaries are potentially sub-region specific.

Within the macrophyte data set, other than the extremely eutrophic and industrially polluted Cape Flats wetland Lot06 (1277 $\mu g.L^{-1}$) and the relatively eutrophic, agriculturally impacted West Coast wetland Vel02 (444 $\mu g.L^{-1}$), no other "Worst" disturbed (HDS) wetlands had eutrophic SRP levels (i.e. >250 $\mu g.L^{-1}$Figures 5.4 and .5.5). West Coast wetland Dar05, with minimal visible anthropogenic impacts, was originally categorized as being in a Reference (HDS) condition, yet had a eutrophic SRP concentration (511 $\mu g.L^{-1}$). The categorization of wetlands as determined by HDS, was closely correlated with environmental condition as given by SRP concentration, in 17 out of 33 wetlands (45%). In a further 15 of these 33 wetlands, the HDS reflected a higher level of disturbance than SRP concentration would suggest. This is likely to be a consequence of land-use impacts that affected the HDS, but did not give rise to nutrient-enrichment. Wetland Dar05 was considered to be eutrophic relative to other wetlands on the West Coast, warranting a change from Reference HDS category to, at the least, a Moderate category of human disturbance.

5.2.2. Total Inorganic Nitrogen

Ammonium is often the greatest fraction of total inorganic nitrogen (TIN) in a habitat with low levels of dissolved oxygen (DO). Increased ammonium concentrations have been reported in impacted wetlands (Malan and Day 2005b). Total inorganic nitrogen is known to vary seasonally as a result of changing concentrations of DO in the water (Maltby *et al.* 1994). The riverine TWQR for TIN (DWAF 1996 and 2002: Table 5.5) were considered by Malan and Day (2005b) to require further refinement before being suitable for application to South African wetlands.

A total of 97 wetlands were surveyed for TIN in the WHI invertebrate study (Bird 2010) and thirty-three of them were assessed in this current macrophyte survey. The nutrient load of each of the "macrophyte wetlands" was therefore assessed within the wider context of endorheic and palustrine wetlands assessed in the WHI in order to assist with the categorization of trophic state. A dendrogram (not shown) of the TIN concentration of these 97 WHI wetlands revealed two obvious outliers: wetlands Pik10 and Lot06, both of which are hyper-eutrophic, with TIN concentrations greater than 9000 µg.L^{-1}. Even without these hyper-eutrophic outliers, the distribution of the TIN data was not normal and the values were therefore log-transformed. After transformation, it was apparent that wetlands Agu12 and Agu22, both of which had extremely low TIN values (4 µg.L^{-1}), were outliers and were excluded from further analyses. Considering all 97 wetlands, the TIN levels on the West Coast were on average the highest and those in the Overberg sub-region were the lowest (Table 5.4). The TIN data for each of the 97 wetlands are presented in Table 2 of Appendix 9.

Table 5.4: Median, mean and maximum values for Total Inorganic Nitrogen (µg N L-1), for wetlands from the different sub-regions in the macrophyte (present study) and invertebrate study (Bird 2010)

	West Coast	**Cape Flats**	**Overberg**
No. of wetlands	32	38	27
Median	772	16	26
Average	1598	315	82
Maximum (excluding outliers)	9280 (5160)	9329 (842)	1269 (1269)
Standard Error	281	245	39
No. eutrophic wetlands: 250-10000 µg.L^{-1} (according to DWAF 1996 values)	8	1	0
No. eutrophic wetlands: >4000 µg.L^{-1} (according to DWAF 2002 values)	4	1	0

The TIN concentration range differed for each sub-region ($F_{2,90}$=10.1, p<0.001) as identified with permutational analysis of dispersion (PERMDISP). Most of this difference is between the wetlands of the West Coast and the Cape Flats (t=4.1, p< 0.001) and West Coast versus Overberg (t=3.8, p<0.001). These differences are partly the result of the greater number of wetlands on the West Coast that were Worst disturbed, relative to those on the Cape Flats and in the Overberg, and also partly to the greater magnitude of disturbance in West Coast wetlands.

A dendrogram of the 95 wetlands, without the extremely low outliers and after log-transformation is shown in Figure 5.6. This dendrogram reveals clusters with boundary separations similar to the riverine TWQR trophic categories (DWAF 1996 and 2002: Table 5.5). In the WHI wetlands a number of breaks were apparent in the TIN concentration data that separate wetlands that are potentially oligotrophic with less than 600µg.L^{-1}[TIN], mesotrophic between 600 and 2000µg.L^{-1}[TIN] and greater concentrations reflecting eutrophic conditions (Figure 5.6 and Table 5.5).

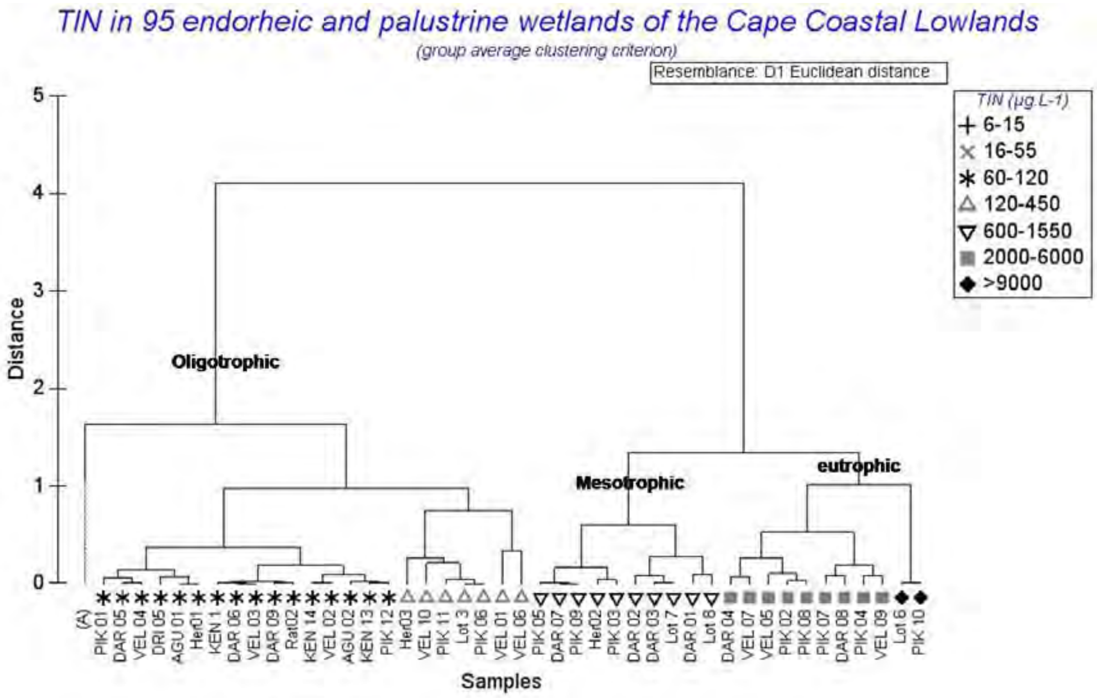


Figure 5.6: Dendrogram, using group average clustering, of the total inorganic nitrogen concentration of palustrine and endorheic wetlands in the Cape Coastal Lowlands. "(A)" represents wetlands with lowest TIN concentration and thus potentially oligotrophic conditions.

Of the wetlands in which macrophytes were sampled, within the HDS-determined Reference category, wetland Dar05 had the highest TIN concentration (106 µg.L^{-1}), well within the limit of what is considered to be oligotrophic, or natural for rivers in this region (DWAF 1996 or 2002). This value of 106 µg.L^{-1} was less than a tenth of the median value of all sampled wetlands in the Darling locality (n=8) or on the West Coast (n=32), confirming oligotrophic TIN concentration. All other wetlands in the Reference group of wetlands assessed for macrophytes, had lower TIN concentration, and could therefore be considered to represent appropriately oligotrophic conditions.

For the Moderate HDS category, Her02 represented the maximum TIN value (1 269 µg.L^{-1}) at almost 50 times greater than the median of all wetlands assessed for TIN in the Overberg (n=27). TIN concentration in the other two wetlands assessed in the Overberg-Hermanus locality, Her01 (88 µg.L^{-1}) and Her03 (139 µg.L^{-1}), represented 3 and 5 times the median TIN concentration for wetlands in the Overberg area (Table 5.4). The high TIN value for wetland Her02 suggested that this wetland should represent a different HDS category of disturbance than wetlands Her01 and Her03. All of these wetlands were in close proximity and had similar HDS. After Her02 the next highest TIN concentration in the Moderate HDS category for macrophytes were in Dar03 (851 µg.L^{-1}) and Dar01 (693 µg.L^{-1}). Dar03 represented the median TIN value for the West Coast Darling locality and was a little higher than the median for the West Coast (Table 5.5). These two wetlands (Dar01 and 03) thus appeared to be suitably classified as "Moderately disturbed" by the HDS.

Table 5.5: Iterative reviews of the TWQR for total inorganic nitrogen concentrations as trophic state category boundaries for riverine ecosystems of South Africa. Best guess default for wetlands based on a review of the TWQR (Malan and Day 2005b) and concentration boundaries suggested by the WHI study as appropriate for palustrine and endorheic wetlands of the West Coast and Cape Flats sub-regions of the Cape Coastal Lowlands** (values in µg.L^{-1}).

DWAF 1996	**DWAF 2002**	**Malan and Day 2005b*** **	**WHI – this study**
Derived for "aquatic ecosystems" (mainly rivers)	**Derived for rivers**	**Derived for wetlands**	**Derived for wetlands**
TIN ($NH_3 + NH_4^+ + NO_2^- + NO_3^{-2}$)		**TIN ($NH_4^+ + NO_2^- + NO_3^{-2}$)**	
	Natural ≤ 250	800	
Oligo- ≤ 500	Good 250-1000		Oligo- < 600
Meso- 500-2500	Fair 1000-4000		Meso- 600-2000
Eutro- 2500-10000	Poor > 4000		Eutro- >2000

**The Overberg represents a sub-region in which these boundaries are all potentially too high.

***'Best Guess' default water quality objective for inland wetlands

Within the macrophyte wetlands of the "Worst HDS category", only the extremely impacted outlier, wetland Lot06 (9329 µg.L^{-1}) exhibited a eutrophic concentration (*sensu* aquatic ecosystem nutrient boundaries suggested in Table 5.5) and was the only wetland assessed in the macrophyte survey to do so. The next highest TIN concentration in the macrophyte wetlands with Worst HDS category was Lot03 (170 µg.L^{-1}) exhibiting an oligotrophic TIN value.

5.2.2.1. Summary of findings for Total Inorganic Nitrogen

Wetlands on the West Coast exhibited significantly different TIN concentration from the wetlands of the Cape Flats or the Overberg (Table 5.4). The Overberg represents an area in which the riverine TWQR for TIN concentration (DWAF 1996 and 2002) are perhaps too high, with most wetlands exhibiting apparently oligotrophic status despite the fact that some of them (n=5) are subject to agricultural and recreational land-uses that involved the use of fertilizers. Even the wetlands in the Overberg-Agulhas Plain that were most heavily impacted by agriculture had TIN concentrations <100 µg.L^{-1}, representing oligotrophic conditions (*sensu* Table 5.5). This raises the question as to whether the Overberg is an area in which the riverine TWQR (DWAF 1996 and 2002) and the WHI trophic TIN boundaries proposed by the present study are too high – i.e. not conservative enough.

For the wetlands assessed for macrophytes, the categorization of wetlands as determined by HDS suitably reflected the environmental condition relative to the exhibited TIN concentrations in 12 out of 34 wetlands (35%); in a further 21 of these 34 wetlands the HDS reflects a higher level of disturbance due to other stressors that do not influence nutrient levels. The riverine TWQRs for TIN (DWAF 1996 and 2002) were unsuitable discriminators between wetlands with different degrees of human disturbance, except in cases with extreme eutrophication relative to other wetlands in their immediate vicinity, such as described for wetlands Lot06 and Her02. Relative to other wetlands in the Overberg, the considerably higher TIN concentration in Her02 suggested this wetland be changed from Moderate to a Worst HDS category.

5.2.3. A summary of nutrient results from the water-column and their effects on disturbance categories

In summary, the West Coast represents a sub-region with far higher SRP and TIN concentrations in endorheic pans and palustrine wetlands than the Cape Flats sub-region; concentrations are generally even lower in the Overberg. The riverine TWQR for TIN

(DWAF1996 and 2002) appears to be too high to distinguish unimpacted, oligotrophic wetlands from impacted, mesotrophic or eutrophic wetlands in the Overberg. For SRP, concentrations greater than 250 µg.L^{-1} suggest eutrophic conditions in these palustrine and endorheic wetlands of the Cape Flats and West Coast (Table 5.3). The HDS predominantly determined suitable categories of environmental condition relative to these independent measures of water-column nutrient concentration and related trophic states. Outliers indicative of eutrophic conditions were apparent in HDS categorized Reference and Moderate wetlands Dar05 and Her02; which were therefore re-categorized as Moderate and Worst disturbed respectively.

5.3. Nutrients in wetland sediments

In wetlands where nutrients are scarce, the concentration, typically of phosphorus, and occasionally of nitrogen, is recognized as the limiting factor affecting plant growth and productivity (Smith *et al.* 1999, Adamus and Gonyaw 2000, Mack *et al.* 2000, Verpraskas and Faulkner 2001, Brock 2003), (see Section 2.8.5.1).

In the dryland conditions of the Cape Coastal Lowlands, soil nutrient concentrations have been shown to vary naturally between the soil types found at different locations (Milewski 1982, Mitchell *et al.* 1984, Witkowski and Mitchell 1987, Stock and Allsopp 1992). This creates conditions that result in broad differences in vegetation type on each soil (e.g. Stock and Allsopp 1992, Rebelo *et al.* 2006). Thus it is hypothesised that soil nutrient values that represent trophic boundaries appropriate for all these soil types may be not be attainable. An analysis of the variation of soil variables is therefore performed below (Section 5.3.1) in order to test this hypothesis.

5.3.1. Values of wetland sediment parameters

5.3.1.1. Sediment parameter variation among and within wetlands

An average value was calculated for each soil variable (e.g. particle size, nutrient concentrations, etc.; a list of variables is presented in Table 5.6) per wetland. These values were obtained by calculating the mean from all the measurements from a given wetland. The values varied considerably between sub-regions ($F_{2,57}$=9.8, p<0.0001) as identified with

PERMDISP. Constrained ordination of the wetland average of all sediment variables (variables listed in Table 5.6), using Canonical Analysis of Principle coordinates (CAP: Anderson and Willis 2003), successfully classified 81.36% of the wetlands as belonging to a specific locality (*sensu* localities in Table 4.2) and revealed significant difference in these variables between the different localities (tr:(Q_m'HQ_m)=3.8, p<0.0001), (Figure 5.7). The Berg River wetlands, most of the Darling wetlands and three of 11 Lotus wetlands were misclassified as the CAP grouped these wetlands as having characteristics of wetlands from a different locality. The misclassification of Darling wetlands is not surprising given the broad range of soil variable values in this locality, as is evident from their dispersion or spread in Figure 5.7. The apparent separation between values for sediments of the Driftsands area in this constrained ordination is a result of samples being taken in two specific areas along the Kuils River floodplain, namely the Driftsands and Mfuleni areas, both of which are labelled as Driftsands in Fig 5.7.

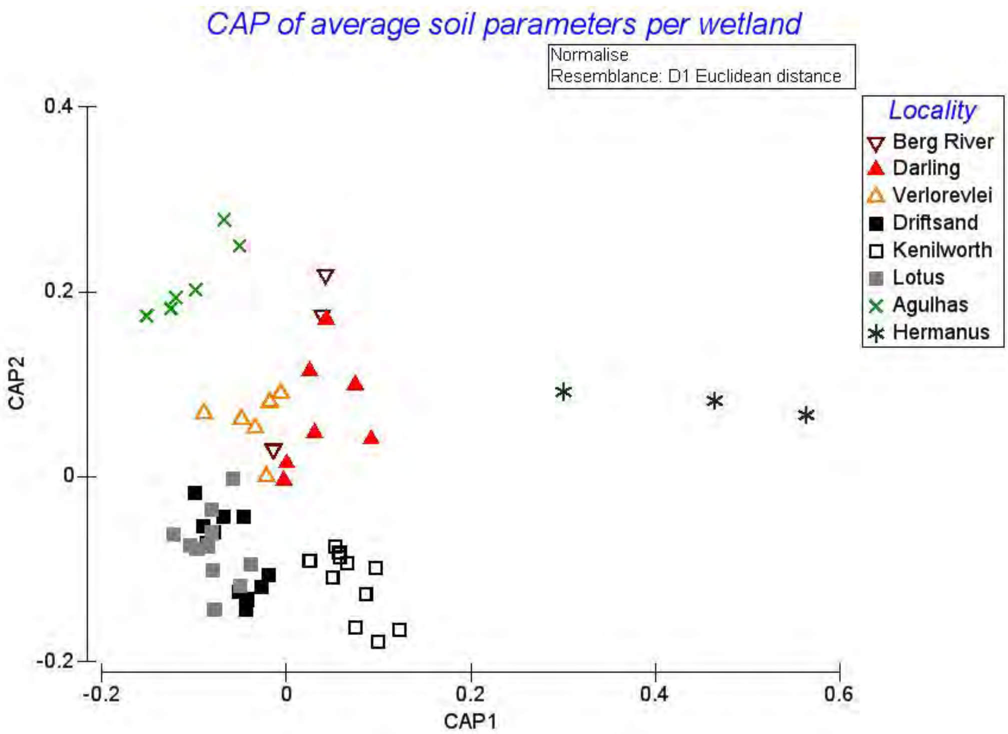


Figure 5.7: Canonical Analysis of Principal coordinates of average wetland sediment variables (as listed in Table 5.6) for each wetland in the different localities of the West Coast, Cape Flats and Overberg sub-regions. Axis CAP1 is correlated at 95.65% with the wetland average sediment variables (δ_1 = 0.9565 and δ_1^2 = 0.9149). Axis CAP2 is correlated at 93.69% (δ_2= 0.9369, δ_2^2= 0.8779). Triangles represent West Coast wetlands, squares are Cape Flats wetlands and crosses and stars are Overberg wetlands.

Differences between the soil characteristics of each locality (Darling, Berg River, Verlorevlei, etc.) are expressly recognized in the classification of dryland vegetation surrounding the wetlands sampled in the present study as each are associated with a given soil type and geology (Rebelo *et al.* 2006; See Appendix 7). For instance, wetlands in the Driftsands (including Mfuleni) locality are surrounded by Cape Flats Dune Strandveld, as are most of the wetlands in the Lotus locality (Section 4.5.2). However, south-western Lotus wetlands (Lot10 to 13) and all of those in the Kenilworth locality are surrounded by Cape Flats Sand Fynbos (Rebelo *et al.* 2006). From the CAP analysis, sediment nutrients of the wetlands assessed in the present study can be seen to vary considerably between localities, probably as a result of their association with different soil types. It is thus inappropriate to compare trophic boundaries between localities unless they have similar or the same soils.

In attempting to identify the effects of anthropogenic impacts on the trophic states of soil (i.e. elevated phosphorus or nitrogen due to fertilizer application), it is necessary to avoid confusion with any natural variation. As shown above, this exists between localities, and is known to exist between soil types associated with different vegetation types (Rebelo *et al.* 2006). The significant natural differences between localities, as shown in Fig 5.7, suggest it would be more informative to search for anomalies/outliers to natural concentration of nutrients within each locality. The innate heterogeneity of soil types further suggests that, within each locality, it may be better to search for anthropogenically-derived anomalies in soil nutrient concentration within Fynbos, Strandveld or Renosterveld vegetation types.

5.3.1.2. Trophic states identified from wetland nutrient values

Examination of average wetland values for soil phosphorus (P) revealed that wetlands Ber02 and Ken20 exhibited eutrophic soil condition despite HDS categorization as Moderate and Reference environmental condition respectively. This is shown in dendrogram Figure 5.8. All of the other wetlands with >25 $mg.kg^{-1}$ of phosphorus were already categorized as worst disturbed using the HDS (as depicted in Fig 5.8). Wetland Ber02 had marginally enriched and thus eutrophic levels of P relative to other soils in its immediate locality. Wetland Ken20 had considerably higher concentrations of P relative to all other wetlands assessed.

The wetland average soil nutrient concentrations are, however, not always accurate for categorization of the environmental condition for the vegetation assemblage of the whole wetland. For instance, only one of the two samples from wetland Ber02 exhibited eutrophic concentrations of P relative to other wetlands in its vicinity (Ber02_5: 90 mg.kg^{-1} vs. Ber02_3: 7 mg.kg^{-1}). Whilst examination of the average concentration did not mask this eutrophic condition, it does mask the state in different sections of the wetland. This could result in the incorrect association of vegetation in eutrophic soils with inappropriately averaged and apparently mesotrophic values. This would, in turn, prevent accurate validation of the HDS categories of disturbance. Thereby reducing, firstly, the ability to accurately compare environmental conditions to vegetation assemblages; and secondly, hindering the identification of useful metrics for phyto-assessment. Examination of the variability between the individual vegetation plot measures of all soil variables was therefore performed in the following section (5.3.2) to determine whether wetland average values were accurate as a representation of the nutrient concentration for the entire vegetation assemblage.

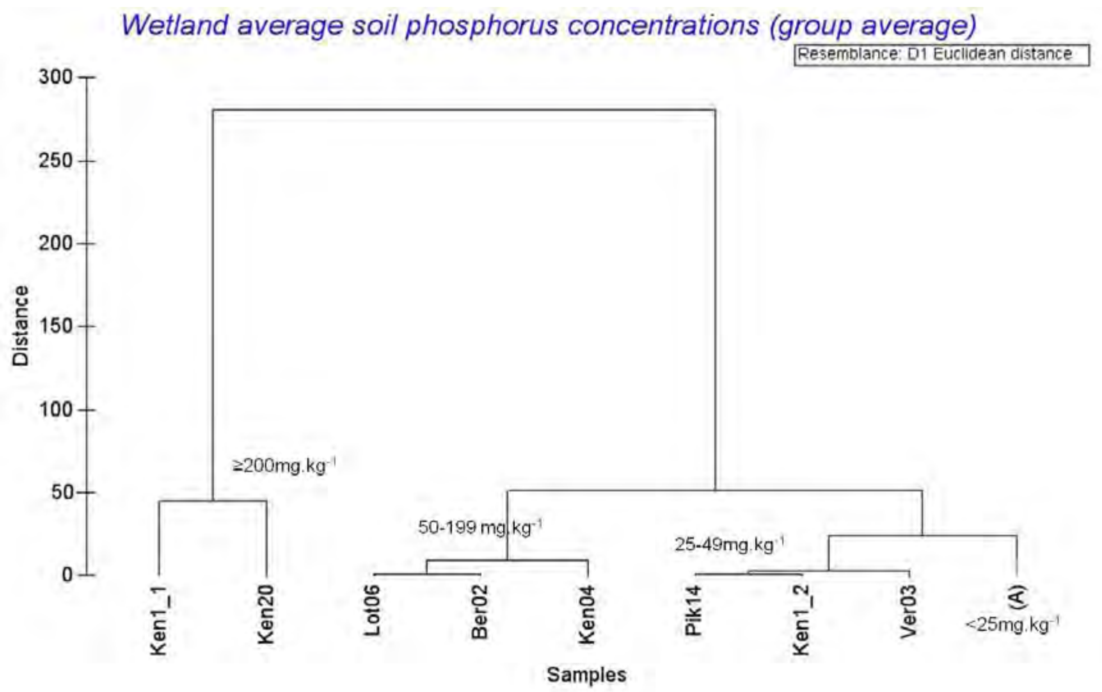


Figure 5.8: Dendrogram, using group average clustering, of the average phosphorus concentration of wetlands assessed in the SWm. "(A)" represents all of those wetlands with less than 25 mg.kg^{-1}, which, are not considered to represent eutrophic phosphorus concentrations.

5.3.2. Soil values for vegetation samples

Environmental variables that vary as much or more within wetlands than amongst them should be measured at the vegetation plot scale to determine their impact on plant species distribution or assemblage pattern within wetlands (Sections 2.8.5.4 and 3.5.6). For the development of accurate metrics for phyto-assessment, this fine spatial scale of examination is a potentially necessary expense.

To determine whether there was generally more variation within, than between wetlands, a test of the range of measurement of a given variable within (i.e. between each sediment sample from a given wetland) relative to between wetlands was performed. This test was performed using permutational analysis of dispersion, as based on spatial medians (PERMDISP: Anderson 2006: See Section 2.10.5), on the Euclidean-distance derived resemblance matrices for each individual variable (i.e. for water-soluble calcium, or for phosphorus only). In situations with considerable within-, relative to between-wetland variation, the test statistic is significant, suggesting heterogeneity of dispersion (Anderson 2006). The accuracy of the PERMDISP test for groups in which fewer than 5 samples have been measured is unknown (Anderson *et al.* 2008). Thus this test was initially limited to those wetlands with a minimum of five soil samples. It was apparent on re-analysis with the addition of those wetlands with at least four samples that significance levels were unaltered and in this way a greater number of the wetlands sampled in the current study were included in the tests for homogeneity of dispersion.

The analyses were performed within each sub-region, rather than across the whole SWm data set due to the differences that were shown in Section 5.3.1.1 to exist between sub-regions. For each variable, a Euclidean distance matrix was created. Normalization, standardizing the range of unit variation between variables, was unnecessary as each variable was analysed independently.

5.3.2.1. Soil nutrient variation within and between wetlands

On the Cape Flats, where the greatest number of wetlands with more than four or five soil samples were assessed, there is a considerable range of soil nutrient variability within wetlands (as demonstrated by the significant difference or heterogeneity of dispersion in Appendix 11). For the Cape Flats therefore, the values for environmental variables from individual vegetation plot soil samples would facilitate more accurate interpretation of plant response to nutrient enriching disturbances and environmental differences. In both the West Coast and the Overberg, relative to the Cape Flats, there was less difference in soil nutrient variation within, relative to between, wetlands. The percentages of soil nitrogen and carbon content were the only parameters for which wetland averages would be acceptable across all sub-regions. These variables showed insignificant variation between samples within wetlands of all sub-regions (Table 5.6).

These results suggested that measurement of soil nutrient concentrations should generally be done per vegetation plot when developing phyto-assessment metrics, at least for the wetlands in the Cape Coastal Lowlands. More accurate interpretation of the patterns of macrophyte species response to soil nutrients would therefore be obtained by comparison of data measured at the plot level rather than a measure per wetland from the average of a few samples. This is contrary to the recommendation of the Biological Assessment of Wetlands Working Group (BAWWG) (US EPA 2002c) (Section 2.8.5.4); yet supports the standard botanical opinion (Kent and Cocker 1992) and approaches used for development of autecological information. The experience of wetland ecologists and botanists working in South Africa also corroborates this considerable variation in many environmental parameters within wetlands (Sieben 2003, Low and Pond 2003, Collins 2005, Ractliffe and Corry 2008). Variation is perhaps to be expected in wetlands, due to the impacts of variable an-/aerobic conditions, resulting in oxidation or reduction reactions that cause fluctuations in nutrient availability between hydrological zones (Verpraskas and Faulkner 2001).

5.3.3. Soil nutrient trophic status of individual vegetation samples

Classification of trophic states and comparison to HDS categories was carried out for soil P and N within each sub-region, and each location, on the basis of nutrient concentrations measured within individual vegetation relevés.

5.3.3.1. Plant-available soil phosphorus

Plant-available phosphorus (P) is the inorganic and water-soluble fraction of phosphorus found in soils. In un-impacted soils at a neutral pH (6-7), more phosphorus is typically available to plants than in alkaline soils, in which insoluble phosphates can form (see Section

Table 5.6: Permutational analysis of dispersion of environmental variables measured at the vegetation sample (sample) scale between the spatial medians of wetlands as a grouping factor. Significant dispersion suggests greater variation of soil variables within rather than between wetlands. X = non-significant.

Variable	**West Coast**	**Cape Flats**	**Overberg**
Potential water depth	significant	significant	significant
Soil depth	significant	constant	no test, too few replicate samples
Aspect	x	significant	x
Slope	x	significant	significant
ph Field measurement	x	significant	significant
Soil Redox	x	significant	x
Soil Variables: laboratory analysed soil parameters sampled in each vegetation sample			
% Clay particles	significant	significant	significant
% Sand particles	x	significant	x
% Silt	x	significant	x
Bulk Density	x	x	no test, too few replicate samples
pH (KCl)	x	significant	x
Resistance	x	significant	x
Exchangeable cations of H+	significant	significant	significant
Exchangeable cations of Na+	significant	significant	x
Exchangeable cations of K+	x	significant	x
Exchangeable cations of Ca++	x	significant	x
Exchangeable cations of Mg++	x	significant	x
Phosphorus	x	significant	x
Potassium	x	significant	x
% Nitrogen	**x**	**x**	**x**
% Carbon	**x**	**x**	**x**
T-value	x	significant	x
Cation exchange capacity	x	x	no test, too few replicate samples
Na water soluble	significant	significant	no test, too few replicate samples
K water soluble	x	significant	no test, too few replicate samples
Ca water soluble	significant	significant	no test, too few replicate samples
Mg water soluble	significant	significant	no test, too few replicate samples

2.8.5.3). A concentration of between 0.1 and 0.2 $cmol_c$ $P.kg^{-1}$ (or <62 mg $P.kg^{-1}$) is considered typical for most natural soils in South Africa (Van Huysteun, Cornie pers. comm. 2010). Organic, insoluble-phosphorus is contained in the soil organic matter content (SOM). Phosphorus was significantly correlated with SOM content (Pearson r (284) = 0.247, $p < 0.05$) in the soil samples from all vegetation plots of this study; with variable but significant correlations in each sub-region:

- On the West Coast there was a significant correlation of soil P with SOM, r (46) = 0.65, $p < 0.01$.
- On the Cape Flats, without hyper-eutrophic outliers (described below), there was a significant correlation of soil P with SOM, r (203) = 0.37, $p < 0.01$.
- On the Overberg there was a significant correlation of soil P with SOM, r (31) = 0.44, $p < 0.01$.

There was no difference in the range of values for P concentration in combination with SOM content between sub-regions, as identified with PERMDISP. Similarly, no range or dispersion difference was apparent for the P concentration alone, but *posteriori* pair-wise analysis (Anderson *et al.* 2008) revealed significant difference between West Coast and Cape Flats P concentrations ($t = 2.5$, $p < 0.01$). This difference remained unchanged when the nutrient-poor and nutrient-enriched (eutrophic/hyper-eutrophic) outliers (in the resemblance matrix) were removed A search for samples with apparently anomalous and eutrophic concentration of P was therefore performed at the sub-regional scale to see if any wetlands that had been categorized (by the HDS) as being in a “Moderate” or “Reference” environmental condition included any eutrophic samples that would more appropriately reflect a “Worst” environmental condition.

Within sub-regions, examination of these values was further subdivided into examination within localities (e.g. on the West Coast between Berg River, Darling and Verlorevlei) and within units of Fynbos, Strandveld or Renosterveld associated wetlands (*sensu* Rebelo *et al.* 2006) as identified to be appropriate in Section 5.3.1.1.

5.3.3.1.1. West Coast

The average P and SOM content of sediment from the West Coast wetlands from each vegetation unit per locality are presented in Table 5.7. In general, higher P concentration was associated with higher SOM.

Table 5.7: Sediment phosphorus concentration (mg.kg^{-1}) and soil characteristics of West Coast wetlands. Values are averages of sediment measurements, ± standard error, from all wetlands associated by locality and upland vegetation unit.

Vegetation unit and locality	**n**	**Inorganic Phosphorus*** (mg.kg^{-1})**	**Organic Matter** (%)**	**pH**
Fynbos, Darling (Dar05)	2	10.5	0.38	6.9
Fynbos, Berg River (Ber02, Vel02)	3	33.3 ± 28.4	2.5 ± 1.5	8.03 ± 0.3
Fynbos, Verlorevlei	8	20.5 ± 5.9	2.8 ± 0.7	5.7 ± 0.5
Renosterveld, Darling	30	12.6 ± 1.7	1.7 ± 0.2	6.8 ± 0.2
Strandveld, Berg River (Ber01)	1	5	0.7	8
Strandveld, Verlorevlei (Ver01b)	2	4	1.24 ± 1	8.2 ± 0.6

***Bray No. 2 or Olsen depending on pH

**Multiply by 10 000 to convert to parts per million, which is equivalent to mg.kg^{-1}

Clustering and ordination of the P concentrations from individual vegetation samples (rather than the locality averages in the above table (5.7)) revealed two anomalous samples, Ber02_5 (90 mg.kg^{-1}) and Ver03_2 (56 mg.kg^{-1}). The rest of the samples grouped into three clusters, with soil P concentrations less than 11, 11 to 22 and 24 to 36 mg.kg^{-1} (Figure 5.9).

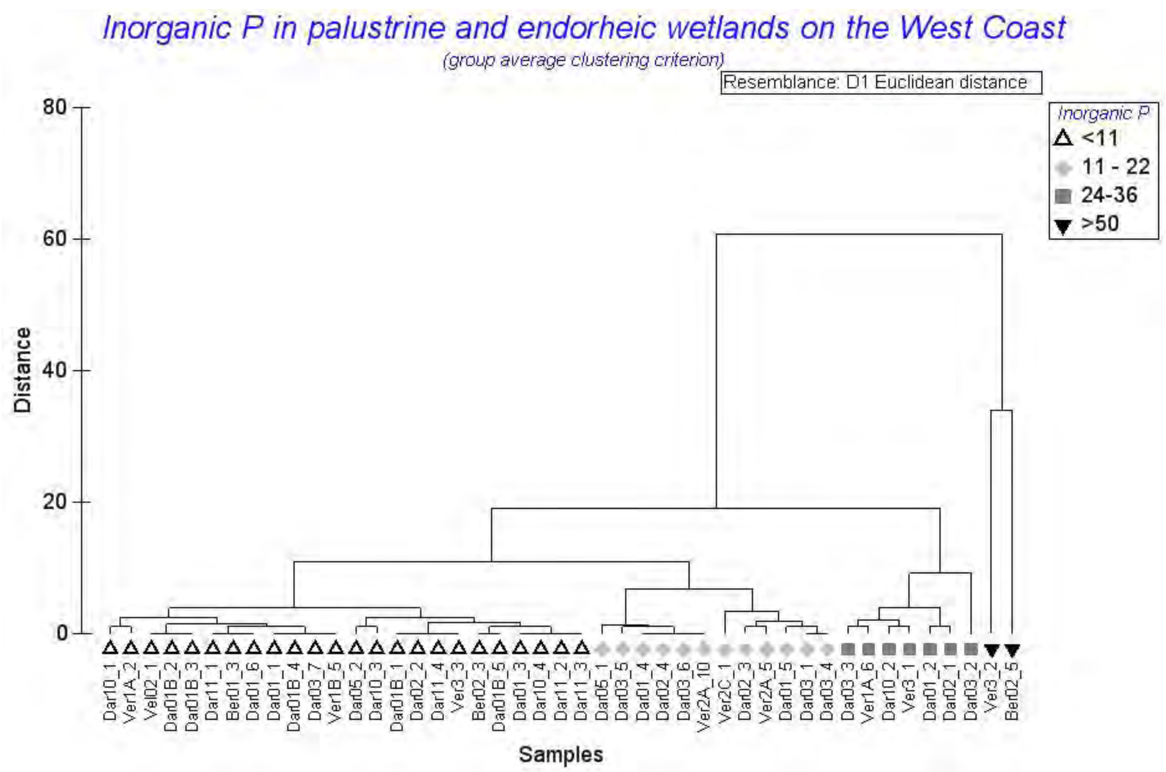


Figure 5.9: Dendrogram, using group average clustering, of inorganic phosphorus levels for sediment samples (in mg.kg^{-1}) from palustrine and endorheic wetlands on the West Coast.

Of importance for the current work is that the two outliers have anomalously high P values. The small number of samples from the West Coast makes it difficult to assess what trophic condition these outliers represent. Both of these samples were in wetlands surrounded by Fynbos vegetation. Previous research into P concentration in West Coast, Fynbos-associated winter-saturated or wetland soils suggests that the high P concentration in samples Ber02_5 and Ver03_2 perhaps represent mesotrophic to eutrophic conditions (Table 5.8).

Winter-waterlogged soils in Strandveld vegetation on the West Coast of the Cape Coastal lowlands have been shown to hold an order of magnitude more soluble phosphorus (68 ± 6 $mg.kg^{-1}$) than soils of lowland Fynbos vegetation (2.9 ± 0.7 $mg.kg^{-1}$) from the same locality (Melkbosstrand: Witkowski and Mitchell 1987: Table 5.8). The reverse was apparent in the wetland sediments from the Berg River and Verlorevlei localities assessed in the present study, with, greater P evident in Fynbos than Strandveld wetland sediments (compare Tables 5.7 *vs.* 5.8). Whether these discrepancies are the result of measurement of different soil types or sediments with different hydrological regimes is unclear from the present data.

Table 5.8: Characteristics and nutrients of soil types from the West Coast of the Cape Coastal Lowlands[1 and 2]. These measurements from winter-waterlogged sediment are associated with seasonal zones of saturation in wetlands (Collins 2005). Values are averages of measurements for that soil type ± standard error.

Vegetation unit, locality, soil type	Inorganic Phosphorus*** ($mg.kg^{-1}$)	Organic Matter** (%)	pH
[1]Fynbos, Pella, Lamotte	2.1 ± 0.1	3.4	4.5 ±0.1
[1]Fynbos, Pella, Westleigh	4.6 ± 0.2	2.2	4.8 ±0.1
[1]Fynbos, Pella, Longlands	3.6 ± 0.9	1.8	4.6 ±0.1
[2]Fynbos, Melkbosstrand, Fernwood	2.9 ± 0.7	2.4	5.2 ± 0.2
[2]Strandveld, Melkbosstrand, Fernwood	68 ± 6	1.4	6.6 ±0.1

After [1]Mitchell *et al.* 1984 and [2]Witkowski and Mitchell 1987

***Bray No.2

**Multiply by 10 000 to convert to parts per million which, is equivalent to $mg.kg^{-1}$

Wetlands Ber02 and Ver03 were classified as Moderate and Worst disturbed by the HDS. The potentially anthropogenically elevated P concentrations therefore support this designation, although Ber02 may better represent the Worst disturbed category. In general, the sediment phosphorus concentrations of individual samples on the West Coast do not suggest any difference from the HDS-determined categories of disturbance.

5.3.3.1.2. Cape Flats

In the Cape Flats wetland sediment samples, higher phosphorus concentrations were exhibited by Fynbos rather than Strandveld soils, even when the most eutrophic samples were excluded (Table 5.9).

Table 5.9: Soil characteristics and sediment phosphorus concentrations for Fynbos and Strandveld associated wetlands from different localities on the Cape Flats as sampled in the present study. Values are averages of measurements for the locality ± standard error.

Vegetation unit and locality	n	Inorganic Phosphorus*** ($mg.kg^{-1}$)	Organic Matter** (%)	pH
Fynbos, Kenilworth (no eutrophic)*	58	14.3 ± 2.4 excl. 6 eutrophic	2.37 ± 0.16	4.6 ± 0.11
Fynbos, Kenilworth (all)	64	64.4 ± 20.7 incl. 6 eutrophic	2.37 ± 0.16	4.7 ± 0.11
Strandveld, Kuils River	55	5.6 ± 0.8	2.21 ± 0.1	8.2 ±0.03
Strandveld, Lotus River	85	8.3 ± 1.8	2.35 ± 0.14	7.6 ± 0.1

***Bray No. 2 or Olsen depending on pH

**Multiply by 10 000 to convert to parts per million which, is equivalent to $mg.kg^{-1}$

* Note: All eutrophic samples were excluded from the analysis

A dendrogram (Figure 5.9) of the P concentrations of individual wetland vegetation samples revealed four main clusters with large differences in P concentration. A set of six samples were outliers from the rest of the Cape Flats soils. These six samples, contained in two wetlands of the Kenilworth locality, had five to ten (or more) times the average P concentration for the Kenilworth wetlands, suggesting hyper-eutrophic conditions. Across a distance of 100 metres, wetland Ken20 exhibited apparently natural P concentration ranging from 6 to 24 $mg.kg^{-1}$ in drier western samples and hyper-eutrophic concentrations of 640 to 720 $mg.kg^{-1}$ in three micro-depressions on the eastern side. To its immediate south, wetland Ken01_1 also had apparently hyper-eutrophic P concentrations in three samples (309, 367 and 541 $mg.kg^{-1}$).

The causes of elevation in Ken01, a waste-water-treatment wetland, are likely to be from effluent and cleaning products from the adjacent equestrian quarantine stables. The cause of higher concentrations of sediment P measured in Ken20_6/7/8, some 100 meters to the north were not apparent. Relative to previously measured Fynbos or Strandveld soil phosphorus concentrations of the Cape Coastal Lowlands, admittedly from the West Coast

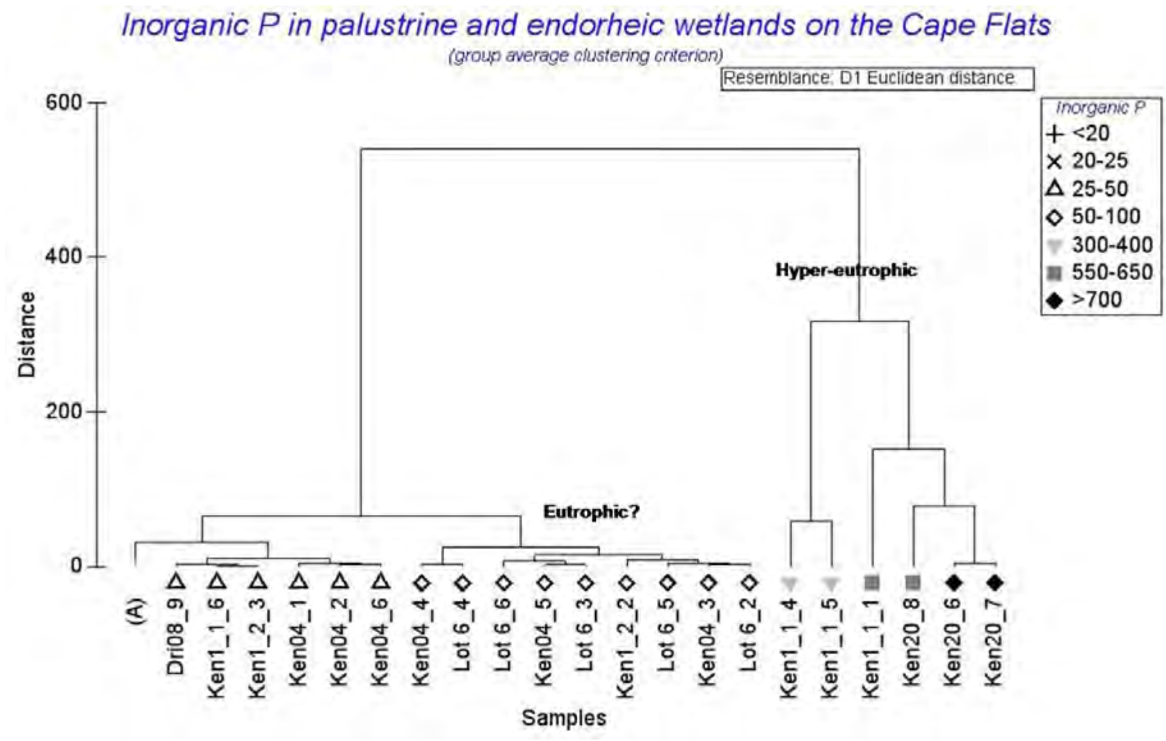


Figure 5.10: Dendrogram, using group average clustering, of the resemblance of the soil phosphorus concentration for Strandveld and Fynbos associated wetland vegetation samples of the Cape Flats. “(A)” represents 184 samples that had P concentration less than 25 $mg.kg^{-1}$ and were considered oligo- to mesotrophic.

(Table 5.9), these six Fynbos Kenilworth samples represent hyper-eutrophic conditions. Wetland Ken01_1 was categorized as having a Worst disturbed environmental condition by the HDS and so the eutrophic phosphorus concentrations do not alter this classification. Wetland Ken20 was, however, categorized as Reference by the HDS. The grouping of HDS Reference samples (Ken20_6/7/8) in the hyper-eutrophic cluster (Figure 5.10) suggested that these samples should be re-categorized as “Worst disturbed”. For purposes of comparison of the plant species assemblages of the different samples the three Ken20 samples with hyper-eutrophic P concentration were re-categorized as Worst disturbed. For the purpose of the wetland average analyses performed in Chapter 6, Ken20 was re-categorized as Moderate.

5.3.3.1.3. Overberg

In the lowlands of the Overberg, Hermanus wetland sediments had, on average, greater quantities of phosphorus and organic matter than wetland sediments on the Agulhas Plain (Table 5.10).

Table 5.10: Sediment phosphorus concentration and soil characteristics from Overberg wetland vegetation samples in the present study. Values are averages of measurements for the locality ± standard error.

Vegetation unit and locality	n	Inorganic Phosphorus*** (mg.kg^{-1})	Organic Matter** (%)	pH
Fynbos, Hermanus	25	17 ± 2.06	11.77 ± 1.84	3.8 ± 0.15
Fynbos, Agulhas Plain	6	2 ± 0.55	2 ± 0.57	7.1 ± 0.43
Renosterveld, Agulhas Plain	1	2	1.86	6.1

***Bray No. 2 or Olsen depending on pH
**Multiply by 10000 to convert to parts per million which, is equivalent to mg.kg^{-1}

A dendrogram of P in sediment from individual vegetation samples showed one set of outliers with considerably higher P concentration (Figure 5.11). These Overberg-Hermanus outliers had lower levels of P than were exhibited by outlying and eutrophic samples on the West Coast or Cape Flats. In this outlying group, whilst Her01_11 had the same or slightly lower P than Her02_14 and _15, it also had more than three times the SOM content, suggesting anthropogenic nutrient enrichment in samples Her02_14/15 but not in Her01_11 (Table 5.11).

The gradation of P concentration was closely associated with SOM. None of the samples appeared to represent eutrophic nutrient levels. It is possible that all of the Hermanus samples represented a mesotrophic concentration of P, since no samples from reference or “un-impacted” wetlands were collected in this locality. The HDS categorization of environmental condition in Hermanus was Moderate due to the presence of a golf course with associated impacts of fertilization, irrigation, channelization and drainage.

5.3.3.1.4. Summary: Plant available phosphorus

In contrast to previous studies in low-lying, winter-waterlogged soils (Table 5.8), Fynbos soil samples of the present study showed greater P concentration than Strandveld soils. On the West Coast and the Cape Flats, eutrophic and hyper-eutrophic soil phosphorus

Table 5.11: Sediment parameters for individual Overberg-Hermanus samples in outlying group from Figure 5.11. Ordered by decreasing ratio of P:SOM

Sample	Inorganic Phosphorus (mg.kg^{-1})	Organic Matter %	pH
Her02_15	41	7	3.8
Her02_14	37	10	4
Her02_6	34	14	4.9
Her01_11	37	34	3

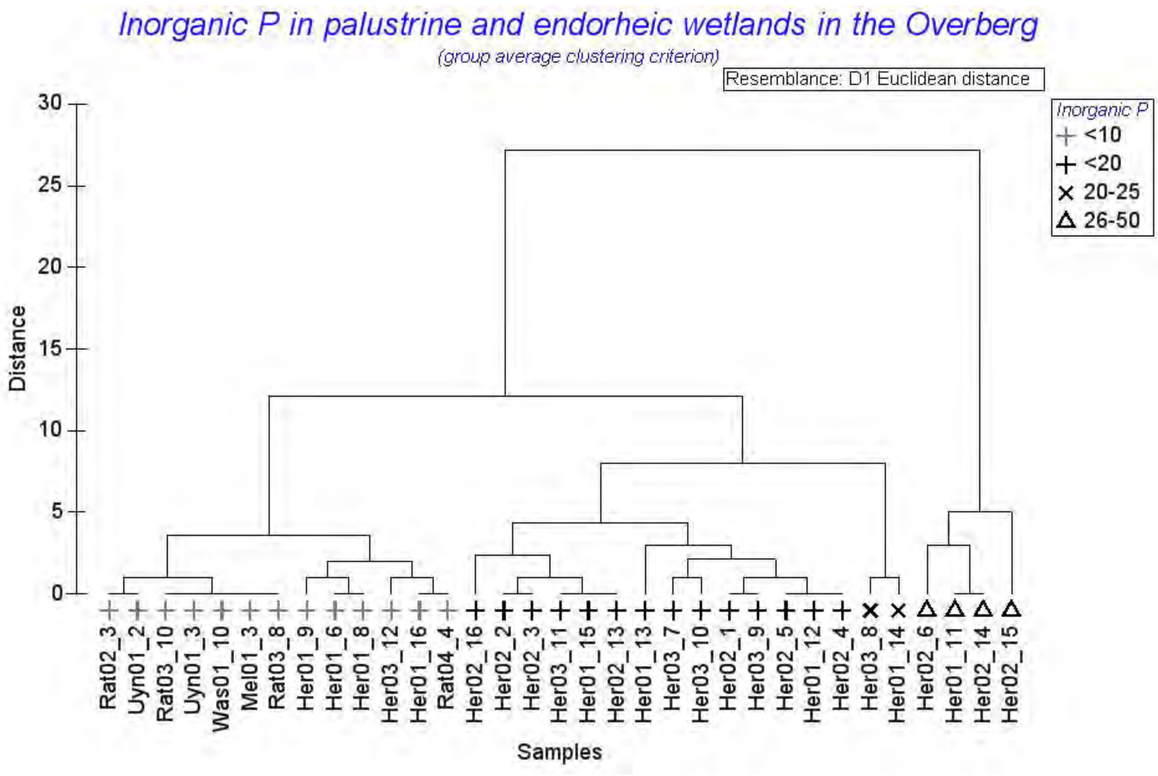


Figure 5.11: Dendrogram, using group average clustering, of the soluble phosphorus concentration (mg.kg^{-1}) in sediments from vegetation stands in predominantly Fynbos associated wetland of the Overberg (Was01 = Renosterveld associate).

concentrations were exhibited in a number of samples. In general, the HDS categorization accurately reflected disturbance classes that matched trophic states suggested by soil phosphorus concentrations. The meso- to eutrophic sample Ber02_5 (90 mg.kg^{-1}) warrants changing the categorization from Moderate to Worst. The hyper-eutrophic samples in the Kenilworth locality of the Cape Flats necessitated adjusting the categorization from Reference to Worst disturbed for samples Ken20_6/7/8.

5.3.3.2. Plant available nitrogen

Studies conducted by Low (1983) and Stock and Lewis (1986) in winter-saturated Sand Plain Lowland Fynbos soils showed significant monthly variations in soil organic and mineral (inorganic) nitrogen. The ammonium (NH_4^+) and (typically smaller) nitrate (NO_3^{-2}) fractions of mineral nitrogen showed no pronounced accumulation in the above studies. The lack of seasonal accumulation of mineral nitrogen in these soils was thought to result from plant nitrogen uptake equalling nitrogen produced by mineralization (Mitchell et al.1987). This was postulated to be indicative of an undisturbed natural ecosystem, limited by low nitrogen levels.

In the present study, only total nitrogen the largest part of which is insoluble organic nitrogen was measured in sediment samples. This measurement is not directly informative about the availability of soluble inorganic nitrogen (nitrate and ammonium) for plants, however the ease with which N is inter-converted between organic and in-organic forms suggests total nitrogen is a useful measure of the potential availability of N in the sediment.

A summary of the measured total nitrogen data is presented below; the raw data are presented in Appendix 9. Nitrogen availability in soils is intricately linked to SOM and these parameters are thus often reported in combination (e.g. Whitehead 2000, Ashma and Puri 2002). In the soils of the present study there was significant correlation between N and SOM in each sub-region as identified by Pearson correlation: West Coast 91.7% (Pearson r (46) = 0.917, $p < 0.01$), Cape Flats 65% (r (203) = 0.653, $p < 0.01$), and Overberg 79% (r (31) = 0.79, $p < 0.01$). Considering that total nitrogen was measured in the present study, the largest fraction of which is organic nitrogen, the strong correlation of N to SOM is to be expected. Significant differences occurred in the range of values (dispersion) for N and SOM between each of the sub-regions ($pseudo\text{-}F_{2,297}$=90.996, p=0.001) (as identified with PERMDISP). Significant dispersion differences were also apparent between the sub-regions using only N ($pseudo\text{-}F_{2,297}$=8.2, $p<0.005$), with most difference apparent between the Cape Flats and Overberg (t=3.8, p=0.01).

Cluster analysis of the log-transformed sediment N content per square meter in the top 25 centimetres of the soil, (determined as described in Section 3.5.6) revealed two outliers with high N (Figure 5.12).

The values of these two outlying samples Ken11_6 (1167 g $N.m^{-2}$) and Lot05_3 (857 g $N.m^{-2}$) are both high relative to previously measured soil nitrogen content at similar depth in “Sand Plain Lowland Fynbos” (27 g $N.m^{-2}$) (Stock and Lewis 1986). Examination at the sub-regional scale and between associated sets of soils as identified by classification of dryland vegetation types – Fynbos, Strandveld and Renosterveld is more informative.

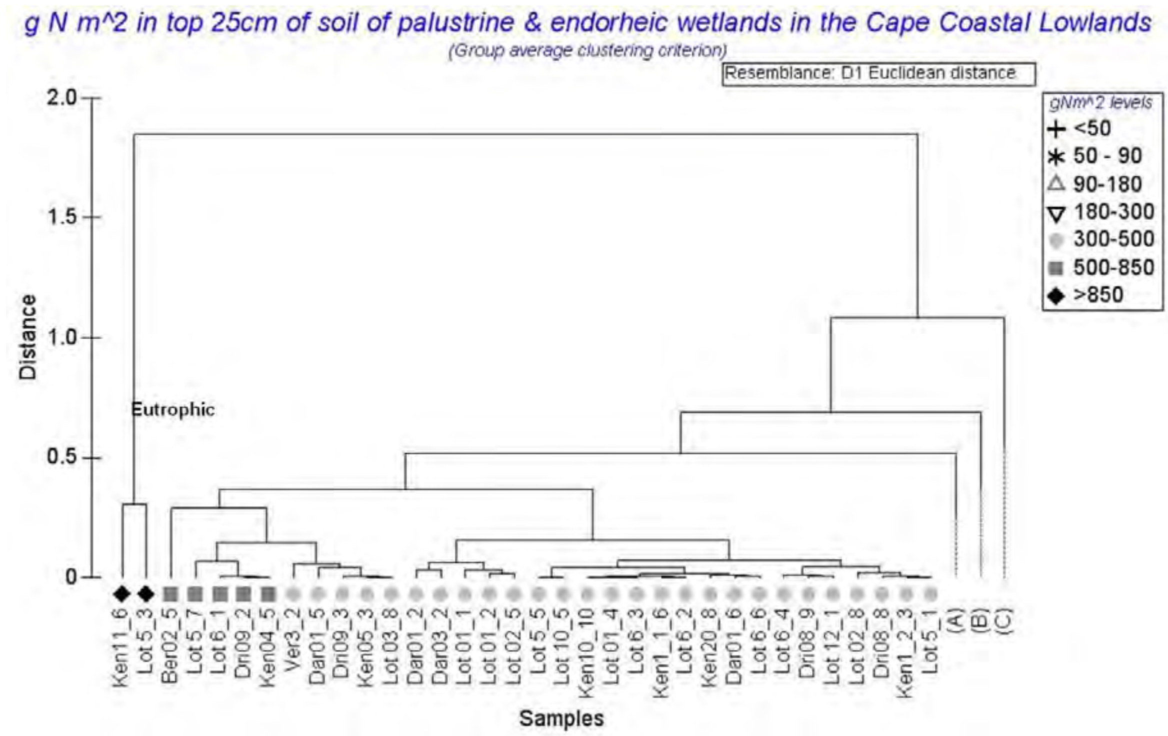


Figure 5.12: Dendrogram, using group average clustering, of the nitrogen content per square meter in the top 25 cm of sediments from vegetation stands in the Western Coastal Slopes region of the Cape Coastal Lowlands. A, B and C represent 219 samples with less than 300 g N.m^{-2} that are considered oligotrophic.

5.3.3.2.1. West Coast

On the West Coast, Soil N increased with increasing SOM content (Table 5.12). There was significant dispersion difference in the range of N and SOM values between Renosterveld and Fynbos samples ($t=3.6$, $p<0.01$). However, with greater pertinence to the present project, cluster analysis revealed no anomalies/outliers in the range of N content in the top 25 cm of soil that were suggestive of eutrophication. Sample Ber02_5 (637 g N.m^{-2}) did

Table 5.12: West Coast: Percentage soil nitrogen, soil organic matter as well as g N.m^{-2}. Values are averages of percentages for the vegetation unit in each locality ± standard error.

Vegetation unit and locality	n	Nitrogen (N) %	Organic Matter (%)	g N.m^{-2}
Fynbos, Darling (Dar05)	2	0.042 ± 0.006	0.76 ±0.1	61 ±8.1
Fynbos, Berg River (Ber02 and Vel02)	3	0.179 ± 0.115	2.52 ± 1.54	144±187
Fynbos, Verlorevlei,	8	0.108 ± 0.026	2.47 ± 0.68	229±68
Renosterveld, Darling	30	0.088 ± 0.009	1.65 ± 0.23	194±23
Strandveld, Berg River (Ber01)	1	0.056	0.68	92
Strandveld, Verlorevlei (Ver01b)	2	0.073 ± 0.044	1.24 ± 1.04	119±78

have higher N content than the average Fynbos related sediments sampled on the West Coast, suggesting potentially mesotrophic conditions. This mesotrophic condition is in keeping with the result identified for sediment P in this sample (Section 5.3.3.1.1)

5.3.3.2.2. Cape Flats

On the Cape Flats, there was a similar range (homogeneity of dispersion) of the N and SOM values between Strandveld and Fynbos samples (Table 5.13). The outliers Ken11_6 (1167 g $N.m^{-2}$) and Lot05_3 (857 g $N.m^{-2}$) (Figure 5.12) are from Sand Plain Fynbos and Dune Strandveld vegetation units and each represent apparently eutrophic N content.

Whilst Lot05_3 was categorized by the HDS as “Worst”, Ken11_6 was categorized as “Reference”. There were no apparent disturbance influences that could explain the elevated N content at Ken11_6, however, this high N content is considered to result from an unidentified unnatural influence.

Table 5.13: Percentage soil nitrogen and soil organic matter in soils of the Cape Flats. Values are averages of percentages for the locality ± standard error.

Soil Type	n	Nitrogen %	Organic Matter (%)	g $N.m^{-2}$
Fynbos, Kenilworth	64	0.101 ± 0.012	2.37 ± 0.158	174±20
Strandveld, Lotus River	85	0.114 ± 0.006	2.38 ± 0.146	210±14
Strandveld, Kuils River	55	0.109 ± 0.005	2.21 ± 0.104	199±10

5.3.3.2.3. Overberg

In the Overberg, only a single soil sample was assessed from Renosterveld with the remainder (n=31) from Fynbos. The Renosterveld sample had low N and SOM relative to the Fynbos samples (Table 5.14). There was significant dispersion difference in the range of samples between the Fynbos samples from the Hermanus (n=25) and the Agulhas Plain (n=7) localities ($t=3.1$, $p<0.05$). On average higher N and SOM were exhibited by Hermanus samples (Table 5.14). There were no outliers evident in a cluster analysis of the N content in the top 25 centimetres of the sediments from samples measured in the Overberg.

Sediments from the Hermanus hillslope-seep wetlands, Her03 (mean=0.247%) and Her02 (mean=0.211%), exhibited considerably higher nitrogen concentrations than other wetlands assessed in the Overberg but also had higher soil organic matter (mean values: 14.1% and 8.5% respectively) thus implying that the elevated nitrogen concentrations were unlikely to be due to human activities. The elevated organic matter reflects a permanently saturated condition in naturally acidic soils in which organic matter does not break down and results in the development of a peaty substrate, with naturally high carbon and nitrogen content.

Table 5.14: Percentage soil nitrogen and soil organic matter in soils of the Overberg. Values are averages percentages for the locality ± standard error.

Soil Type	n	Nitrogen %	Organic Matter (%)	g N.m^{-2}
Fynbos, Hermanus	25	0.204 ± 0.019	11.77 ± 0.184	-***
Fynbos, Agulhas Plain	6	0.169 ± 0.015	8.2 ± 0.52	207±36
Renosterveld, Agulhas Plain	1	0.072	1.86	116

***Bulk density was not recorded in the Hermanus samples

5.3.3.2.4. Summary: total nitrogen

The apparently eutrophic samples Ken11_6 and Lot05_3 and mesotrophic Ber02_5 were the only obvious indications of elevated N content, suggestive of anthropogenic influence. The propensity, in natural ecosystems, for plant available nitrogen (ammonium and nitrate) to leach, be denitrified and be taken up by plants, suggests that the accumulation of inorganic nitrogen is unlikely in undisturbed soils. Sample Ken11_6 was therefore recategorized as Worst disturbed for purposes of further analyses.

5.3.4. Adjustments to the disturbance categories

Table 5.15 indicates a number of vegetation samples that were re-categorized based on the following analyses: The comparison of the independently measured soil nutrient levels with

Table 5.15: Phosphorus concentration (mg P.kg-1) and nitrogen content (g N.m-2) from sediment samples with mesotrophic to eutrophic states and suggested adjustments of human disturbance categorization. Nutrient loads indicative of eutrophication are in bold text.

Sample	P	N	HDS	HDTS**	Vegetation Unit***	Sub-region
Ber02_5	**90**	**637**	Moderate	Moderate	Fynbos	West Coast
Ken11_3	20	**1167**	Reference	Worst	Fynbos	Cape Flats
Ken20_6	**720**	107	Reference	Worst		
Ken20_7	**717**	86	Reference	Worst		
Ken20_8	**641**	329	Reference	Worst		

**Human Disturbance and Trophic State categories

***Broad association with dryland vegetation type around wetland (Rebelo *et al.* 2006).

HDS categories suggested, on the whole, a successful estimation of the degree of human disturbance. Whilst the correlation is not necessarily indicative of causation, it is at least a useful adjunct to show that human disturbances are causing impacts on nutrient load and thereby on environmental condition of wetlands. The nutrient status of a number of samples did, however, suggest that a change in the HDS category was required if environmental condition was to be suitably categorized.

5.4. Discussion

5.4.1. Trophic boundaries in the water-column of wetlands

Soluble Reactive Phosphorus data recorded previously in South African wetlands have shown greater concentration in impacted than un-impacted wetlands of similar type (Malan and Day 2005b). Since many of these "unimpacted" wetlands would fall into the "eutrophic" category for rivers (DWAF 1996), the trophic state boundaries developed by DWAF for SRP were considered to be inappropriate for palustrine wetlands (Malan and Day 2005b). In their review of the TWQR only "extremely polluted sites" were considered as being impacted (Malan and Day 2005b). Within the present study, 62 percent of wetlands had less than 25 µg $P.L^{-1}$ of SRP, with 80 percent of the wetlands having less than 250 µg $P.L^{-1}$. Of the 19 wetlands with greater than 250 µg $P.L^{-1}$, most had easily-identifiable stressors that were suggestive of an impacted environmental condition. Hence, 250 µg $P.L^{-1}$ consistently indicated a boundary above which wetlands were considered to be eutrophic. Whether this boundary has an ecological manifestation in the vegetation structure is evaluated in Chapters 6 and 7. A concentration range of 25-250 $µg.L^{-1}$ is suggested for eutrophic condition of aquatic ecosystems as calculated from riverine examples (DWAF 1996). The revision of this eutrophic boundary to a "poor status" for river reaches exhibiting more than 125 $µg.L^{-1}$ [SRP] (DWAF 2002) appears to be too conservative for wetlands, for which, a value of more than 250 $µg.L^{-1}$ appears to be more appropriate based on the WHI data set of wetlands in the Cape Coastal Lowlands. The riverine TWQRs (DWAF 1996 and 2002) thus underestimate the phosphorus loads representative of different trophic states within palustrine and endorheic wetlands measured in the present study as is apparent from an examination of Table 5.3.

In contrast to the SRP levels the TIN levels that appear to represent eutrophic conditions in palustrine and endorheic wetlands of the present study are similar to those proposed by DWAF in 1996; whilst the TWQR proposed in 2002 (DWAF 2002) are however too high (Table 5.5).

The levels of nutrient enrichment that suggest different trophic states/conditions appear to be sub-region-specific for both water and soil. Considerable differences were apparent between sub-regions in terms of the range of nutrient measurements exhibited by wetlands (Section 5.2). Despite considerable agricultural impacts, wetlands in the Overberg did not exhibit concentrations of nutrients that would suggest eutrophication according to the riverine TWQR (DWAF 1996 and 2002). Both TIN and SRP boundary concentrations of the TWQR and of the present WHI study appear to be too high for the Overberg sub-region (Tables 5.3 and 5.5). Whilst this may be because the magnitude of disturbance was generally lower in the Overberg than other sub-regions further investigation is necessary to confirm nutrient loads indicative of different trophic states for the Overberg.

5.4.2. Variation of nutrients in wetland sediments

Within the sediment data, the considerable variation in nutrient concentrations that were detected within wetlands (Section 5.3.1), suggests that a more accurate interpretation of the patterns of plant response to soil nutrients would be obtained by comparison of data measured at the plot level rather than an average value calculated from several samples from a given wetland. This is contrary to the recommendation of the Biological Assessment of Wetlands Working Group (BAWWG) (US EPA 2002c) (Section 2.8.5.4); yet supports the standard botanical opinion (Kent and Cocker 1992) and approaches used for the development of autecological information. This result corroborates the experience of wetland ecologists and botanists working in South African wetlands that suggests that there is often considerable variation in many environmental parameters within wetlands (Sieben 2003, Low and Pond 2003, Collins 2005, Ractliffe and Corry 2008). Considerable internal variation is perhaps to be expected in wetlands, due to the impacts of fluctuating aerobic and anaerobic conditions, which result in oxidation or reduction reactions that in turn cause the nutrient availability to differ between hydrological zones (Verpraskas and Faulkner 2001).

5.4.3. Measurement of inorganic nitrogen in sediments

Wetland sediments could potentially be tested with a 1:2 water extract to determine whether anthropogenic enrichment of soluble inorganic nitrogen is detectable. The natural seasonal/temporal variability in inorganic nitrogen (nitrate and ammonium levels), suggests that such an experiment would need to be conducted within a short time of the sample being taken, with comparable temperature and moisture availability between samples. The storage of the sample after collection and during pre-analysis would need to be carefully considered in order to prevent concentration changes due to denitrification and dehydration.

It is possible that the measurement of soluble inorganic nitrogen in a 1:2 water extract from soil samples would facilitate the measurement of plant-available N in wetlands with no standing water at the time of sampling. This could potentially be used in place of TIN measurements, therefore. The value of determining plant available N in dry wetlands; and the correlation to TIN should be examined in future studies. The use of water-column TIN to check for eutrophication revealed two wetlands with exceedingly eutrophic conditions (>9000 $\mu g.L^{-1}$).

5.4.4. HDS category changes suggested by trophic states

Nutrient concentrations are independent measurements of the impact of human disturbance, and may be used in conjunction with the qualitatively-determined HDS. The measurement of water-column nutrient concentration highlighted the eutrophic state of two wetlands (Dar05 and Her02), and warranted downgrading their HDS-determined environmental condition. Soil nutrient measurements in vegetation samples indicated eutrophication in a number of samples (Ken11_6, Ken20_6/7/8, Ber02_5), in contrast to the Reference or Moderate environmental conditions that had been suggested by the HDS. Extremely elevated water-column or soil nutrient concentrations (relative to surrounding wetlands or soil samples), were used to corroborate or adjust the HDS categorization of wetlands. The number of Reference wetlands was reduced from 17 to 14 as indicated by soil and water nutrient concentrations. The Moderately impacted wetland category retained 24 examples. Worst disturbed wetlands increased from 19 to 22.

Nutrient enrichment is only one of the types of disturbance/stressors that is assessed using HDS. Many of the wetlands exhibiting apparently oligotrophic nutrient concentrations showed evidence of human impacts, such as physical disturbance to the wetland morphology or vegetation cover, that do not impact on nutrient concentration. It should therefore be noted that an oligotrophic trophic state does not always warrant a “Reference” categorization of wetlands using HDS.

5.5. Conclusions and implications for further analyses.

It is apparent from the above analyses that, for many environmental parameters that were measured at the vegetation plot scale within the different localities (different soil types) in each sub-region, there is as much variation within, as among, wetlands (Section 5.3.2). It would seem pertinent therefore to analyse these environmental characteristics at the scale of the vegetation plot to gain as much insight as possible into the drivers behind species distribution and assemblage and thereby to determine indicator species. For example, the vegetation sample scale comparison of soil nutrients made it evident that wetlands Ken20 and Ber02 were not uniformly impacted by elevated phosphorus concentrations. It is therefore apparent that, at least within these two wetlands, more accurate autecological information for indicator species is provided by the analysis of soil nutrients at the scale of vegetation sampling. Examination of wetland average nutrient values, as calculated from the amalgamation of sub-samples (recommended by the BAWWG), would have masked the variation and eutrophication of sub-sections of these wetlands. The greater expense of vegetation plot, rather than wetland average soil samples, revealed two of the 39 wetlands sampled to have considerable internal nutrient variability. Further advantages, in the identification of metrics that specifically reflect different trophic states, are anticipated from the soil sample analysis at the scale of the vegetation plot.

Development of phyto-assessment indices of environmental condition in the United States were based on the comparison of vegetation assemblages of wetlands that were assumed to represent different environmental conditions. The measurement of environmental condition was based on the cumulative amount of human disturbance impacting on each wetland (e.g. Mack *et al.* 2000, Simon *et al.* 2001, Gernes and Helgen 2002). As a direct measure of environmental conditions in each wetland, nutrient concentrations are also typically measured in the soil and water-column of wetlands. Vegetation assemblages are then compared against gradients of human disturbance and of individual nutrients thought to impact on species assemblages and environmental conditions (e.g. US EPA 2002, Section 2.7.2.1). In the present study, the soil and water-column nutrient concentrations were used to corroborate the level of human disturbance in each wetland before any attempt was made to compare vegetation assemblages of wetlands of different environmental conditions. This process facilitates a cross-check on the *a priori* categorization of environmental condition of wetlands, reducing the likelihood of associating species assemblages with incorrectly assigned disturbance categories.

In Chapter 6, a multivariate analysis is performed on the association of plant species and vegetation attributes with disturbance categories. This facilitates the identification of metrics for phyto-assessment.

6. WETLAND SCALE INDEX DEVELOPMENT

This chapter tests Hypothesis number 8 as described in Section 3.4 and outlined in the textbox below:

> **Hypothesis 8:** Wetlands from different categories of human disturbance impact (Reference, Moderate, Worst) are represented by different species assemblages within each sub-unit of vegetation identified in Analysis 1 or 2. The wetlands from each category will have:
>
> a. Characteristic and discriminatory species from each disturbance category. Such species will occur with consistently different weighted-average wetland cover/abundance per disturbance category; and
>
> b. Different diversity measures (species richness, evenness or dominance) per disturbance category.

This chapter thus investigates:

- if any species of plant can be reliably associated with particular categories of disturbance;
- if potential indicators of disturbance (species and other aspects of community assemblage) can be identified; and
- the potential for creating an index of environmental condition for the wetlands under consideration.

The disturbance categories used in this chapter are those developed as HDS scores in Chapter 3 and adjusted according to nutrient status in Chapter 5. They are referred to hereafter as HDTS categories, or simply Reference, Moderate or Worst.

In the present study, both wetland HGM and vegetation types represented more than a single set of similar habitats. Such natural variability may mask associations between species or vegetation attributes and disturbance categories. Permutational Multivariate Analysis of Variance (PERMANOVA) (Clarke and Gorley 2006, Anderson *et al.* 2008), was therefore chosen as an appropriate method for distinguishing between naturally different units of wetland vegetation, and thereafter for searching within each unit for significantly different assemblages of plants per disturbance category. PERMANOVA was used to test the difference in species assemblages within different disturbance categories (Reference, Moderate, Worst) of spatial units (region/sub-regions or locations within sub-regions) shown in Chapter 4 to hold uniform wetland vegetation.

The data set collected during the course of this study, is descriptive of the vegetation and environmental variables for each vegetation plot, within each hydrological zone, and within each wetland (“wetland average”). The present chapter deals specifically with the “wetland average” data. The environmental variables most responsible for the difference between wetlands of different disturbance categories were established using distance-based Linear Modelling (DistLM) (Legendre and Anderson 1999) (See Section 2.10.5.2 for details). The plant species most characteristic of each disturbance category (where these were shown to hold significantly different vegetation) were identified using percentage similarity tests (SIMPER) in PRIMER-E (Clarke 1993, Clarke and Gorley 2006). Thereafter, differences in plant functional diversity between wetlands of different disturbance categories were assessed with the tool DIVERSE in PRIMER-E.

6.1. Sub-regional scale analysis

Having demonstrated (Chapter 4) considerable differences between the vegetation assemblages of the different sub-regions and between different locations within sub-regions, it remains to be determined whether differences are apparent between wetlands exposed to different degrees of human disturbance:

a) within a given locality; and/or
b) whether differences are consistent between among the localities within sub-regions.

6.1.1. Multivariate analysis of the biotic assemblage

Permutational Multivariate Analysis Of Variance (PERMANOVA: details in section 2.10.5.2) was used to test for significant differences in vegetation assemblages of wetlands from different categories of disturbance in each sub-region and in each locality. Partitioning the data set such that the various localities are nested components of each sub-region ensures that comparison made between disturbance categories is restricted to those wetlands within sub-regions and or within localities. Furthermore the nested samples will not be permutated outside of the sub-region in which they occur ensuring that spatial autocorrelation between sub-regions does not invalidate the permutational generation of significance tests (Legendre 1993).

As in Chapter 4, the Bray-Curtis resemblance-matrix of the 4^{th}-root-transformed weighted average species cover per wetland, was used.

A 3-way nested PERMANOVA design of sub-regions crossed with localities (nested within sub-regions) crossed with HDTS categories of disturbance served to compare the species assemblages between:

1. The sub-regions;
2. Disturbance categories across the whole Western Coastal Slope (SWm) region;
3. Localities as nested components of each sub-region;
4. The categories of disturbance in each sub-region; and
5. The categories of disturbance within localities as subsets of the sub-regions.

This hierarchy for the analysis of resemblance between wetland vegetation assemblages from different subsets of the data for the SWm is displayed in Table 6.1.

In the PERMANOVA the measurement of the impact of the HDTS disturbance on vegetation assemblages was performed as the interaction of a fixed-effect “disturbance” factor within each sub-region or within localities as nested components of sub-regions. The spatial hierarchy of the data available in each category of disturbance, in each locality, and as a subset of each sub-region is displayed in Table 6.1

Table 6.1: Spatial hierarchy of number of wetlands within each disturbance category in localities from each sub-region of the Western Coastal Slopes (SWm) wetland region.

			HDTS Categories		
Sub-region	**Locality**	**Code**	**Reference**	**Moderate**	**Worst**
West Coast	Berg River	Ber	-	1	2
	Darling	Dar	1	4	2
	Verlorevlei	Ver	-	4	2
Cape Flats	Driftsands	Dri	2	6	3
	Kenilworth	Ken	2	5	4
	Lotus River	Lot	5	1	5
Overberg	Hermanus	Her	-	2	1
	Agulhas	Agu	5	1	1

6.1.2 Comparison of vegetation assemblages in different disturbance categories

PERMANOVA of the vegetation assemblage data shows significant differences among wetlands of the different sub-regions, among the different localities (nested in sub-regions) and among wetlands of different disturbance categories in localities (Table 6.2). The design is unbalanced, however, with only a single wetland representing the Reference condition on the West Coast and no Reference wetlands in the Hermanus locality of the Overberg sub-region (See Table 6.1). The unbalanced design does not, however, invalidate the PERMANOVA result.

Table 6.2: Test statistics for PERMANOVA of the 4th root of Bray-Curtis measure of resemblance between weighted average vegetation assemblage data of each wetland between sub-regions, disturbance categories and localities as subsets of the sub-regions. Relationships that are significant at $p<0.05$ are marked *.

Species cover/abundance Analysis #		d.f.†	Sum of Squares	Mean Squares	Pseudo-F	p-value
1	Sub-Region	2	17494	8747.1	1.4	0.04*
2	Disturbance	2	7234	3616.7	1.2	0.3
3	Locality(Sub-region)	5	37538	7507.5	3.1	0.0001*
4	Sub-regionxDisturbance	4	14673	3668.2	1.2	0.3
5	Locality(Subregion)xDisturbance**	6	19539	3256.5	1.3	0.02*
	Residual	39	95601	2451.3		
	Totals	58	2.14E+05			

d.f.†=Degrees of Freedom (number of wetlands in analysis less one. In nested analyses: #'s 3 and 5 although six and seven wetlands are respectively incorporated, only the labels of those that belong to a sub-regional subset are permuted for each sub-region).

**Term has one or more empty cells (Locality lacks one of the categories of disturbance [Locality "Hermanus" – no Reference]; or has only a single sample in a disturbance category: [Locality "Darling" has only a single Reference wetland]). This information is summarized in Table 6.1.

From Table 6.2 it is clear that

1. each of the West Coast, Cape Flats and Overberg sub-regions holds significantly different assemblages of wetland vegetation (pseudo-F=1.4, $p<0.05$);
2. across the whole collective of wetlands sampled, the disturbance categories do not hold significantly different vegetation assemblages;
3. each location (as nested within sub-regions) holds a significantly different wetland vegetation assemblage from every other location (pseudo-F=3.06, $p=0.0001$);
4. within sub-regions, no significant difference was apparent between the collective vegetation assemblage of the wetlands from different disturbance categories. This suggested that there were no typical and consistent plant responses to different degrees of disturbance amongst the different localities of a sub-region; and
5. within each locality, differences in vegetation between disturbance categories are apparent (pseudo-F=1.3, $p=0.02$). However, a *posteriori* pair-wise PERMANOVA between disturbance classes within each locality revealed that only wetlands in the Lotus River locality exhibited significantly different vegetation assemblages between

disturbance categories, specifically between Reference and Worst (t=1.9, p=0.01: Table 6.3).

Using hydrological habitat subsets of the wetland weighted-average species cover data, further PERMANOVA analyses were performed to test whether the above result was consistent when looking only at the littoral or the supralittoral vegetation. The results of these two further PERMANOVA tests revealed the same result, namely that only the Lotus wetlands of Reference and Worst condition held significantly different vegetation (supralittoral (t=1.4) and littoral (t=1.7) habitats ($p<0.05$, 126 unique permutations)). Similar results were found when analysing the species presence/absence inventory data (t=1.4, $p<0.05$) rather than the cover/abundance date determined from vegetation samples and when using the functional group data base in place of species cover/abundance (t=1.6, $p<0.05$). The functional groups used were those listed in appendix 8. Within the Lotus River locality, wetlands in a Reference condition exhibited unequivocally different species assemblages from those wetlands that had been most (Worst) disturbed. The single Moderate wetland from the Lotus wetlands had vegetation that was not significantly different from either Reference or Worst Lotus wetlands. Lotus River is the only locality assessed in which significant difference was apparent at this wetland weighted average cover scale of investigation.

Table 6.3: *A posteriori* pair-wise tests in PERMANOVA between vegetation assemblages of wetlands of different disturbance categories within locality “Lotus River”. P(MC) represents Monte Carlo p-values that are necessary to use in tests with less than 100 possible permutations. P-values < 0.01 are marked *

Groups	t-value	p-value (perm)	Unique permutations	p(MC)
Worst, Reference	1.8873	0.01*	126	0.01*
Worst, Moderate	1.03	0.5	6	0.4
Moderate, Reference	1.13	0.3	6	0.3

As is apparent from the *a priori* spatial hierarchy of the disturbance-locality units displayed in Table 6.1, that the number of Reference and Worst wetlands compared at the Lotus wetlands was greater than in any of the other localities assessed. At other localities, the relatively small range in degree of disturbance might be responsible for the lack of discernable differences between vegetation assemblages in each disturbance category. A discussion of the above results and their implications for phyto-assessment development is presented in Section 6.5. The following sections describe the potential for developing metrics based on the differences established between Reference and Worst wetlands of the Lotus locality.

6.1.3. Lotus River: differences in vegetation assemblage

The differences in vegetation assemblages between Reference and Worst-disturbed conditions in the Lotus River wetlands may reveal potential indicator species and metrics. Multi-Dimensional Scaling ordination (MDS) of the vegetation assemblages based on 4^{th}-root transformed wetland-average per species cover values in each wetland showed separation between Reference and Worst disturbed wetlands (Figure 6.1a). Wetland Lot01, with a Moderate level of disturbance, was included in this ordination to show its relationship with wetlands of the other categories of disturbance. The assemblage in Lot01 was not significantly different from that of either the Reference or Worst wetlands. A dendrogram (cluster analysis) of the same data revealed that the species composition of Lot02 and 03 was approximately 55% similar, despite the two wetlands falling into Reference and Worst-disturbed categories respectively (Figure 6.1b). Similarity between these two wetlands

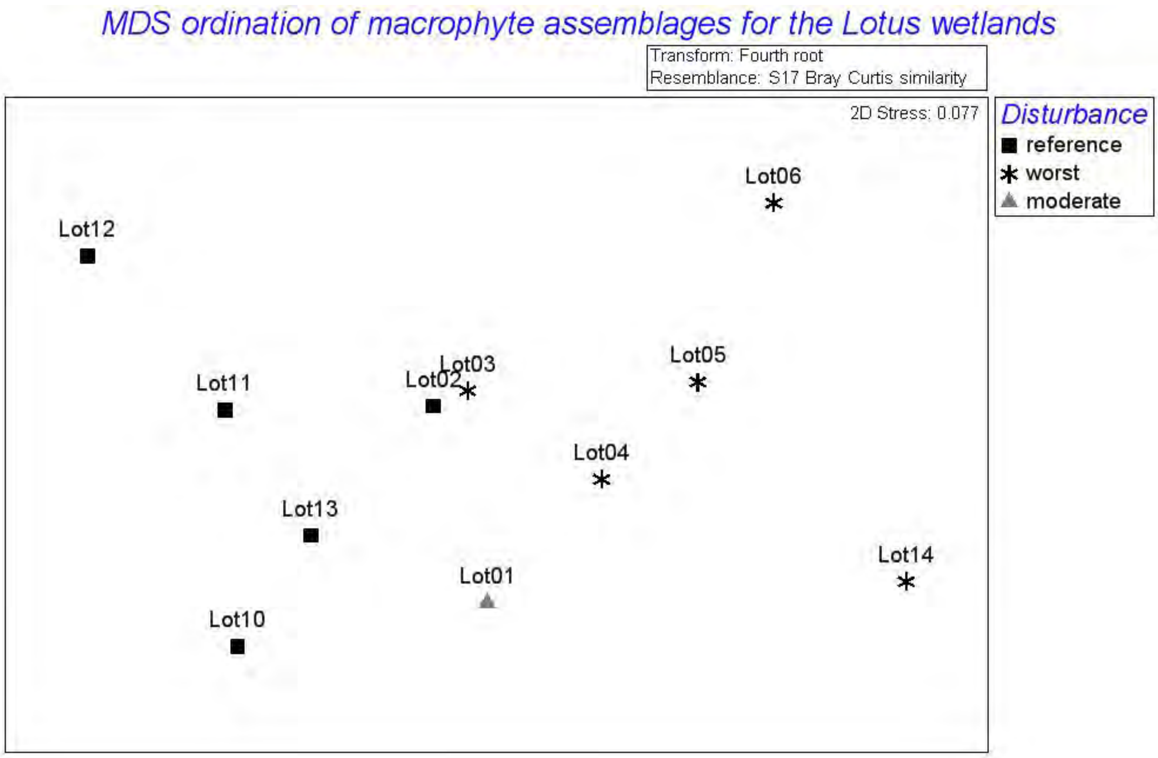


Figure 6.1a: Multi-Dimensional Scaling ordination plot of the 4^{th} root transformed Bray-Curtis resemblance of vegetation assemblage as measured by average cover per species per wetland. The Moderately disturbed wetland Lot01 is included in this ordination in order to show its relationship to the wetlands of the Reference and Worst categories of disturbance.

suggests that disturbance has had little effect on the vegetation or that there is a time-lag in response to change, since both of these wetlands were in the process of rehabilitation. Wetlands Lot12 and Lot14 are obvious outliers from these two disturbance groups (Fig. 6.1a and b). Wetlands Lot01 to 03 and 10 to 14 were within the Rondevlei Nature Reserve, whilst Lot04 to 06 are located east and slightly north in urban and agricultural landscapes.

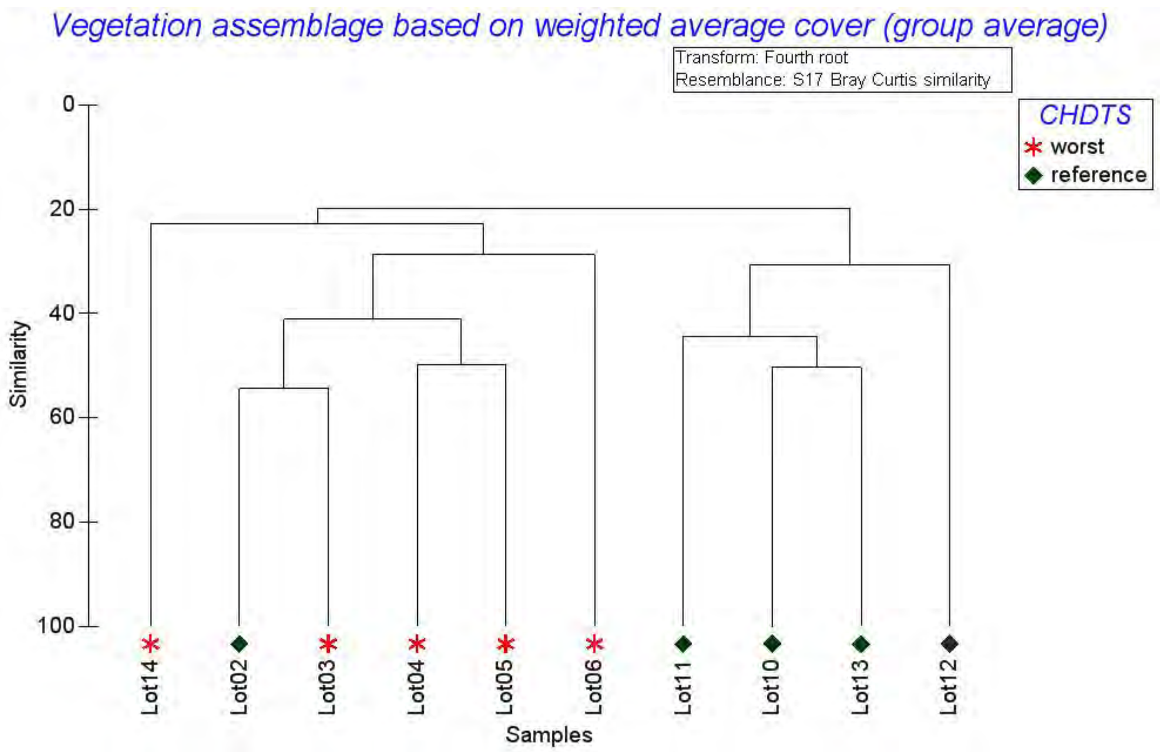


Figure 6.1b: Dendrogram, using group average clustering criterion, of 4th root transformed Bray-Curtis similarity of vegetation assemblages as measured by average cover per species per wetland.

The Reference wetlands of the Lotus River had, on the whole, greater total water capacity (volume) (Table 6.4). This greater volume and, by proxy, longer residence time of standing water, provides a greater diversity of potential vegetation habitat than is available in the Worst-disturbed wetlands. This physical difference suggests a potentially natural and large source of difference between the wetlands of Reference and Worst HDTS categories. Exploration of the environmental data is needed to confirm whether human impacts or other, potentially spatially related, environmental parameters are responsible for the observed difference in vegetation assemblage between Reference and Worst disturbed Lotus wetlands.

6.2. Biotic affiliation with environmental variables

Distance-based Linear Modelling (DistLM) (Legendre and Anderson 1999, McArdle and Anderson 2001: see Section 2.10.5.2) was used to determine which environmental variables were most responsible for the difference in the Bray-Curtis resemblance of the vegetation assemblages of Reference and Worst disturbed Lotus wetlands. The DistLM, matches biotic assemblage patterns to environmental variables to determine which variables explain most of the variance in the assemblage pattern between Reference and Worst wetlands. As no numerical alteration to the HDS was made in determining HDTS categories for the Lotus wetlands in Chapter 5, the qualitatively determined HDSs were used to quantify anthropogenic disturbance as a measure of likely ecosystem condition and was included in the DistLM as a numerical variable. The contribution of natural environmental variation and human disturbance in influencing the biotic assemblage distribution were thus simultaneously assessed.

Ordinations and dendrograms were used to search for any obvious environmental or vegetation data that should be removed before these variables are compared in the DistLM. Wetland Lot06 was an obvious outlier in the environmental data set, being categorized as eutrophic (see Chapter 5 and Table 6.4). This wetland was therefore removed from the model to prevent the high nutrient levels from skewing the model outcome. Wetlands Lot12 (Reference) and Lot14 (Worst) were outliers in the vegetation dataset in terms of their species assemblages (Fig. 6.1a and b) and were therefore also excluded from the DistLM.

Dissolved Oxygen was not measured in wetland Lot13 and SRP concentration was not measured in wetlands Lot05 and Lot13. To facilitate the inclusion of these variables in the DistLM, conservative estimates of these missing values were made, based on nearest and most similar wetland neighbours. A second DistLM was performed without these estimated variables to assess if any different results would be apparent (See results below Fig 6.2).

Attempts to perform DistLM without first removing variables whose average value was not significantly different between Reference and Worst wetlands led to the DistLM process selecting variables that did not differentiate between Reference and Worst Disturbed wetlands. Soil particle size and depth, wetland size, slope, aspect and altitude as well as climatic variability are all similar between the wetlands of the Lotus River data set. A total of seven environmental variables out of the 54 that were measured in the Lotus River wetlands showed different average value between the Reference and Worst wetlands (Table 6.4). Statistical differences of these seven variables between Reference and Worst Lotus River wetlands were determined using pair-wise PERMANOVA in a one-way analysis within the factor "Disturbance".

None of the seven variables were collinear at or greater than 95% (or ≤ -95%) although dissolved oxygen concentrations decreased with increasing human disturbance (HDS) and these variables were inversely collinear at -93.05%.

Table 6.4: Environmental variables with different average values (± S.E.) in the Reference and Worst disturbed wetlands of the Lotus River.

	Unit	Reference	Worst	t-value	p-value
Wetland Water Volume	(score)	6.8 (0.8)	3.5 (0.4)	3.5	0.008
Soil Redox	(mV)	-38.8 (5.3)	-15.3 (9.8)	4.4	0.004
Dissolved Oxygen	($mg.L^{-1}$)	7.9 (0.5)	5.6 (1.1)	4.8	0.006
SRP[†]	($mg.L^{-1}$)	2.0 (0.5)	3.6 (0.9)	4.2	0.05
Soil Phosphorus	($mg.kg^{-1}$)	3.1 (0.4)	4.7 (1)	3.4	0.05
Water soluble calcium	($mg.L^{-1}$)	71.2 (1.6)	129.1 (33)	2.9	0.05
HDS	(score)	39.8 (16)	106 (19)	5.7	0.006

SRP[†] = Soluble Reactive Phosphorus or ortho-phosphate as measured in water column.

6.2.1. DistLM results and constrained ordination

HDS was shown in the analysis to be the variable that best described the difference between Reference and Worst wetlands (explaining 30% of the model fitted variation). The distance-based linear model based on Adjusted R^2 selection criterion showed that each of the retained variables (Table 6.4) independently explained a considerable and often significant ($p<0.05$) percentage of the variation between the vegetation assemblages of the Reference and Worst disturbed wetlands (Marginal tests, Table 6.5).

Collectively, looking at the multivariate interaction, the solution that explained most of the variability (92.69%) between Reference and Worst wetlands was the combination of soil redox potential, soluble reactive phosphorus, water volume, soil phosphorus and water-soluble calcium (Best Solutions #1, variables 3-7 Table 6.5). Considering that dissolved oxygen was almost the inverse of human disturbance (HDS) this correlation perhaps influences the linear model (r=-0.93 or -93%), and these variables are likely to be used interchangeably by the DistLM and/or dbRDA procedures. Other than the first and aforementioned solution that explained most of the variation, all potential best solutions to the model (with less than two adjusted-R^2 units difference from the first solution (Variables 3-7)) included human disturbance or dissolved oxygen.

Table 6.5: Test statistics for Distance-based Linear Model analysis based on "best" selection procedure and the adjusted R^2 selection criterion for the average vegetation assemblage in Reference and Worst disturbed Lotus River wetlands. SS = Sum of Squares, RSS = Residual Sum of Squares, R^2 = RSS/SS. Significance at p<0.05 marked *.

MARGINAL TESTS:				
Variable	**SS(trace)**	**Pseudo-F**	**p-value**	**% of total variation**
(1) Dissolved oxygen	5451.2	3.8	0.001*	42.896
(2) HDS	5328.6	3.68	0.009*	41.93
(3) Soil redox potential	5061.2	3.38	0.007*	39.827
(4) S. R. P.	4871.8	3.18	0.01*	38.337
(5) Water volume	4092.3	2.4	0.04*	32.204
(6) Soil phosphorus	3605.8	1.98	0.08	28.375
(7) Water soluble calcium	2781.7	1.4	0.2	21.89

BEST SOLUTIONS					
#	**Variable Selections**	**AdjR^2**	**RSS**	**R ^{2}**	**% of total variation**
1	3-7	0.5616	928.51	0.92693	92.69
2	2-5, 7	0.55479	942.95	0.9258	92.58
3	2, 4-7	0.54674	960	0.92446	92.45
4	1, 3, 4, 5, 6	0.54296	967.99	0.92383	92.38
5	1, 3, 5, 6, 7	0.53772	979.08	0.92295	92.295

A distance-based Redundancy Analysis (dbRDA) plot of the DistLM separation of the different wetlands is presented in (Figure 6.2 with a vector overlay of the environmental variables that contributed to the species assemblage pattern. Only vectors with Pearson Rank correlations *r>0.1* to the species assemblage pattern were selected to be displayed. The lengths of each vector reflect the univariate importance of their impact in explaining the modelled vegetation assemblage pattern (Ter Braak 1990). The dbRDA is a constrained ordination based on the multivariate relationship of the best linear combination of environmental variables that explains the separation of vegetation between Reference and Worst wetlands. Increasing soil redox (0.822), water soluble calcium (0.422) and SRP (0.354) best explain separation from left to right between Reference and Worst wetlands along the primary or x-axis (dbRDA 1), collectively explaining 45.07% of total variation. Increasing water volume (0.594), water-soluble calcium (0.562) and soil phosphorus (0.341), as well as decreasing soil redox (-0.368), best explain the separation between wetlands from bottom to top along the y-axis (db RDA 2), explaining 18.35% of total variation. These two axes explained 68.4% of the model fitted variation and 63.4% of the total observed variation in the combined environmental and vegetation assemblage data sets.

Without the “estimated dissolved oxygen and SRP values” increasing human impacts (0.88), soil redox (0.429) and water soluble calcium (0.204) best explained the separation from left to right or Reference to Worst along the x-axis, explaining 44.1% of total variation. Increasing water volume (0.64), water soluble calcium (0.52), soil phosphorus (0.444), and decreasing soil redox (-0.349) best explain separation from bottom to top along the y-axis. Collectively these variables described 62.5% of the total variation of the Bray-Curtis resemblance of the species assemblages between Reference and Worst disturbed wetlands in the Lotus River data set without estimated variables.

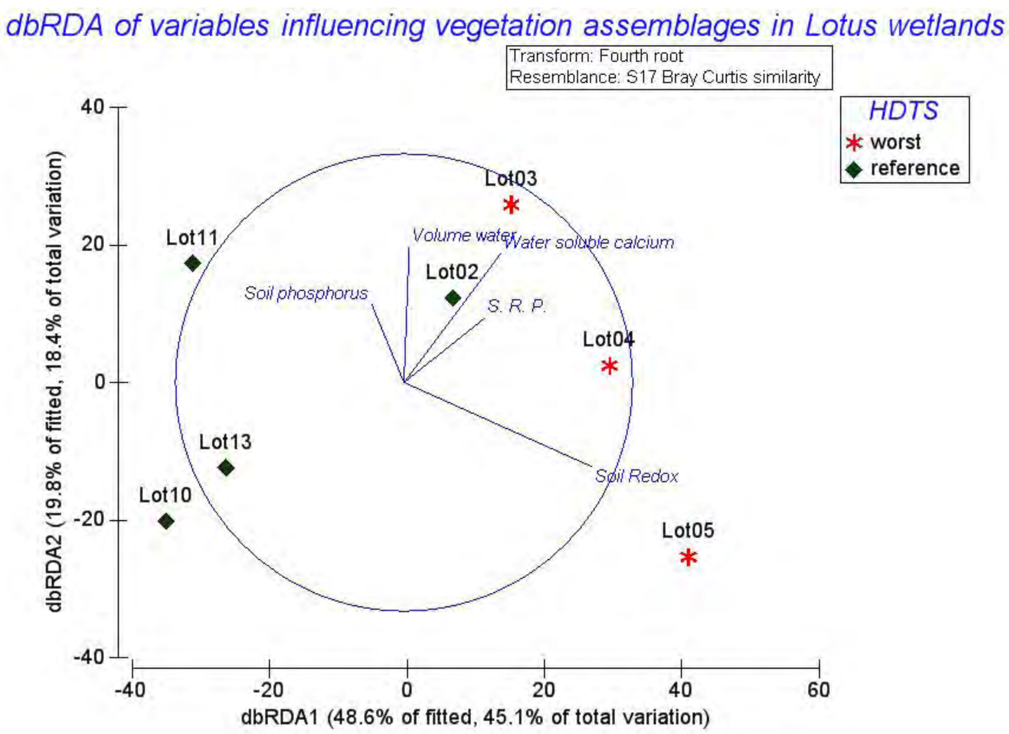


Figure 6.2: Distance based Redundancy Analysis diagram of the most influential environmental variables in determining different vegetation assemblages in Reference and Worst disturbed wetlands of the Lotus River locality as determined by distance Linear Modelling. Vectors are Pearson correlations.

Essentially the DistLM and dbRDA procedures confirm that the impact of human disturbance was paramount in separating the vegetation assemblages that were apparent in Reference relative to Worst disturbed Lotus wetlands.

6.2.2. Univariate comparison of individual gradients of impact

The gradients of human disturbance that were considered in the construction of the HDS revealed that the Worst wetlands in the Lotus River were on average more impacted than the Reference wetlands in a number of ways (Table 6.6). Comparisons were performed as one-way PERMANOVA of disturbance for all of the Reference and Worst Lotus wetlands; within each comparison there were 8 degrees of freedom due to the inclusion of five Reference and five Worst wetlands. Monte Carlo asymptotic p-values were used to determine significance due to the limited potential number of permutations.

Table 6.6: Impact gradients that proved with univariate t-tests to occur with different average value (± S.E.) in the Reference and Worst wetlands of the Lotus River. Monte Carlo asymptotic p-values were used due to limited potential number of permutations. All comparisons are significant.

	Reference	Worst	t-value	p-value
Hydrological	-2.8 (3.5) Drier	14.8 (6.03) Wetter	2.5	0.03
Water Quality	15 (1.6) Better	61.8 (5.4) Worse	8.3	0.001
Physical	15.4 (4.8) Less	39.8 (3.3) More	4.2	0.003
Buffer Width	1.2 (0.5) Broad	2.6 (0.3) Narrow	2.6	0.04
HDS	41.4 (9.2) Less	129.8 (6.9) More	7.7	0.001

6.2.2.1. Hydrological Impacts

The Worst wetlands had a considerably greater number of impacts that seem to have made them wetter than they would have been in a natural state (t=2.5, p<0.5) and all but one Lot04) held increased water volumes from stormwater drains feeding into them. The Reference wetlands did not have stormwater drains and all suffered from a slight decrease in water volume in comparison to the Worst wetlands. The difference in impact between Worst and Reference wetlands was small (small t-value). As each of these wetlands is of a different size and volume the hydrological impact does not mean that the Worst wetlands held more water for longer than the Reference wetlands. In fact, the Reference wetlands all had larger maximal capacities (Table 6.4).

6.2.2.2. Water Quality

The Worst disturbed Lotus wetlands were affected by a considerable number of land-use activities believed to negatively impact on water quality (t=8.3, p<0.001). This gradient was most responsible for high HDS values (Table 6.6), a feature that appeared to be validated by the DistLM analysis selecting SRP and water-soluble calcium as considerable components of the separation between Reference and Worst wetlands.

6.2.2.3. Physical Impacts

The Worst disturbed wetlands had been subject to considerable anthropogenic impact, leading to more physical disturbance of the substratum and natural vegetation cover than was found in the Reference wetlands (t=4.2, $p<0.003$).

6.2.2.4. Buffer Width

Narrow buffers (narrower on average than 50 m) were apparent around all of the Worst wetlands. Whilst the other three disturbance types were qualitatively assessed on the basis of the impacts of numerous land-uses, the score for buffer width was a measure of the average width and condition of the surrounding dryland vegetation. Buffer width has the lowest possible score and thus the least numerical influence of all of these gradients of disturbance on the HDS (See Section 3.5.4).

The most degraded Lotus wetlands were thus physically disturbed, with only narrow buffers to restrict the influx of surface-borne pollutants and were considerably influenced by impacts on water quality.

6.2.3. Conclusion: disturbance vs. natural environmental differences

Impacts of human disturbance, including low levels of dissolved oxygen and positive soil oxidation-reduction potentials, play a significant role in differentiating between Reference and Worst assemblages of vegetation assessed in the Lotus wetlands. Increasingly negative redox values reflect increasingly reducing conditions typical of the anoxic soils of many wetlands (Ellis and Mellor 1995). Under reducing conditions, soluble phosphorus may be released from organic material and mineral sediments and thus become available for plant uptake (section 2.8.5.3 and Cronk and Fennessy 2001; Keddy 2000). Since redox potentials were largely lower in Reference than in disturbed wetlands, soluble phosphorus (Olsen) levels in soils of Reference wetlands could potentially be higher than in disturbed ones. The reverse was in fact observed, with elevated soil phosphorus in Worst-disturbed wetlands, indicating that the high nutrient levels in the soils of disturbed wetlands are of anthropogenic origin. The greater concentrations of dissolved oxygen in the waters of the Reference wetlands indicates that these waters are oxic, thus suggesting that they should hold lower SRP concentration, which indeed they did. In all of the Worst disturbed wetlands, average dissolved oxygen was lower and SRP higher than in the Reference wetlands (Table 6.4). The association of human disturbance with elevated phosphorus levels in the soils supports the finding of Batchelor *et al.* (2002), who suggested that anthropogenic elevation of phosphorus concentrations would be more discernable in the soil solution than the water column. The DistLM analysis suggested that the redox and phosphorus concentrations were partially responsible for the difference in species assemblages of the Reference and Worst disturbed wetlands in the Lotus River wetlands (Best Solutions (Variables 3-7), Table 6.5).

The change in the water-soluble calcium concentration, although not significant when taken on its own (Marginal Tests, Table 6.5), did influence vegetation assemblages in combination with the other predictors, as revealed by all but the 4th of the "Best Solutions". Elevated calcium is not a spatially related phenomenon, there being no common spatial gradient to the change that is evident between different wetlands in the Lotus River area. The Reference condition Lotus wetlands generally contained more water and had significantly more negative soil redox potentials and lower water-soluble calcium levels; on average soil pH values were low but not significantly so. Wetland water volume played only a minor role in separating wetlands, most differences being between Reference and Worst wetlands (Figure 6.2). In general it can be said that anthropogenic disturbances were mostly responsible for the difference in species assemblages recorded in the Lotus River. The considerably higher concentrations of water soluble calcium in Worst disturbed Lotus wetlands is perhaps also indicative of some anthropogenic influence but further research would be required to verify this.

6.3. Discriminatory species in the Lotus River

SIMPER Analysis (Clarke 1993) of percentage similarities of the vegetation assemblages was used to determine which species were characteristic of, and which discriminated between, Reference and Worst-disturbed wetlands in the Lotus River wetland set. In these analyses the average (Bray-Curtis) dissimilarities between all pairs of samples (i.e. Reference *vs*. Worst wetlands) are broken down into separate contributions from each species, divided by the dissimilarity between each disturbance category (see Section 2.10.5.2 for details).

6.3.1. Indicator species for Worst and Reference wetlands: Lotus River

The species determined by an analysis of percentage similarity to occur with consistently discriminatory cover/abundance in the Reference and Worst Lotus River wetlands are presented in Figure 6.3. Wetlands Lot06, Lot12 and Lot14 were included in this analysis because the species representative of these outlier wetlands were considered useful indicators of disturbance. The inclusion of Lot06 revealed that the dominant species in this extremely eutrophic wetland were also those that indicated disturbance in other wetlands. Lot12, a small, shallow and shrub-dominated wetland was scored (HDS) as being in a Reference condition. The inclusion of data for this wetland suggested *Senecio halimifolius* as an important indicator of the reference condition. It was also recommended as an indicator of Reference condition in the SIMPER analysis. The inclusion of Lot14, a small artificial wetland created by a channel for stormwater drainage, perhaps over-emphasises the importance of *Typha capensis* as an indicator of Worst-disturbed environmental conditions. The artificial nature and impacted condition of Lot14 is however common to many wetlands in the Lotus River locality that were not sampled in the present study.

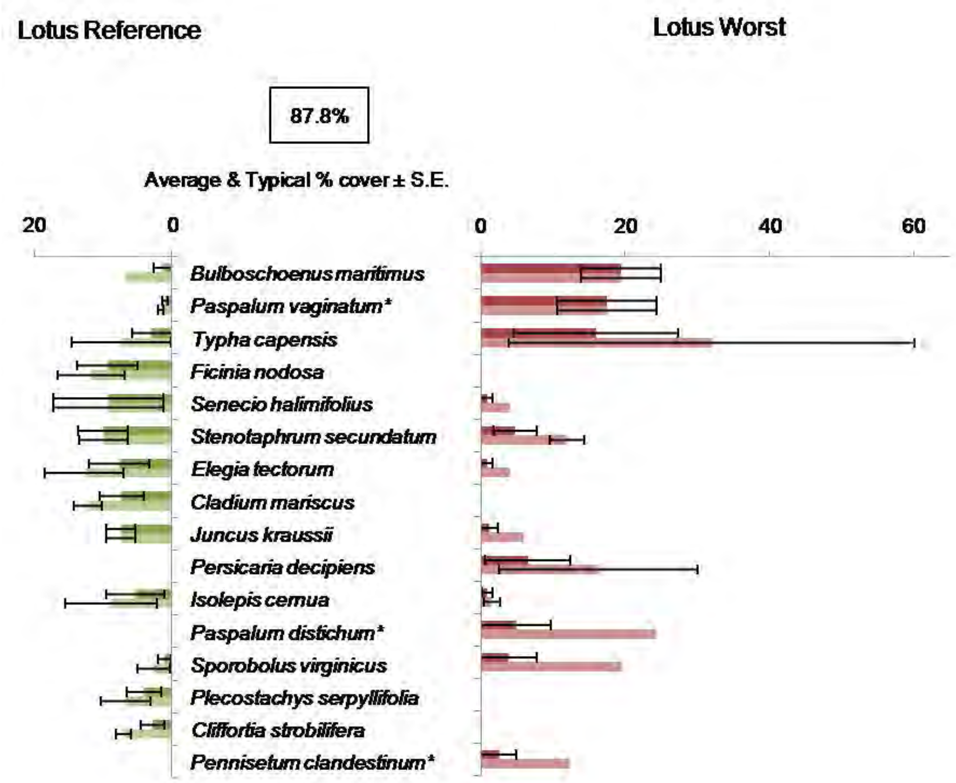


Figure 6.3: Species with discriminatory cover (± standard error) between the Reference (n=5) and Worst (n=5) disturbed Lotus River wetlands. Dissimilarity percentage between the species resemblance of the different disturbance categories is indicated at 87.8%. In species where no error bar is apparent the standard error was zero. Species that are non-indigenous and have C4 photosynthesis are marked with *. Dark bars mark average cover and light bars mark typical cover per wetland.

Whilst the average cover-value (± S.E.) provided some concept of how much cover of each species occurred in each disturbance category, not every species was recorded in each wetland. For any species absent from in all wetlands, the average value per disturbance category is deflated to a level considerably below that which was typically encountered in wetlands where one of these species occurred. Hence the characteristic species chosen by the SIMPER analysis based on average cover were sometimes impractical for discriminating between Reference and Worst wetlands. In order to improve resolution, the typical cover-value (± S.E.) was generated, based on the average cover in all wetlands where the species were encountered, thus more accurately representing 'typical' species cover-values encountered in these wetlands. These values, along with the average cover values are reported in Table 6.7. Both average and typical cover values were used to generate Figure 6.3, which indicates the species most characteristic of Reference and Worst wetlands in the Lotus locality.

Table 6.7: Species with characteristic association with either Reference or Worst Lotus wetlands.

Species	Reference wetlands			Worst wetlands		
	#	**Average cover**	**Typical cover**	**#**	**Average cover**	**Typical cover**
Bulboschoenus maritimus	**1**	1.3 (1.3)	6.7 (0)	**5**	19.4 (5.5)	19.4 (5.5)
*Paspalum vaginatum**	**3**	1 (0.4)	1.6 (0.3)	**5**	17.5 (6.8)	17.5 (6.8)
Typha capensis	**2**	2.9 (2.9)	7.3 (7.2)	**3**	16 (11.4)	32 (28.2)
Ficinia nodosa	**4**	9.4 (4.4)	11.7 (4.8)	**0**	0	0
Senecio halimifolius	**5**	9.2 (7.9)	9.2 (7.9)	**1**	0.8 (0.8)	4 (0)
Stenotaphrum secundatum	**5**	10 (3.6)	10 (3.6)	**2**	4.8 (3)	11.9 (2.4)
Elegia tectorum	**3**	7.6 (4.4)	12.7 (5.7)	**1**	0.8 (0.8)	4 (0)
Cladium mariscus	**3**	7.3 (3.2)	12.2 (2.1)	**0**	0	0
Juncus kraussii	**5**	7.4 (2.1)	7.3 (2.1)	**1**	1.2 (1.2)	5.9 (0)
Persicaria decipiens	0	0	0	2	6.5 (5.9)	16.3 (13.8)
Isolepis cernua	3	5.3 (4.2)	8.8 (6.6)	3	0.9 (0.7)	1.5 (1.1)
*Paspalum distichum**	0	0	0	1	4.9 (4.9)	24.4 (0)
*Sporobolus virginicus**	2	1.1 (1)	2.7 (2.3)	1	3.9 (3.9)	19.5 (0)
Plecostachys serpyllifolia	3	4 (2.6)	6.7 (3.6)	0	0	0
Cliffortia strobilifera	2	2.8 (1.7)	7 (1.1)	0	0	0
*Pennisetum clandestinum**	0	0	0	1	2.5 (2.5)	12.4 (0)

= number of wetlands

The four species that are reported in shaded rows in the above Table 6.7 occurred in only one or two of the Lotus wetlands. It is important to recognize that the development of indicator or characteristic species relies not only on cover values but also on frequency of occurrence. Species recorded in only one or two wetlands thus represent less reliability and suggest potentially spurious association with a disturbance category. It is noteworthy that whilst these species were present in only one or two wetlands, their presence may have been recorded in numerous vegetation stands in these wetlands (See Chapter 7); thereby corroborating their association with disturbed conditions. A brief explanation of some of these potentially spurious examples is provided below. Also included is the reasoning as to why the reported values for other species were considered useful for the development of metrics for phyto-assessment.

6.3.2. Species contributions

*Bolboschoenus maritimus, Paspalum vaginatum** (asterisks denote alien species) and *P. distichum*, Sporobolus virginicus*, Typha capensis, Persicaria decipiens* and *Pennisetum clandestinum** occurred with considerably greater cover in Worst disturbed wetlands. Typical cover values for these species occurred with a difference between Reference and Worst disturbed wetlands of more than one Braun Blanquet rank (Section 3.5.8.2; Table 3.6). *Paspalum vaginatum** and *P. distichum*, Sporobolus virginicus*,* and *Pennisetum clandestinum** are all alien grasses with a C4 photosynthetic pathway for carbon fixation. C4 grasses have lower water requirements than C3 grasses and produce greater bulk or biomass. The genus *Paspalum* is alien to the Western Cape (Goldblatt and Manning 2000) and these lawn-forming grasses are invasive (Bromilow 2001); hence, their association with disturbance is not surprising (Chapter2, Table 2.6). *Sporobolus virginicus** and *Pennisetum clandestinum** are also alien lawn-forming grasses, both frequently creating dominant stands to the exclusion of other species. *Paspalum distichum** and *Pennisetum clandestinum** were recorded in only one wetland each, reducing the certainty with which these species can be reported as characteristic of the disturbed or Worst category of environmental condition. *Bolboschoenus* and *Typha*, both indigenous graminoids were also observed to produce large and dominant (superficially monospecific) stands in wetlands. All of these species that occur with considerably greater cover in the worst disturbed wetlands are therefore considered as taxa that are tolerant of disturbance in\ the Lotus River wetlands. As is apparent from Figure 6.3, the standard error (S.E.) of the typical cover with which *Typha* occurred in both Reference and Worst wetlands was as large as its typical cover value; making it perhaps an unreliable indicator. In combination with other metrics of disturbance, though, increased coverage of *Typha* should prove to be a reliable indicator of disturbance.

The graminoid taxa *Ficinia nodosa* and *Cladium mariscus,* which did not occur in the disturbed wetlands at all, are potentially good indicator species of the Reference condition. These two species can be classed as taxa that are sensitive to disturbance in the Lotus River area. In the wetlands in which these species occurred, their typical cover was greater than 10%. The herb *Plecostachys serpilifolia* (n=3) and shrub *Cliffortia strobilifera* (n=2) were also recorded only in the Reference wetlands, typically occurring with 5-15% and >5%

cover respectively. *Cliffortia strobilifera* is reported to increase in abundance in eutrophic conditions, forming large, often monospecific, stands (*pers. obs*. and Boucher, C pers. comm.). Represented in only two Reference samples, *Cliffortia strobilifera* had a median cover of 18% and 88%, suggesting that this species can equally be abundant under relatively natural conditions.

The shrub *Senecio halimifolius,* the restio *Elegia tectorum* and (in comparison the relatively miniature) sedge *Isolepis cernua* typically occurred with greater cover in the Reference (5 - 12.5%) than the Worst (<5%) wetlands, suggesting potential indicators of disturbance. *Senecio halimifolius* and *E. tectorum* occurred in Worst conditions with a typical cover value that represents the Braun Blanquet level of between 2 to 10 specimens per wetland. *Isolepis cernua* occurred in Worst wetlands with typical cover of less than 2% suggesting 1 to 10 individual specimens.

The difference in average cover of the grass *Stenotaphrum secundatum* and the rush *Juncus kraussii* between Reference and Worst disturbed Lotus River sites was 5%. In the wetlands in which these last two species occurred, their typical cover was equivalent to the same rank on the Braun Blanquet scale (5-12.5% cover), and the difference is not large enough to be of consistent value as indicator species or metrics in an index.

The typical cover values of these tolerant and sensitive species are indicative of wetlands at different ends of the spectrum of environmental condition. The species can be used independently and/or potentially collectively as “total tolerant” or “total sensitive species cover” metrics of environmental condition in a phyto-assessment index.

6.4. Univariate analysis of species diversity for Lotus wetlands

An exploration of the species diversity variables of the vegetation of the Lotus River wetlands with Reference and Worst environmental condition has the potential to reveal vegetation based metrics for use in the development of an index of environmental condition for this locality. To this end an analysis of the various measures of species diversity was performed using DIVERSE in PRIMER-E (Clarke and Gorley 2006; as described in Section 2.10.6 of this volume). The following diversity measures were determined per wetland:

- Total number of species (S);
- Species richness as determined by Margalef’s index (d) (Margalef 1975);

- Species diversity: Shannon-Weiner (H') (Shannon and Weaver 1949) and/or Simpson's diversity index (Dominance (λ) or Evenness (1- λ)) (Simpson 1949).

These values for each wetland were then used in pair-wise tests to determine diversity differences between Reference and Worst wetlands.

6.4.1. Diversity measures: Lotus River

A total of 91 species were recorded in the Lotus River locality; 72 of these occurred in both the Reference and the Worst-disturbed wetlands and at least one other wetland from the Western Coastal Slopes wetland region. Wetlands Lot12 and Lot14, which had atypical vegetation assemblages (Fig. 6.1a and b), were excluded from these analyses. The Reference wetlands had more species, with similar cover but greater diversity as revealed by lower species dominance than the Worst disturbed wetlands (Table 6.8). For this and all subsequent Lotus River species diversity comparisons Monte Carlo asymptotic significance values were used (Hope 1968).

Table 6.8: Differences in aspects of diversity between Reference and Worst Lotus wetlands. Values in disturbance categories represent the average per category (±S.E.) for all Lotus wetlands.

Diversity variable	Taxon type	Reference	Worst	t-test	p-value
number	All species	23.2 (3.4)	14.3 (2.3)	2.5	0.05
cover	All species	94 ± 1%	95 ± 2%	-	n.s.
dominance (Simpson)	All species	0.15 (0.04)	0.2 (0.02)	2.9	0.05
number	Aliens	6.5 (0.9)	4.8 (0.9)	2.96	0.05
cover	Aliens	15 ± 4%	40 ± 7%	-	n.s.
richness (Margalef)	Aliens	2.15 (0.36)	1.02 (0.21)	2.6	0.05
Number	FW to OW††	8.5 (0.9)	5.5 (0.7)	8.5	0.05
Cover	FW to OW	40 ± 8%	60 ± 7%	-	n.s.
Richness (Margalef)	FW to OW	1 (0.16)	0.3 (0.2)	2.35	0.05
Number	Graminoids	13 (1.5)	8 (0.8)	2.9	0.05
Cover	Graminoids	78 ± 5%	86 ± 3%	-	n.s.
Dominance (Simpson')	Graminoids	0.15 (0.02)	0.23 (0.02)	3.1	0.05
Number	Woody	4.5 (0.7)	0.6 (0.2)	5.4	0.001
Cover	Woody	8 ± 2.5%	1 ± 0.8%	2.5	0.05
Biodiversity (Shannon-Wiener)	Woody	0.88 (0.2)	0	4.5	0.01

††Species with Facultative to Obligate affinity for the wetland habitat (*sensu* Reed 1999) Based on Glen

Greater total cover of alien species was observed in Worst-disturbed wetlands, despite lower average number of alien species and consequently lower alien species richness than wetlands in a natural condition. There were a greater number and greater species richness of obligate wetland plants in Reference than in Worst wetlands. A greater number of graminoid plants, with insignificantly low total coverage, were recorded in Reference wetlands, which were less dominated by single or a few species than the Worst disturbed wetlands were. A greater number, cover and diversity of woody plants were contained in the Reference than in Worst disturbed wetlands. These diversity measures therefore provide potentially useful metrics for development of an index for the determination of environmental condition in the Lotus River wetlands.

6.5. Discussion

There are significant differences in the vegetation assemblages ($t=1.9$, $p<0.01$) of the Reference and Worst-disturbed wetlands from the Lotus River locality of the Cape Flats. The Lotus River is the only one of the eight localities assessed in which significant differences were apparent at this scale (weighted average cover) of investigation. A PERMANOVA analysis, using both the functional group and inventory data revealed similar results to those based on the weighted-average cover/abundance values. No other instances of significant differences between wetlands in *a priori* determined disturbance categories (Reference, Moderate, Worst) were found. The clumping of supralittoral and littoral habitat zones into a single average value per species per wetland may have masked some differences between disturbance categories. However, on independently assessing the average values of vegetation assemblages from each hydrological zone (supralittoral and littoral), no greater differentiation was apparent between *a priori* determined environmental condition categories. An investigation of the difference between disturbance categories as based on individual vegetation sample values representative of each wetland is therefore carried out in the following chapter (Chapter 7).

For the Lotus River, human disturbance, dissolved oxygen content of the water-column, soil redox potential and related SRP and soil phosphorus concentrations, as well as water-soluble calcium concentration, have been shown to differentiate and, in part, to explain the difference between the vegetation assemblages of Reference and Worst disturbed wetlands. Differences in diversity at the level of both species and vegetation community that appear to be characteristic of Reference and Worst wetlands have been identified. It may be that they can be used for the development of metrics for the phyto-assessment of environmental condition in this locality. The geographical extent over which these metrics would be accurate would be limited to the Lotus River. This does not meet the objective of a regional, or even a national assessment tool.

In the Lotus locality, a considerable range of disturbance was included from extremely eutrophic and physically degraded agricultural and urban impacted wetlands to those in a natural and actively conserved state (Nature Reserve). Thus, considerable contrast can be expected between the Reference and Worst disturbed wetlands. The paucity of Reference wetlands sampled at all localities other than Lotus, as shown in Table 6.1 does suggest that Lotus wetlands provided the greatest potential contrast between Reference and Worst disturbance. The narrow range of disturbance at other localities may be responsible for the lack of discernable differences in vegetation assemblages at these localities. On the Cape Flats, the Kenilworth locality contained what were originally categorised as Reference (n=2) and Worst (n=4) wetlands yet it emerged on enquiry with previous land managers of this area that all of the wetlands were artificially created. Thus, what appeared to represent Reference conditions is not necessarily truly representative of a natural state. Subsequent land use around these wetlands does appear to have promoted the development of some less disturbed, potentially Reference wetlands. At the Driftsands locality on the Kuils River floodplain on the Cape Flats, after adjustment of the initial HDS disturbance ranking by the independently measured nutrient concentrations (creating the HDTS categories), only two wetlands were considered to be Reference. Similarly, of all wetlands sampled on the West Coast, only a single wetland was considered Reference. In the Overberg there were no Reference wetlands in the Hermanus locality and no Worst disturbed wetlands in the Agulhas Plain sample set. The paucity of samples in many of the locations (Berg River (n=3) and Hermanus (n=3)) also reduced the possibility of contrasts. The Moderate category of intermediately disturbed wetlands was also included in the PERMANOVA analyses. No significant differences in vegetation assemblage were apparent when compared to the Worst and/or Reference wetlands within each locality.

At the outset of the study, the little existing information about wetland vegetation in the region suggested that, at least at the sub-regional scale, wetlands of similar HGM type (such as depressions) dominated by Cape Lowland Freshwater (CLF) vegetation should contain relatively homogenous species assemblages under natural or reference conditions (Mucina *et al.* 2006a). Some local differences due to variation in soil type were expected, but the driving forces of waterlogging and associated mineral concentration were expected to dominate and create similar azonal vegetation features in the landscape. The assumption was therefore that the sub-regions would hold enough comparable wetlands of different disturbance categories to facilitate comparison across strong contrasts of disturbance. In reality, the differences between the natural or minimally impaired vegetation of each locality within each sub-region (such as between the Lotus and Driftsands *Strandveld* associated wetlands of the Cape Flats) is so considerable that these localities cannot be combined in order to indentify useful metrics for phyto-assessment. This significant difference between localities reduced the comparable data sets to the locality scale and thus in some cases to too few samples for sufficient contrast between disturbance categories. This is an important finding, suggesting that the determination of ecoregions with similar vegetation is very important for facilitating collation of comparable data sets.

The lack of difference in species assemblage in wetlands with different intensities of human disturbance can also potentially be explained by the incorporation of too wide a range of wetland vegetation types, HGMs and habitat types into the data set. Within the Cape Flats data set, CLF vegetation dominated endorheic depressions were however the predominant wetland type (n=28 of 32 wetlands) yet each locality still held considerably different wetland vegetation communities. Local geology, soil types and climatic differences that determine the distribution of zonal vegetation are likely to have greater influence than previously believed on the distribution of supposedly “azonal” vegetation such as in the wetlands of the present study.

The investigation into differences in soil parameters within and among wetlands, revealed that investigation of environmental influence would best be conducted at the scale of sample plots rather than at the scale of whole wetlands. The influence of disturbance at this finer spatial scale of investigation (per vegetation plot) is described in Chapter 7.

7. RELEVÉ SCALE ANALYSIS

7.1. Multivariate analysis of vegetation relevés

The numerous localities and disturbance categories within the Western Coastal Slopes dataset suggests considerable natural variability that can only be separated out by use of multivariate analysis procedures. Using data for each vegetation sample (relevé), this chapter examines significant differences in vegetation assemblages for all the sub-regions and the localities within them, as well as differences between degrees of disturbance. The disturbance categories that were used were those derived after cross checking Human Disturbance Score (HDS) against the impact of eutrophication in Chapter 5 and were thus based on the combination of the Human Disturbance and Trophic state Scores (HDTS).

The results of the PERMANOVA analyses are shown in Table 7.1. The following conclusions can be drawn:

1. each of the West Coast, Cape Flats and Overberg sub-regions hold significantly different assemblages of wetland vegetation (pseudo-F=1.6, $p<0.01$);
2. across the whole collective of vegetation relevés that were sampled, the disturbance categories do not hold significantly different vegetation assemblages;
3. each locality (nested within sub-regions) holds a significantly different wetland vegetation assemblage from every other locality (pseudo-$F_{2,8}$ =3.7, p=0.001);
4. within each sub-region, no significant difference was apparent between the collective vegetation assemblages of the wetlands from different disturbance categories. This suggested that there were no typical and consistent plant responses to different degrees of disturbance amongst the different localities of a sub-region; and that differences between disturbance categories must be dealt with independently for each locality.
5. within each locality, as isolated subsets of each sub-region [locality(sub-regions)xdisturbance], differences in vegetation relevés between human disturbance categories are apparent (*pseudo-$F_{2,6}$* = 2.24, $p < 0.001$).

These results reiterate the findings reported in the previous chapter, namely that the vegetation of each disturbance category within each locality (as a subset of sub-region) is significantly different from that in other disturbance categories and in other localities. These analyses add no new information to that already established from the wetland average data set. However, *posteriori* pair-wise analysis (Table 7.2) revealed that using the relevé data, significant differences are apparent between the disturbance categories in many more localities than was apparent when using the wetland average data. The vegetation sample data therefore undoubtedly provide greater discriminatory power between disturbance categories than was provided by the wetland average data set.

Table 7.1: PERMANOVA of the vegetation sample data for the Western Coastal Slope wetland region. Relationships that are significant at $p < 0.01$ are marked with *.

Species cover/abundance		Degrees of freedom	Pseudo-F	p-value
#1	Sub-Region	2	1.6	0.006*
#2	Disturbance	2	1.2	0.3
#3	Locality(Sub-Region)	8	3.7	0.001*
#4	Sub-RegionxDisturbance	4	1.3	0.3
#5	Locality(SubRegion)xDisturbance**	6	2.2	0.001*
	Residual	376		
	Total	399		

**Term has one or more empty cells (locality lacks one of the categories of disturbance [i.e. in Overberg – no Worst])

Table 7.2: Localities that held significantly different vegetation communities in different disturbance categories (p<0.05). Disturbance category comparisons with the greatest difference in any locality are underlined.

Sub-region	Locality	Groups	t-test	p-value
West Coast	Berg River	Worst *vs.* Moderate	1.6	0.004
	Darling	Worst *vs.* Moderate	1.3	0.04
		Worst *vs.* Reference	1.4	0.006
Cape Flats	Driftsands	Worst *vs.* Moderate	1.3	0.05
		Worst *vs.* Reference	1.6	0.006
		Moderate *vs.* Reference	1.4	0.02
	Kenilworth	Worst *vs.* Moderate	1.7	0.004
		Worst *vs.* Reference	1.9	0.001
		Moderate *vs.* Reference	1.4	0.01
	Lotus River	Worst *vs.* Moderate	1.4	0.02
		Worst *vs.* Reference	2.2	0.001
		Moderate *vs.* Reference	1.6	0.001
Overberg	Hermanus	Worst *vs.* Moderate	1.7	0.001
Overberg	Ratels	Moderate *vs.* Reference	1.3	0.04
Overberg	Waskraalvlei	Worst *vs.* Reference	1.2	0.02

In all of the Cape Flats and Darling wetlands, the greatest difference is apparent between the most extreme comparisons of disturbance, as evidenced by greater significance levels (lower p-values) between Reference and Worst categories. The tests including the ‘intermediate’ or Moderate category also reveal greater difference than was apparent using the wetland average data in chapter 6. Within localities, it would be possible to search for metrics of difference between all of these significantly different categories of disturbance compared in Table 7.2. Metrics developed from more precisely defined habitat units are likely to provide stronger metrics for phyto-assessment than can be determined from either the wetland average data in Chapter 6 or the hydrologically undifferentiated vegetation sample comparisons in Table 7.2. The hydrologically undifferentiated sample comparisons were done by simply amalgamating all the vegetation sample data together, and then comparing them.

7.1.1. Multivariate analysis of vegetation relevés in different hydrological habitats

Table 7.3a lists the PERMANOVA results after subdividing the data from each locality into supralittoral and littoral habitats. It is apparent that the two habitats hold significantly different vegetation when the whole Western Coastal Slope dataset is assessed (*pseudo-F*=2.2, p<0.03: 2nd row of Table 7.3). An examination of the difference between the vegetation relevés of littoral and supralittoral habitats in each locality as nested units of each sub-region [locality(sub-regions)xhabitat] revealed significant difference between habitats in most of the localities that were sampled (*pseudo-F* $_{2,7}$ = 2.09, p < 0.001: Table 7.3b).

Table 7.3a: PERMANOVA of the vegetation sample data after being allocated to different hydrological habitats. Relationships that are significant at p < 0.01 are marked *.

Species cover/abundance	Degrees of freedom	Pseudo-F	p-value
Sub-region	2	1.7	0.002*
Habitat	1	2.2	0.03*
Locality(Sub-region)	8	3.7	0.001*
Sub-regionxHabitat	2	1.4	0.1
Locality(Sub-region)xHabitat	7	2.09	0.001*
Residual	376		
Total	395		

This analysis suggests that generally, the plant assemblage of the supralittoral habitat is significantly different from that of the littoral habitat within most of the localities that were sampled. This is an important finding as it suggests that considerable natural variability should be expected between the assemblages subject to different hydroperiod. Failure to recognize this difference as natural, and to account for this difference within analyses (by separately analyzing the naturally different habitat sample sets), may mask differences in attributes of species or assemblages between disturbance categories.

A posteriori PERMANOVA (Table 7.3b) revealed significant differences between supralittoral and littoral habitats in almost all localities. Exceptions were the hillslope seeps of Hermanus, and in two wetlands in the Agulhas area. In these latter wetlands, however, either the magnitude of hydrological difference between the relevés assigned to different hydrological habitats was small (Hermanus), or the number of relevés representing one of the habitats was too small (Melkbos wetlands).

Table 7.3b: Localities that held different vegetation communities in supralittoral *vs.* littoral hydrological zones as determined using t-tests.

Sub-region	Locality	t-test	p-value
West Coast	Berg River	1.74	0.001**
	Darling	1.32	0.014*
	Verlorevlei	1.67	0.001**
Cape Flats	Driftsands	1.91	0.001**
	Kenilworth	1.95	0.001**
	Lotus	2.03	0.001**
Overberg	Hermanus	1.04	0.3
	Melkbos – Agulhas Plain	0.93	0.5
	Ratels – Agulhas Plain	2.02	0.001**
	Waskraalvlei – Agulhas Plain	1.33	0.002**

Results of these analyses suggest that the determination of difference in species assemblages between disturbance categories should be dealt with independently in each locality and in each of the littoral and supralittoral hydrological habitats. To re-iterate, the separation into different habitats within each locality should make the detection of unnatural or anthropogenic influence more feasible.

7.1.2. Multivariate analysis of hydrological habitats per disturbance category

Separation of all relevés into supralittoral and littoral subsets within each locality facilitated comparison between disturbance categories. A four-way PERMANOVA revealed significant differences between the vegetation relevés from different hydrological habitats and different disturbance categories in each locality of the sub-regions [locality(sub-region)xdisturbancexhabitat] (*pseudo-F* $_{2,\ 5}$ = 1.3, $p < 0.003$). The localities that held significantly different species assemblages in each disturbance category within hydrological habitats were revealed with *a posteriori* PERMANOVA and are presented below (Table 7.4).

The PERMANOVA partitions the localities as subsets of the sub-regions. The groups compared in the above analysis are therefore the relevés within a hydrological zone (littoral or supralittoral) and a disturbance category (Reference, Moderate or Worst) per locality. Within these groups (Table 7.4), heterogeneity of dispersion (i.e. a considerable range of variability in vegetation cover) existed between each of the disturbance categories compared in Table 7.5.

Table 7.4: Localities that held different species assemblages per hydrological zone in different disturbance categories. Disturbance category comparisons with the greatest difference in any locality are underlined.

Sub-region	**Locality**	**Habitat**	**Disturbance categories**	**t-test**	**(p-value)**
West Coast	Verlorevlei	Littoral	Worst *vs.* Moderate	1.39	0.023
Cape Flats	Driftsands	Supralittoral	Moderate *vs.* Reference	1.5	0.04
		Littoral	Worst *vs.* Moderate	1.7	0.004
		Littoral	Worst *vs.* Reference	1.6	0.004
		Littoral	Moderate *vs.* Reference	1.4	0.05
	Kenilworth	Supralittoral	Worst *vs.* Moderate	1.4	0.004
		Supralittoral	Worst *vs.* Reference	1.6	<u>0.001</u>
		Supralittoral	Moderate *vs.* Reference	1.5	0.002
		Littoral	Worst *vs.* Moderate	1.5	0.04
		Littoral	Worst *vs.* Reference	1.7	<u>0.008</u>
	Lotus	Supralittoral	Worst *vs.* Reference	1.5	<u>0.006</u>
		Supralittoral	Moderate *vs.* Reference	1.6	0.002
		Littoral	Worst *vs.* Moderate	1.5	0.04
		Littoral	Worst *vs.* Reference	2.05	<u>0.001</u>
Overberg	Hermanus	Supralittoral	Worst *vs.* Moderate	1.7	0.001
	Ratels	Supralittoral	Moderate *vs.* Reference	1.4	0.04

For these three locality habitat groups in Table 7.5, the significant differences exhibited between the units of the *a posteriori* PERMANOVA comparison (in Table 7.4) may therefore be an artefact of unequal dispersion (different range of cover value differences) between disturbance categories. Results from an analysis performed with groups that do not have homogenous dispersions between disturbance categories, could lead to the incorrect acceptance that the relevés representing these categories of disturbance are significantly different from one other. These differences in dispersion may not be substantial enough to inflate the error rates of the PERMANOVA test, which is robust to some heterogeneity of dispersion (Anderson *et al.* 2008); however, the small sample size especially at Verlorevlei perhaps negates this and ordination is required in order to be able to determine if significant dispersion differences do exist.

Table 7.5: Heterogeneity of dispersion between relevés of the different disturbance categories from habitats within localities.

Locality + Habitat	Disturbance categories (n= # relevés)	t-test	p-value
Verlorevlei – littoral	Moderate(n=12) *vs.* Worst (n=4)	3.1	0.05
Driftsands – supralittoral	Reference (n=8) *vs.* Moderate (n=19)	2.6	0.04
Lotus – littoral	Reference (n=19) *vs.* Worst (n=20)	2.8	0.01

Ordination of these groups reveals:

- Considerably greater dispersion is apparent in the Moderate than Worst Verlorevlei littoral relevés; suggesting that the difference in dispersion does invalidate the PERMANOVA result.
- Considerably greater dispersion is apparent in the Driftsands supralittoral Moderate than Reference relevés, suggesting that the difference in dispersion does invalidate the PERMANOVA result.
- A strong locational difference is apparent between the Lotus littoral Reference and Worst relevés; suggesting the differences determined by PERMANOVA is an accurate representation of different assemblages in Reference *vs.* Worst relevés.

Thus 14 units of comparison that hold different vegetation assemblages (those in Table 7.4 other than Driftsands-supralittoral and Verlorevlei-littoral) can potentially be used to define phyto-assessment metrics. In each habitat and locality combination (e.g. littoral Kenilworth), it is necessary to determine whether the vegetation differences are indeed the result of human disturbance rather than of natural environmental variability. In the previous chapter this determination was successfully achieved using distance linear modelling (DistLM) in the Lotus River data set (Section 6.2). The same method was therefore employed in Section 7.2.

7.2. Identification of metrics for each hydrological habitat within each locality

In situations in which human disturbance does appear to be the cause of difference between vegetation relevés, attributes of the vegetation that were characteristically associated with different disturbance categories were identified as measurements (metrics) for phyto-assessment purposes. These metrics were based on discriminatory species, or diversity differences (community attributes) between the vegetation assemblages of different environmental condition or disturbance categories (HDTS). Where different vegetation communities were shown to exist between disturbance categories (Table 7.4), separate determinations were done of the discriminatory species or community attributes that might be used to create metrics for phyto-assessment. This process was done for each of the habitat-locality combinations. The following is a brief outline of the method for ascertaining significant impacts of human disturbance and the characteristic species associated with each disturbance category.

7.2.1. Method for metric determination

The description of the statistical techniques used in this part of the study, is provided in Section 2.10.5.2 and only a brief synopsis of the steps taken in the determination of metrics is therefore provided here:

1. Remove any outliers apparent in ordination and dendrograms,
2. as well as one of any pair of collinear variables as determined using multivariate Pearson correlation,
3. and those variables that did not show any significant difference between disturbance categories,
4. Perform a multivariate determination of the environmental variables most responsible for the differences between vegetation assemblages in wetlands of each disturbance category, using distance-based redundancy analysis (dbRDA) within Distance Linear Modelling (DistLM) (Legendre and Anderson 1999, McArdle and Anderson 2001).

This procedure is a multivariate multiple regression that identifies the environmental variables that best explain the distribution of biotic relevés between categories. DistLM performs partitioning between disturbance categories, tests which linear combination of variables is important, and chooses alternative combinations of environmental variables that best explain the species distribution between categories of disturbance. The dbRDA provides a constrained ordination of the best fit of the linear combination of the environmental variables that explain the greatest variation within the vegetation relevés from different categories of disturbance (Anderson et al.2008).

In situations in which anthropogenic disturbance appears to be the cause of the significant differences shown to exist (Table 7.4) between relevés from disturbance categories within a habitat of a given locality, discriminatory species were identified using similarity percentage analysis (SIMPER – see Section 2.11.5 for details). Whilst the SIMPER analysis reveals the "average" cover values of a species per disturbance category within a given a habitat of a given locality (e.g. the average percentage cover of *Typha capensis* within all Worst disturbed littoral relevés from Kenilworth), this average value under-represents the cover values in the actual relevés. Typical cover was therefore ascertained from the average of cover within relevés within which a species was found (but excluding those where the species was not present – see Section 6.3.1). Typical cover values are shown together with the average cover values calculated by SIMPER in Figures 7.2, 7.5, 7.8, 7.11, 7.14 and 7.17. These "average" and "typical" cover values per disturbance category within a given habitat of a locality were then used to identify possible metrics of disturbance. The use of either "average" or "typical" measures each require a different approach in the way a metric is used for phyto-assessment purposes.

Differences in various aspects of diversity between disturbance categories were determined for a number of attributes using both DIVERSE (PRIMER-E) and some basic interrogation of the data set. DIVERSE generates diversity indices from the vegetation relevés data. In the current study, species counts (number), cover/abundance, species richness, and dominance or evenness were focused on as described in Chapter 6 (Section 6.4). Standardized cover values were used such that each taxa subset represents a proportion of total cover. As plants may occur at multiple strata within a sample plot, this can result in more than 100% total cover being reported, so the cover value is standardized by total cover. These diversity indices and species counts or cover determinations were made using the relevés data. Thereafter, significant differences between disturbance categories were established with pair-wise t-tests using the relevés as permutable replicates for each disturbance category within a habitat of a given locality. The use of t-tests, with significance values generated using permutation, removes the need to "jack-knife" the sample values before determining significant differences. These t-tests are therefore relatively robust even at limited sample sizes.

The diversity indices were determined for the entire set of species within a habitat-locality dataset and for species subsets determined by growth form (e.g. graminoids or shrubs), structural aspects of plant form (woodiness), life history attributes (annuals *vs.* perennials), affinity to the wetland habitat (obligate or facultative affiliation to wetland habitat (*sensu* Reed 1988)), and origin (indigenous *vs.* alien). The existence of species with different degrees of tolerance to disturbance (sensitive *vs.* tolerant taxa) and their potential as possible indicator species was not explored for each locality dataset but rather for the whole Western Coastal Slope set of data and will be described in Section 7.3.2. All alien species are marked with an asterisk in all figures and text where they are referred to by name.

7.2.2. Hermanus supralittoral: Moderate versus Worst

Three wetlands were sampled in Hermanus with a total of 16 relevés in a Moderate category of disturbance and 10 in a Worst category. The Hermanus wetland vegetation relevés are all from hillslope seeps of similar landform. They are dominated by Cape Lowland Freshwater vegetation and sandy soils with small percentages of clay and silt. None of the Hermanus supralittoral vegetation relevés (relevés) or the collective of environmental variables for each sample are obvious outliers in dendrograms or ordinations.

A number of the environmental variables were collinear at >0.95 or <-0.95:

- TIN was inversely collinear with SRP (-0.998);
- the percentage of silt and sand were inversely collinear (-0.96);
- Soil pH was inversely collinear to soil redox (-0.999); and
- Soil potassium was collinear to exchangeable potassium cations (0.999).

Soluble reactive phosphorus, sand, soil redox and potassium were therefore removed from the environmental dataset.

Six of the 54 environmental variables that were measured in the Hermanus wetlands showed different average values between the Moderate and Worst vegetation relevés (Table 7.6). The average values of Total Inorganic Nitrogen (TIN) concentration in the water column, percentage silt, soil pH, exchangeable hydrogen and calcium cations and human impact (HDS) were different between Moderate and Worst vegetation relevés. Statistical differences of the six variables between Moderate and Worst Hermanus relevés were determined using pair-wise PERMANOVA in one-way analyses within the factor “Disturbance”. The results of these tests are shown in Table 7.6.

Table 7.6: Environmental variables that occur with different average value (± S.E.) in the Moderate and Worst disturbed relevés of the Hermanus wetlands.

Parameter	Unit	Moderate (n=16)	Worst (n=10)	t-test	p-value
TIN	μgL^{-1}	107 (7)	1269 (0)	140.5	0.001
Silt	%	4.5 (0.8)	7.4 (0.7)	2.6	0.01
pH soil	pH	3.3 (0.13)	4.5 (0.14)	6.39	0.001
Exca H^+	$cmol(^+).kg^{-1}$	6.02 (1.2)	2.6 (0.4)	2.51	0.02
Exca Ca^{++}	$cmol(^+).kg^{-1}$	1.33 (0.3)	3.7 (0.8)	3.14	0.005
HDS	Score	83 (1)	100 (0)	14.81	0.001

Exca H^+ is the exchangeable hydrogen cations

Whilst for each environmental variable, the averages (± S.E.) from the Moderate or Worst relevés are presented above; individual sample values are however used by the pair-wise PERMANOVA generation of t-tests and in the multi-dimensional DistLM procedure below.

7.2.2.1. Hermanus DistLM Results

The distance-based linear model showed that of six the retained environmental variables (Table 7.6), TIN in the water column, silt, soil pH, HDS and exchangeable cations hydrogen and calcium each independently explained a considerable and significant percentage ($p<0.05$) of the variation between Moderate and Worst disturbed vegetation relevés (Marginal tests, Table 7.7).

Looking at the multivariate interaction, the best solution (the linear combination of variables that best explain the variable association of biotic assemblages to different disturbance categories), which explained 29.16% of the variability between Moderate and Worst wetlands, was the combination of TIN, soil pH, human disturbance and percentages of silt (Best Solutions: (1,3,5,6) Table 7.7). All potentially best solutions to the model (with less than two Adjusted R^2 units difference from the first solution) included TIN concentration and human disturbance. An order of magnitude more TIN was recorded in the Worst disturbed samples at wetland Her02, which was categorized as Worst disturbed than in the Moderate samples of wetlands Her01 and Her03, both of which were categorized as Moderate. It must be remembered that TIN was measured as an average value per wetland and not separately measured per individual sample as was the case for soil nutrients. The high TIN concentrations and other HDS-determined disturbance impacts in the Worst disturbed samples in the Hermanus locality, all point to anthropogenic influence in causing difference

Table 7.7: Test statistics for DistLM analysis based on "best" selection procedure and the Adjusted R^2 selection criterion for the average vegetation assemblage in Moderate and Worst disturbed Hermanus vegetation relevés. SS = Sum of Squares, RSS = Residual Sum of Squares, R^2 = RSS/SS. Significance at p<0.05 marked *.

MARGINAL TESTS:				
Variable	**SS(trace)**	**Pseudo-F**	**p-value**	**% of total variation**
(1) TIN	12165	3.1	0.001*	12.89
(2) pH soil	10722	2.7	0.003*	11.36
(3) HDS	11471	2.9	0.001*	12.16
(4) Exca Ca^{++}	8637	2.1	0.007*	9.15
(5) Exca H^{+}	5366	1.3	0.2	5.69
(6) % Silt	4487	1.05	0.4	4.75
		Best Solutions		
Variable Selections	**Adj R^2**	**RSS**	**R^2**	**% of total variation**
1,3,5,6	0.13415	66852	0.2916	29.16
1,2,5,6	0.12423	67618	0.2835	28.35
1,2,3,5,6	0.12256	63976	0.3221	32.21
1,3,6	0.11966	71747	0.2397	23.97

between the vegetation assemblages of the Worst and Moderate supralittoral vegetation samples. It is apparent that TIN and HDS are also strongly collinear (0.94), whilst marginally less than the suggested 95%, this was not excluded by searching for collinear variables in the PRIMER package. The inclusion of both variables perhaps affected the model and rerunning the model without TIN and including some of the variables which had average values that were not different between disturbance categories and were not collinear, still selected HDS as the most important variable separating Moderate from Worst vegetation samples (Figure 7.1).

A distance-based Redundancy Analysis (dbRDA) plot of the DistLM separation of the different supralittoral relevés is presented below (Figure 7.1) with Pearson Rank correlation vector overlays of the variables that contributed to the distribution pattern (r>0.4). Vector length reflects the univariate importance of each variable in explaining the modelled pattern and interaction effects are excluded (Ter Braak 1990). In the dbRDA plot, based on the multivariate relationship of environmental variables to species cover, increasing HDS (0.894), soil pH (0.354) and resistance (0.264) best explain separation from left to right between Moderate and Worst relevés along the primary or x-axis (dbRDA 1). This axis explained 14.1% of total variability between relevés. Decreasing resistance (-0.524), soil pH (-0.483), silt (-0.402) and increasing exchangeable hydrogen cations (0.495) best explain the separation between wetlands from bottom to top along the y-axis (dbRDA 2). The y-axis explained 6.6% of total variation. Together these two axes explained 67.1% of the model fitted variation and 20.7% of the total observed variation in the combined environmental and vegetation assemblage data sets.

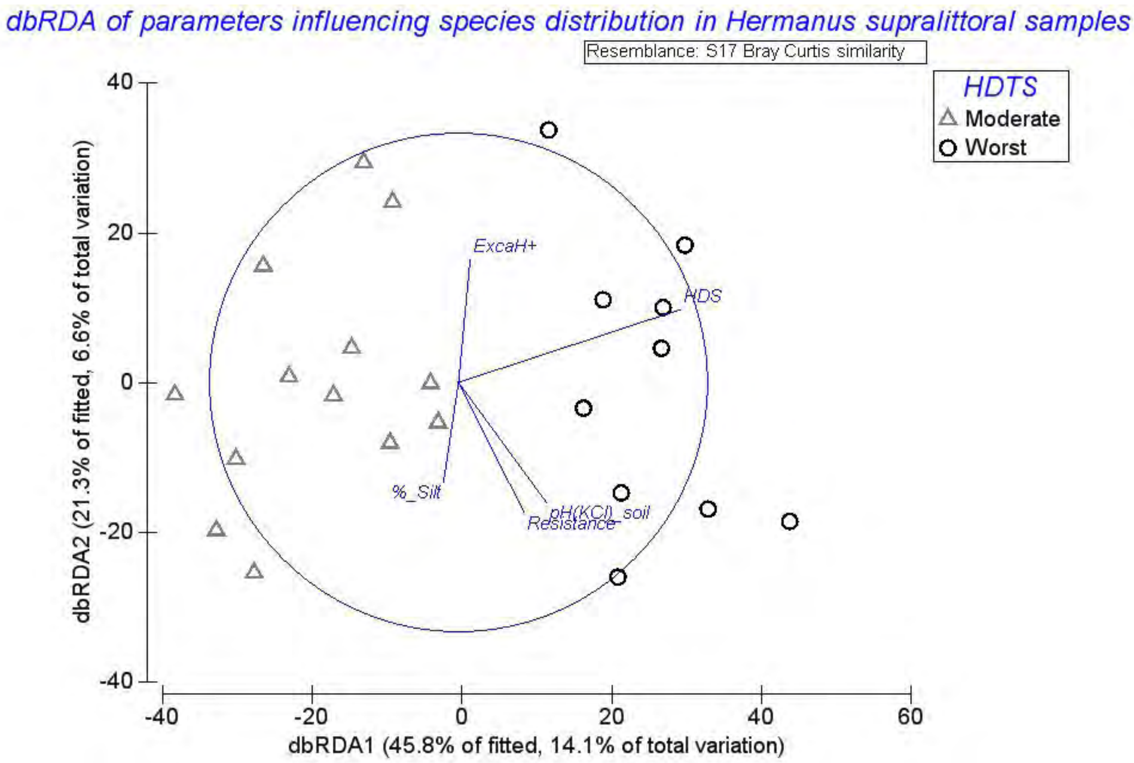


Figure 7.1: Constrained ordination by the most influential environmental variables in determining different vegetation assemblages in Moderate and Worst disturbed vegetation relevés of the Hermanus locality determined with dbRDA, after excluding TIN. Vectors are Pearson correlations (r=0.4). Exca H^+ is the exchangeable hydrogen cations.

As determined by the qualitative HDS assessment process, the Worst impacted relevés at Hermanus had been subjected to a greater level of physical impact (t=21, p=0.001), greater water quality disturbance (t=9.7, p=0.001) and marginally more water loss than the Moderate relevés. A relatively similar buffer width was apparent around all wetlands. In combination with the dbRDA outcome, it appears therefore that human disturbance is responsible for observed differences in vegetation between the disturbance categories. Species that best represent each disturbance category were therefore sought as a means of determining metrics for phyto-assessment purposes. This process is described below.

7.2.2.2. Discriminatory species in Hermanus seeps

Analysis of the percentage similarity (SIMPER: as described in Section 6.3) of the vegetation assemblages was performed to determine which species discriminated between Moderate and Worst disturbed vegetation relevés in the supralittoral habitat of the Hermanus seeps. The species determined by SIMPER to occur with consistently discriminatory cover/abundance in the relevés of Moderate and Worst categories are presented in Figure 7.2. This figure is a graphical representation of the average and typical cover values of species that occur with discriminatory or consistently greater or lesser value in relevés from each disturbance category.

Typical cover values, based on the average cover in all relevés where each species was encountered (see Section 6.3.1), more accurately represent cover-values encountered in the field than do the average cover values generated from all relevés in the SIMPER analysis procedure. Typical and average covers represent different values that can potentially both be used to determine metrics. Large differences between 'typical' and 'average' cover occurs when a species does not occur frequently in a community ("low fidelity" in phytosociology). The use of species with low fidelity as indicator species is therefore

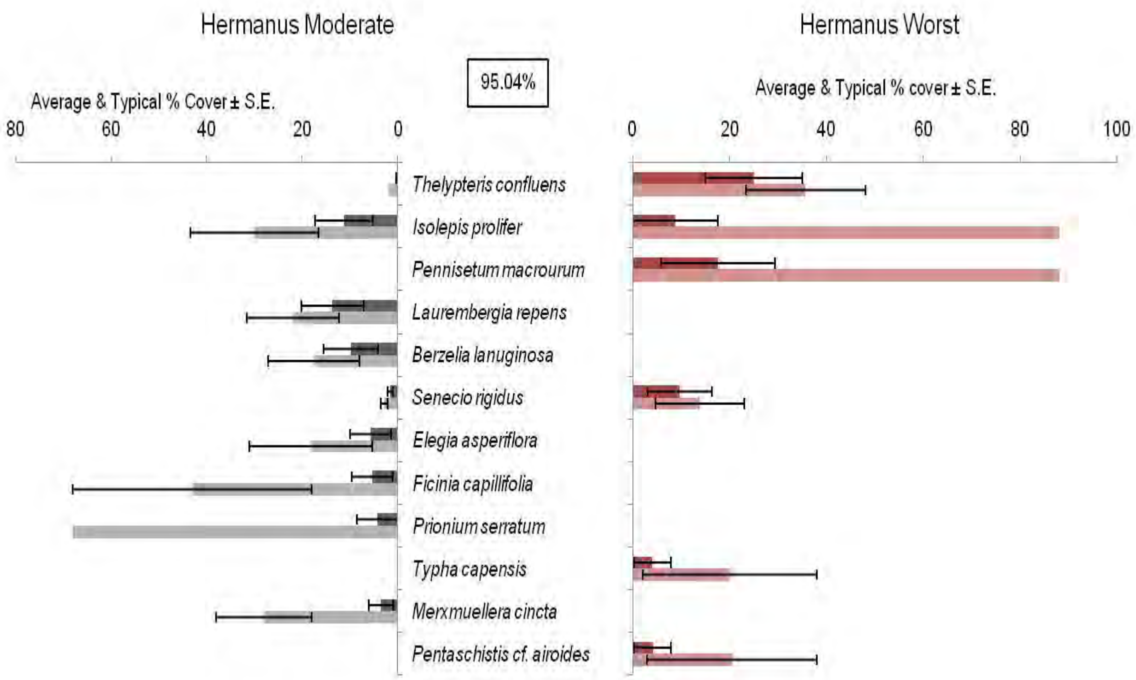


Figure 7.2: Average (dark bars) and typical (light bars) species cover (± standard error) in the Moderate and Worst disturbed supralittoral-Hermanus vegetation relevés. Species without error bars have standard error of zero (often meaning they were present only in a single sample). The dissimilarity percentage between the communities of the different disturbance categories is presented in the rectangle at the top of the graph.

questionable. However, as targeted sampling chooses representative vegetation stands, even species with apparently low fidelity in the data set are characteristic of a given community of plants found within the overall assemblage for the wetlands of a locality (See Section 8.7.6).

A total of 16 Moderate and 10 Worst disturbed vegetation relevés were compared. The use of a minimum of 30 comparable wetlands is recommended (to reduce effects of spatial autocorrelation) for the identification of reliable metrics for any region with uniform vegetation (US EPA 1998c; Section 2.7.2.1). Thus conclusions based on the limited number of Hermanus relevés (taken from only three wetlands) must be treated only as a preliminary investigation, since spatial autocorrelation between relevés within the Hermanus locality may be influencing the outcome.

Figure 7.2 shows that within the Hermanus supralittoral data set, the fern *Thelypteris confluens*, the pioneer sedge *Isolepis prolifer,* the post-fire pioneering herb *Senecio rigidus* the grass *Pentaschistis airoides* as well as the mega-graminoids *Pennisetum macrourum* (low fidelity) and *Typha capensis,* all typically occurred with greater abundance in Worst disturbed than in Moderate relevés. The mega-graminoid *Merxmuellera cincta* exhibited the reverse trend, occurring with greater typical abundance in relevés with Moderate disturbance. The sedge *Ficinia capillifolia, the* restio *Elegia asperifolia,* the creeping herb *Laurembergia repens, the* shrub *Berzelia lanuginosa* and the mega-sedge *Prionium serratum* (low fidelity) also occurred with greater abundance in Moderately than in Worst-impacted relevés.

Without further samples (relevés) to validate the findings of this comparison, the affiliation of any of these species with different disturbance categories should be treated with caution. Comparison with the discriminatory species from the supralittoral habitat of other localities, however, may reveal species that have consistent affiliation with disturbance categories throughout the Western Coastal Slope.

7.2.2.3. Differences in diversity between Moderate and Worst relevés

A total of 79 species were recorded in the Hermanus supralittoral relevés. In Moderate relevés a total of 58 species with an average of 11.7% cover per species were sampled relative to 31 species with 16% mean cover per species in the Worst relevés. Fewer species with greater average cover per species in the Worst than Reference relevés is perhaps indicative of a greater frequency of disturbance events or degree of impact in the Worst than

Reference relevés. Comparisons of the measures of diversity of various groups of taxa are presented in Table 7.8. Statistical differences between Moderate and Worst relevés were determined using pair-wise PERMANOVA in one-way analyses within the factor "Disturbance". The results of these tests are shown in Table 7.8.

Table 7.8: Differences in diversity between Moderate and Worst Hermanus supralittoral vegetation relevés. Values in disturbance categories represent the average per sample (±S.E.). Pairwise t-tests performed in PERMANOVA.

Diversity variable	Taxa type	Moderate (n=16)	Worst (n=10)	t-test	p-value
Number	Annuals	0.13 (0.1)	1.2 (0.3)	3.6	0.001
cover	Annuals	3 (0)	66 (0.4)	2.3	0.04
diversity***	Annuals	0 (0)	0.3 (0.1)	2.3	0.03
number	Sclerophyllous shrubs	1.25 (0.3)	0	3.3	0.006
number	Woody	3.1 (0.5)	1.2 (0.3)	2.96	0.01
diversity***	Woody	0.8 (0.1)	0.2 (0.1)	2.9	0.008

***Shannon Wiener diversity ($\log_e$)

- A greater number, total cover, and diversity of annuals, were recorded in Worst than Moderate relevés. Two annual taxa with a mean cover of <2% were recorded in the Moderate relevés. Seven annual taxa were recorded in the Worst relevés with a mean cover of 6% for the category. The greater number and mean cover of annuals in the Worst than Moderate disturbed category provide potentially useful metrics.
- 10 indigenous sclerophyllous shrubs – typical of fynbos shrubland – were recorded in the Moderate relevés, and none in the Worst disturbed relevés.
- 16 indigenous and four alien woody taxa were recorded in the Moderate relevés whilst four woody taxa (all indigenous) were recorded in the Worst relevés. A greater mean number and mean diversity of woody taxa were recorded per Moderate than per Worst sample. A look at the "typical" number of woody species (*as derived from the average value from only those relevés in which these species are recorded*) reveals a similar picture to that derived from the average number per sample (Table 7.8). The "typical" number of woody species is greater in Moderate than in Worst relevés (Reference (3.5 ± 0.4) *vs.* Worst (1.7 ± 0.2)). The greater total number of woody taxa in Moderate than Worst categories provides the most intuitively simple metric to calculate based on species per wetland. The "typical" number per sample also appears to offer some potential for metric development at the sample (relevé) scale.

7.2.3. Ratelsvlei supralittoral: Reference vs. Moderate

Only 12 relevés are contained in the Ratelsvlei supralittoral habitat data set. Four of these were categorized as being in Moderate condition, with the rest being Reference. The vegetation of the Moderate samples is however classified as Cape Inland Salt Pan whilst the Reference relevés are from Cape Lowland Freshwater (Mucina *et al.* 2006a). Furthermore, the relevés of the Moderate category are all from a Floodplain Flat landform whilst the Reference relevés are all from depressions (SANBI 2009). The supralittoral relevés from the depressions are from the depressional edge that is susceptible to annual inundation with rising winter water levels. The Floodplain relevés are from ground that is more typically only saturated in winter months with potential for short periods of shallow inundation. Hence, considerable natural variability between these relevés from each disturbance category suggests no comparative value for the purposes of developing metrics for phyto-assessment. No attempt to determine metrics was therefore pursued.

7.2.4. Driftsands littoral assemblages

The littoral relevés from Driftsands are all from depressions (n=11) in sandy substrate with similar soil depths. A total of 11 Reference, 13 Moderate and 15 Worst-disturbed relevés were assessed in the Driftsands littoral habitat. There is indistinct separation between the Driftsands littoral relevés of each disturbance category as shown by unconstrained ordination (MDS Figure 7.3). This suggests that, despite differences between relevés of each HDTS category shown using PERMANOVA (Table 7.4), it will be difficult to ordinate these as distinctly different relevés using dbRDA.

The level of potential annual inundation depth (cm) in Moderate relevés (56±7) was more than double that of Reference (16±4) and Worst (24±3) relevés. Considering sample choice was based on homogenous stands of vegetation (Section 3.2.4), these depth differences are an apparently natural source of difference. Increasingly greater land-use disturbances causing water loss (HDS is qualitatively scored and not measured) were recorded as having impacted Reference (-18±1), Moderate (-24±1) and Worst (-27±0.4) categories of Driftsands wetlands. The moderate wetlands were on the whole inundated to greater depth than the Reference or Worst wetlands, suggesting a natural cause of variability between samples from these wetlands.

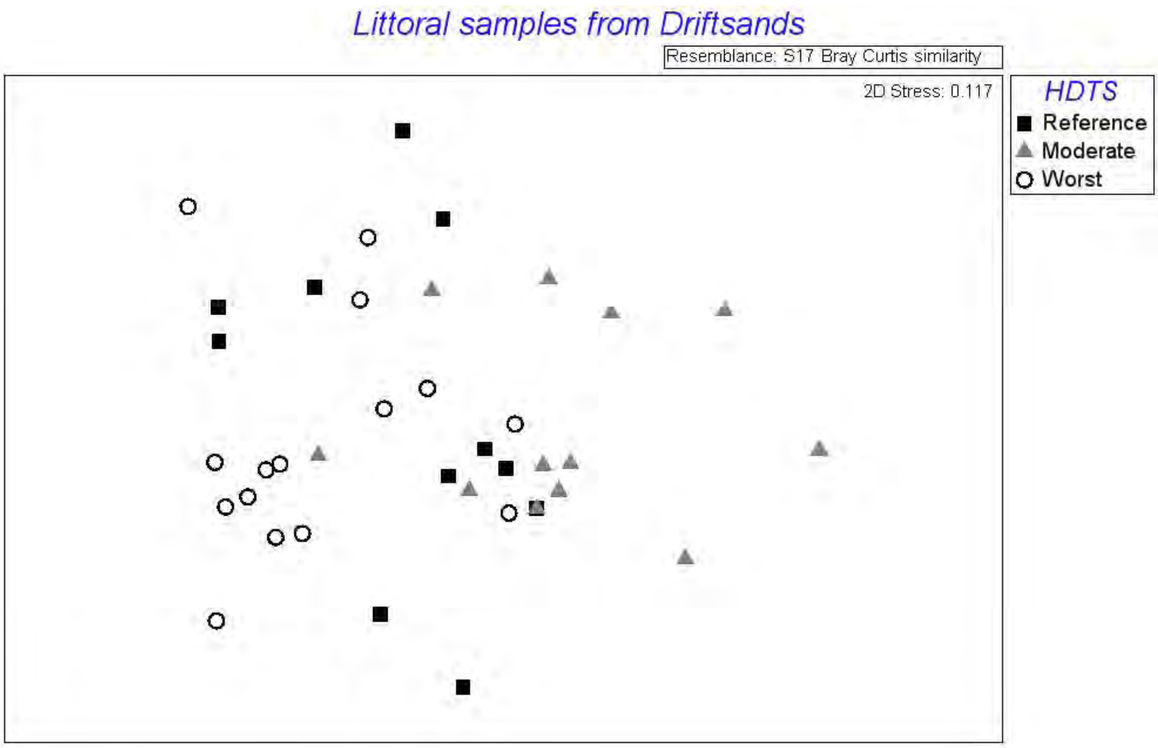


Figure 7.3: Multi-Dimensional Scaling ordination of the littoral Driftsands vegetation relevés.

The qualitatively assessed HDS suggested that the differences in land-use had caused greater disturbance in Worst than in Reference relevés (Table 7.9). It was therefore decided, for the purposes of metric development, to compare only Reference and Worst relevés. All of the "Worst" relevés are adjacent to an agricultural section of the informal settlement area of Mfuleni Township. These relevés and wetlands are heavily impacted by grazing and reed harvesting, trampling, human and domestic stock effluent and dumping of solid waste.

Table 7.9: Human Disturbance impacts (± S.E.) and their comparison using pair-wise t-tests in PERMANOVA. Significance at p<0.05 marked with*.

	Reference	Worst	Worst Samples	t-test	p-value
Hydrological Impacts	-18 (1.3)	-27 (0.3)	Drier	7.9	0.001*
Water Quality Impacts	34 (0)	79 (3.5)	Worse quality	10.8	0.001*
Physical Impacts	13 (0.2)	32 (0.8)	More impacted	19.1	0.001*
Buffer Width	6 (0.5)	8 (0.7)	less buffered	1.6	0.1
HDS	71 (0.5)	145 (5)	Worse	12.2	0.001*

Physical impacts returned the highest t-test value, suggesting that physical modification was the greatest cause of negative impacts between Reference and Worst samples. These physical disturbances (trampling, vegetation utilization and dumping of solid waste), are likely to exert no significant effect on the chemical signature and perhaps thus have only a limited impact in terms of species distribution.

Not all variables were measured in each relevé and only 10 Reference and 11 Worst relevés were comparable using the environmental data. A number of the environmental parameters (aspect, exchangeable sodium, water soluble sodium, water soluble magnesium, evaporation and exchangeable potassium) were collinear at > 95% or <-95% with other variables as displayed in Table 7.10 and were removed from the environmental dataset before attempting to perform distance linear modelling.

Table 7.10: Collinear environmental parameters in the littoral Driftsands relevés

	aspect	conductivity	turbidity	K	Exca Na^+	Na water soluble	Evaporation
slope	**0.97**						
turbidity	-0.3	0.8					
Exca Na^+	-0.2	**0.96**	0.8	0.88			
Exca K^+	-0.3	0.9	0.8	**0.999**	0.9		
Na water soluble	-0.2	**0.96**	0.9	0.9	**0.98**		
Mg water soluble	-0.2	**0.97**	0.8	0.87	**0.96**	**0.98**	
evaporation	0.3	-0.64	**-0.97**	-0.7	-0.7	-0.8	
TIN	-0.2	0.6	0.9	0.6	0.6	0.7	**-0.95**

Exca stands for exchangeable cations

An important point to note is that, TIN was 93% collinear with turbidity, as well as being inversely collinear with HDS (-94%). TIN was therefore also removed to prevent domination of the outcome of the dbRDA.

Altitude, three variables determining water quality, and nine variables determining soil particle size and/or soil chemistry, all had significantly different value in Reference relative to Worst relevés (Table 7.11).

Qualitatively measured HDS impacts were not mirrored in the quantitative measurements of disturbance to soil and water column parameters. Parameters for which significantly elevated values would suggest disturbance to water column such as conductivity, turbidity and TIN as well as greater levels of soil nutrients (P, K, exchangeable (Exca) Mg^{+}, water-soluble Ca and K) are apparent in *Reference* rather than Worst relevés in Table 7.11. Higher levels of conductivity, turbidity, soil P, and TIN in Reference than in worst-disturbed relevés are all potentially incongruous with these being considered as less disturbed than the Worst relevés. The magnitude of difference for water column TIN and soil P is considerably less than would be considered to cause a change in trophic level (see Section 5.2). Thus, the recorded differences do not suggest the Reference relevés are in any worse condition than the Worst relevés. The considerably higher turbidity in Reference than Worst relevés partially relates to higher clay and silt content (Pearson r=33% and r=52% respectively) in the soils of the Reference relevés, a feature that is not necessarily indicative of human disturbance. The higher water column conductivity, and correspondingly lower electrical resistance in the soils of Reference relative to Worst relevés, suggests a potential source of natural differences between these relevés. In combination with high soluble sodium content, low resistance is indicative of saline conditions. Water-soluble sodium was inversely collinear with conductivity and was thus removed before Table 7.11 was constructed. Examination revealed significantly higher water-soluble sodium in the soils of

Table 7.11: Environmental variables that occur with different average value (± S.E.) in the Reference and Worst littoral relevés of the Driftsands wetlands.

Parameter	Unit	Reference (n=10)	Worst (n=11)	t-test	p-value
HDS	score	72 (0.5)	138 (6)	11.5	0.001
Altitude	m	30.5 (0.5)	27.1 (0.3)	6.3	0.001
Conductivity	$mS.cm^{-1}$	10.4 (2.3)	1.8 (0.2)	3.9	0.001
Turbidity	NTU***	7.9 (0.3)	2.3 (0.12)	18.3	0.001
TIN	μgL^{-1}	99 (0)	29.6 (3.2)	20.6	0.001
% Clay	%	0.54 (0.2)	0.1 (0.03)	2.4	0.03
% Sand	%	96.5 (0.4)	97.8 (0.2)	2.9	0.009
Resistance	$mS.cm^{-1}$	206 (38)	749 (62)	7.2	0.001
Soil phosphorus	$mg.kg^{-1}$	7.8 (1.8)	3.5 (0.3)	2.4	0.025
Soil potassium	$mg.kg^{-1}$	72.5 (7.8)	32.3 (4)	4.7	0.001
exchangeable Mg^{++}	$cmol(^{+}).kg^{-1}$	3 (0.1)	1.5 (0.2)	7.2	0.001
CEC	$cmol(^{+}).kg^{-1}$	3.3 (0.2)	2.45 (0.2)	3.3	0.006
Ca water soluble	$mg.kg^{-1}$	40.7 (5.8)	12.57 (2.1)	4.7	0.001
K water soluble	$mg.kg^{-1}$	137 (22)	81.9 (4.4)	2.6	0.015

The 3 horizontal partitions represent a separation of HDS + landform, water and soil related parameters.

***Nephelometric Turbidity Units

Reference (4837±865 mg.kg^{-1}) relative to Worst (657±106 mg.kg^{-1}) relevés. Thus the Reference samples are saline or brackish whilst those in the Worst condition are not. This difference does not mean that saline wetlands are necessarily impacted by humans and many wetlands especially those near the coast or in marine geological deposits are naturally brackish. Human land-use and related activities that were recorded as having impacted the Worst wetlands are not indicative of increased water influx that could have led to reduction of salinity in these wetlands. If anything, as described below Figure 7.3, more water loss seems to have occurred in Worst than in Reference wetlands. The relevés from Worst wetlands were, on average, collected from situations with 0.10 metre deeper annual inundation than the Reference relevés. It is, however, considered unlikely that reduced water availability would significantly alter salinity.

As mentioned previously, the higher concentration of TIN in Reference compared to Worst category relevés is well within the range of natural variability and is not indicative of anthropogenic disturbance. The same is true for differences in the soil potassium, phosphorus, exchangeable magnesium cations, water-soluble calcium and water-soluble potassium between categories, all of which were measured as being in marginally higher concentration in Reference compared to Worst disturbed relevés. As TIN was inversely collinear with HDS, TIN was removed from the dataset before performing dbRDA.

It seems that the natural salinity difference between Worst and Reference wetlands is responsible for difference in vegetation communities from these relevés. If salinity is the major cause of difference, then constrained ordination using dbRDA should reveal variables that are collinear with sodium and resistance as being vectors that separate saline Reference from non-saline Worst relevés. The parameters in Table 7.11, other than TIN, were therefore used in a distance linear model to predict which parameters explained most of the difference in vegetation assemblages between the Reference and Worst littoral relevés.

7.2.4.1. Driftsands littoral DistLM Results

Distance linear modelling suggests that the soil parameters; percentage sand, concentrations of phosphorus and potassium, water soluble calcium and water soluble potassium and exchangeable magnesium cations; and the water column parameters conductivity and turbidity; and lastly altitude each independently has a significant influence in determining the vegetation communities representative of each disturbance category (Marginal Tests: Table 7.12).

Table 7.12: Test statistics for DistLM analysis based on "best" selection procedure and the Adjusted R^2 selection criterion for the average vegetation assemblage in Reference and Worst disturbed Driftsands littoral vegetation relevés. SS = Sum of Squares, RSS = Residual Sum of Squares, R^2 = RSS/SS. Significance at p<0.05 marked *.

MARGINAL TESTS:				
Variable	**SS(trace)**	**Pseudo-F**	**p-value**	**% of total variation**
(1) %_Sand	11469	2.9	0.002*	13.2
(2) P	10890	2.7	0.002*	12.6
(3) Conductivity	10661	2.7	0.005*	12.3
(4) Ca_water_soluble	10299	2.6	0.004*	11.9
(5) K_water_soluble	9981	2.5	0.005*	11.5
(6) K	10136	2.5	0.002*	11.7
(7) Altitude	8134	2.0	0.02*	9.4
(8) Turbidity	7947	1.9	0.03*	9.2
(9) Exca_Mg	7520	1.8	0.04*	8.7
(10) %_Clay	6064	1.4	0.1	7.0
(11) Resistance	6331	1.5	0.09	7.3
(12) CEC	4357	1.0	0.4	5.0
(13) HDS	5767	1.4	0.2	6.7
Best Solutions				
Variable Selections	**Adj R^2**	**RSS**	**R^2**	**% of total variation**
2,3,5-9,12,13	0.28513	34039	0.6068	60.68
2,3,5,7,8,9,12,13	0.2851	37135	0.5711	57.11
2,3,5,7,8,9,11,12,13	0.2806	34253	0.6044	60.44

In combination DistLM consistently selected higher soil phosphorus concentration, lower percentage sand, higher water soluble potassium, higher altitude, higher turbidity, higher conductivity, higher exchangeable magnesium cations, higher cation exchange capacity (CEC:), and lower human disturbance (HDS) in distinguishing Reference from Worst relevés for the top three best solutions (Best solutions: Table 7.12).

Potassium concentration (more) and resistance (less) are other parameters chosen by the DistLM as influencing the different vegetation assemblages of Reference and Worst relevés. The greater levels of clay and nutrient concentrations (P, K, TIN, exchangeable Mg^+, water soluble K) in Reference relevés are allied to the higher CEC or fertility of the Reference relevés. None of these parameters are particularly indicative of human impacts that cause a deleterious or negative chemical change in soil or water quality.

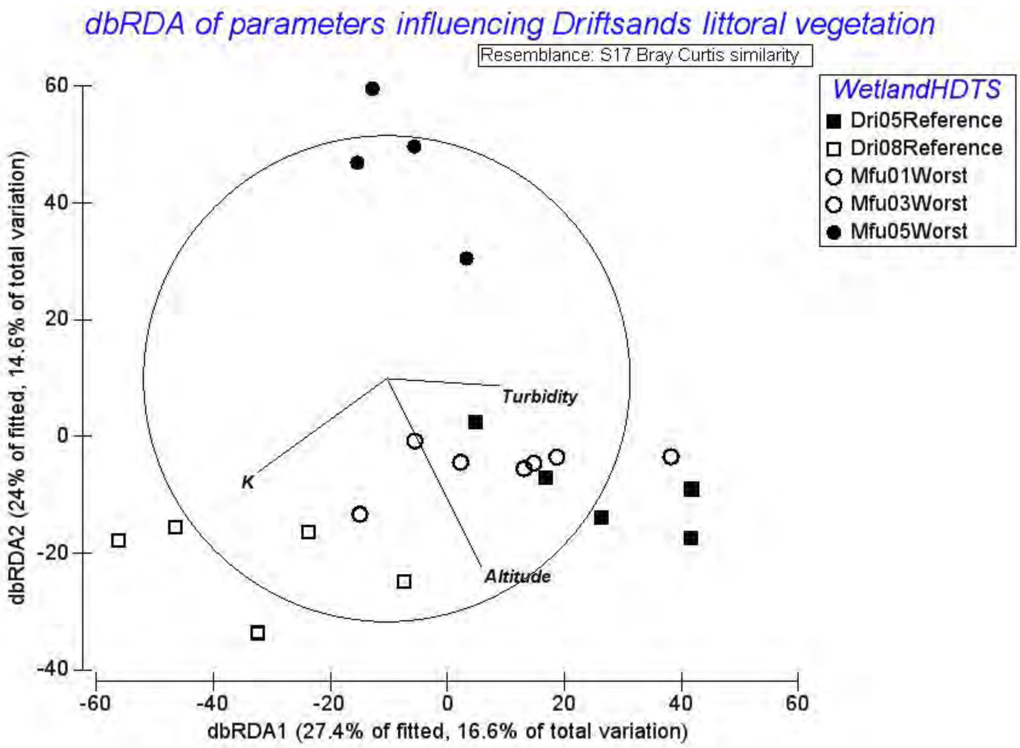


Figure 7.4: Constrained ordination by the most influential environmental variables in determining different vegetation communities in Reference and Worst disturbed littoral-Driftsands relevés determined with dbRDA. Vectors are Pearson correlations (*r>0.35)*.

Indistinct separation is apparent between Reference and Worst relevés in the constrained ordination in Figure 7.4. The position of the Dri08 Reference relevés at the bottom left (indicated by hollow squares in Fig. 7.4) is most determined by soil K. Soil K was inversely collinear to resistance (-76%), and collinear with exchangeable and water soluble sodium (88% and 90% respectively), suggesting that these relevés are the most saline of all in the dataset.\

7.2.4.2. Discriminatory species in Driftsands littoral relevés

Species with discriminatory cover between disturbance categories were sought using SIMPER. The SIMPER analysis revealed 11 species with potential to discriminate between the Reference (n=10) and Worst (n=11) environmental condition in the Driftsands littoral vegetation relevés. As is apparent from Figure 7.5, the lawn grass *Cynodon dactylon* along with limited cover by the alien grass *Polypogon monspeliensis**, the sedge *Schoenus nigricans* (low fidelity), the herb *Falkia repens* and a *Sarcocornia* sp. all occurred with greater average and typical cover in the Reference than Worst littoral relevés at Driftsands. The grass *Imperata cylindrica*, the tall restio *Elegia tectorum*, the mega-graminoid *Typha capensis*, the shrub *Senecio halimifolius*, the mat-forming sedge *Isolepis rubicunda* (low fidelity) and the macroalga *Tolypella glomerata* all occurred with greater average and typical cover in the littoral vegetation categorized as being in “Worst” condition than in vegetation in Reference condition.

The rush *Juncus kraussii* occurred with very similar average cover in the Reference and Worst relevés. The magnitude of average cover difference between Reference and Worst relevés is too small to facilitate differentiation using the Braun Blanquet scale. The typical cover of this species was slightly more useful for this purpose, but the species did not occur with consistently greater cover in Reference than Worst relevés and is thus not useful as an indicator species.

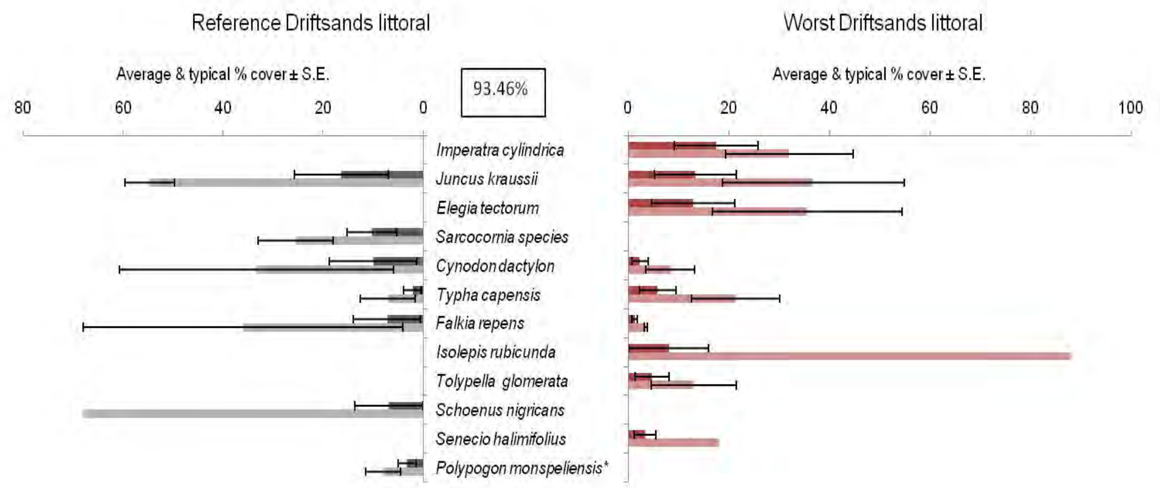


Figure 7.5: Average (dark bars) and typical (light) species cover (± standard error) in the Reference and Worst littoral-Driftsands vegetation relevés. Species without error bars have standard error of zero (often meaning they were present only in a single sample). The dissimilarity percentage between the communities of the different disturbance categories is presented in the rectangle at the top of the graph.

Of the above species, only *Typha capensis* is frequently associated in greater abundance with impacted or disturbed conditions. Increased abundance and dominance of almost monospecific stands of *T. capensis* have been recorded on the Kuils River floodplain as a result of human-derived extension of the season of waterlogging (Hall 1992). The Driftsands wetlands sampled in the present study are located on, or adjacent to, the Kuils River floodplain. The limited cover (<20%) of *T. capensis* in both Reference and Worst littoral relevés at Driftsands does not suggest aseasonal extension of waterlogging in these seasonally inundated wetlands. The area of the Kuils River floodplain impacted by extended flooding is perhaps limited in spatial extent and does not include the wetlands sampled in the present study, which would otherwise have been both perennial and considerably dominated by *Typha.*

7.2.4.3. Differences in diversity between Reference and Worst relevés

A total of 34 species were recorded in the Driftsands littoral relevés but of those, only 27 were recorded in the Reference and Worst relevés. A total of 15 taxa with 21% mean cover per species were recorded in the Reference relevés whilst 21 taxa with average cover of 19% per species were recorded in the Worst relevés. Diversity differences that were significant between the mean sample values in the Reference and Worst disturbance categories are presented in Table 7.13.

Table 7.13: Difference in diversity between the Driftsands littoral Reference and Worst relevés. Values in disturbance categories represent the average per sample (±S.E.).

Diversity variable	Taxa type	Reference (n=10)	Worst (n=11)	t-test	p-value
number	aliens	0.6 (0.2)	0.07 (0.07)	3.1	0.01
cover	alien	3.1 (1.7)	0.07 (0.07)	2.1	0.04
number	leafless graminoids	0.18 (0.1)	0.7 (0.15)	2.7	0.02

- A greater number and cover of alien plants were recorded in the Reference than in the Worst relevés. The presence of a single plant of *Xanthium strumarium**,* with less than 5% cover, accounted for all of the alien cover in Worst relevés. Two alien taxa (*Acacia saligna** and *Polypogon monspeliensis**) with a mean cover of <6%, were recorded in Reference relevés. Interestingly, the Moderate category relevés that were not assessed in the previous analyses had a further two alien taxa (*Lolium perenne** and *Spergularia media**) and a mean alien cover of 25%.
- Leafless graminoids *Ficinia nodosa* and *Elegia tectorum* were recorded in both Reference and Worst relevés, whilst *Scirpoides thunbergii* occurred only in the Reference relevés. The difference in number of these leafless graminoids between disturbance categories is in sufficient to use as a metric.

No other diversity differences were apparent. The difference in number and cover of aliens between Reference and Worst is not large. This is fortunate as it does not confound our ecological understanding that more aliens should be found in disturbed than in undisturbed situations. Thus, n potential diversity metrics could be identified from this dataset.

7.2.4.4. Summary of Driftsands littoral results

Despite differences shown to exist between the Reference and Worst littoral Driftsands relevés by PERMANOVA (Table 7.4), no clear separation is apparent when using either unconstrained or constrained ordinations (Figures 7.3 and 7.4 respectively). Differences between the categories are perhaps at least partly due to the (naturally) brackish conditions in some Reference relevés compared to the fresh-water water quality in some impacted wetlands. Whether these salinity differences are related to human activities is unclear from the recorded dataset.

The diversity differences apparent between relevés from Worst and Reference littoral Driftsands habitat are not useful for metric development. The species differences are potentially more characteristic of differences encountered along a salinity gradient than a disturbance gradient. *Sarcocornia natalensis*[1], *Juncus kraussii* and the alien grass *Polypogon monspeliensis*,* all of which were found to occur with greater cover in the saline relevés, are all known to be important taxa of brackish conditions in coastal lowland wetland habitats dominated by Cape Lowland Freshwater vegetation (Mucina *et al.* 2006a). The determination of plants that indicate brackish or saline wetlands, is not the objective of the current research and such species are identified by Mucina *et al.* (2006a).

7.2.5. Kenilworth supralittoral assemblages

The supralittoral relevés from Kenilworth are predominantly from the flat edge of sandy Depressions but include some relevés from Flats (SANBI 2009). The vegetation is mostly dominated by Cape Lowland Freshwater, but Vernal Pool and zonal vegetation dominate in relevés from the Flat landforms. Ordination of the supralittoral Kenilworth relevés showed relatively distinct separation of Reference (n=18) and Worst (n=11) relevés, although the Moderate relevés (n=11) are intermingled (Figure 7.6). This intermingling suggests indistinct differences between Moderate and other disturbance categories, despite the significant differences between the vegetation of all categories determined with PERMANOVA (Table 7.4: Section 7.1). The fern-dominated sample Ken10_14 (far left in Figure 7.6) is an outlier in the ordination and was thus excluded from further analyses. Only the Reference and Worst disturbed relevés were compared for purposes of phyto-assessment development.

[1] The identity of *Sarcocornia* specimens recorded in the present study were not confirmed by a specialist and are therefore reported simply as *Sarcocornia* species. The most likely species of this genus to have been found in the Driftsands location is however *Sarcoconia natalensis.*

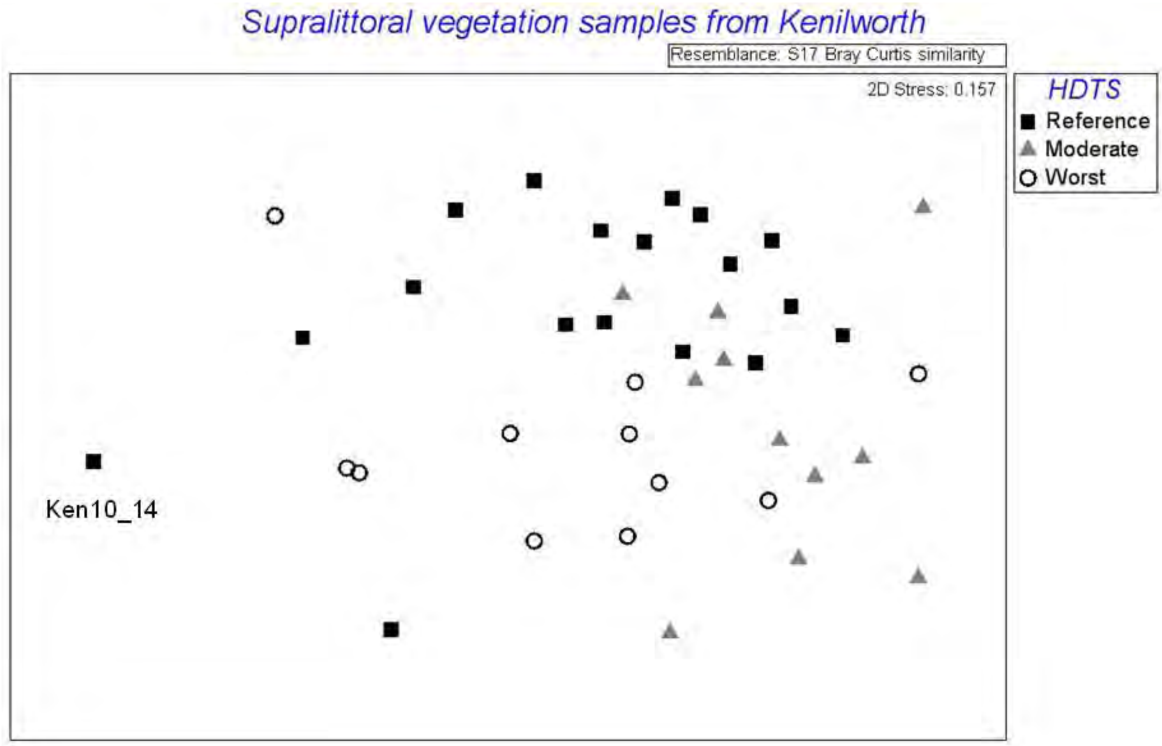


Figure 7.6: Multi-Dimensional Scaling ordination of the supralittoral Kenilworth vegetation relevés.

Human disturbances in the Kenilworth Racecourse area include land use causing water loss in wetlands, poor water quality as a result of stormwater and livestock waste and potentially also due to past and present fertilizer use, and some physical disturbances including road fill and excavation.

Whilst many environmental parameters were measured in this location, the flat and vernal pool samples had no surface water and water parameters were therefore not measured at these two wetlands. These wetland-scale water variables were thus excluded from further analyses as a whole, rather than the exclusion of the relevés from the Flat and Vernal Pool wetlands, facilitating the inclusion of the vegetation sample-based soil environmental parameters for all relevés.

A number of variables (percentage sand, exchangeable K and Na cations and water soluble Mg) were collinear with other variables as presented in the partially complete triangular matrix in Table 7.14 and were removed from the environmental data set.

Table 7.14: Collinear environmental parameters in the supralittoral Kenilworth relevés.

	%_Silt	%_Sand	K	Exca Na	Exca K	Na water soluble
%_Sand	**-0.95**					
K	0.4	-0.4				
Exca Na	0.9	-0.9	0.6			
Exca K	0.4	-0.4	**0.99**	0.6		
Na water soluble	0.8	-0.9	0.6	**0.98**	0.6	
Mg water soluble	0.9	-0.9	0.5	0.9	0.5	**0.95**

Exca stands for exchangeable cations

Kenilworth sample Ken04_5 is an outlier from the rest of the dataset in terms of the environmental parameters that were measured. Relative to other Kenilworth relevés, this sparsely vegetated sample (<35% cover) had high silt (12% *vs.* average of 1.3%), high exchangeable (13% *vs.* 4%) and soluble (1927 *vs.* 293. $mg.kg^{-1}$) sodium content, and low resistance (370 *vs.* 2510 Ohms). These features represent a saline-sodic soil type which is atypical of the Kenilworth wetlands. Ken04_5 was therefore excluded from further analyses, as was Ken10_8, which was deeply inundated and from which no soil sample was taken.

Only seven environmental parameters exhibited significantly different values between the Reference and Worst supralittoral Kenilworth samples (Table 7.15).

Table 7.15: Environmental variables that proved with univariate t-tests to occur with different average value (± S.E.) in the Reference and Worst supralittoral relevés of the Kenilworth wetlands.

Parameter	**Unit**	**Reference (n=17)**	**Worst (n=11)**	**t-test**	**p-value**
Silt	%	1 (0.2)	1.6 (0.2)	2.4	0.03
Soil phosphorus	$mg.kg^{-1}$	8.7 (1.4)	103 (40)	3.1	0.001
exchangeable cations Ca^{++}	$cmol(^{+}).kg^{-1}$	0.62 (0.1)	2.6 (0.6)	4.3	0.001
exchangeable cations H^{+}	$cmol(^{+}).kg^{-1}$	1.5 (0.2)	0.6 (0.1)	3.8	0.002
Ca water soluble	$mg.kg^{-1}$	3.8 (1)	11.5 (3)	2.7	0.005
Hydroregime	score	12 (1)	14 (2)	2.5	0.02
HDS	score	54 (1)	140 (6)	19.7	0.001

The large difference in soil phosphorus content between Reference and Worst category is noteworthy. The relative influence of these environmental variables on the cover and distribution of species in the Reference and Worst supralittoral Kenilworth samples was determined using DistLM.

7.2.5.1. Kenilworth supralittoral DistLM Results

Distance linear modelling suggested that only soil phosphorus, HDS and exchangeable hydrogen cations each independently explained a considerable and significant percentage ($p<0.05$) of the variation between vegetation assemblages of Reference and Worst disturbance categories (Marginal tests: Table 7.16).

The distance linear model consistently selected lower soil phosphorus concentration, lower HDS and higher exchangeable H^+ cations as the three factors best distinguishing Reference from Worst conditions. A drier hydroregime distinguishing Reference from Worst relevés is the factor chosen by the top two, as well as the fourth solutions. Soluble calcium in the Reference and Worst relevés is selected as an important influence by the three best solutions.

Table 7.16: Test statistics for DistLM based on "best" selection procedure and the Adjusted R^2 selection criterion for the average vegetation assemblage in Reference and Worst disturbed Kenilworth supralittoral vegetation relevés. SS = Sum of Squares, RSS = Residual Sum of Squares, R^2 = RSS/SS. Significance at $p<0.05$ marked *.

MARGINAL TESTS:				
Variable	**SS(trace)**	**Pseudo-F**	**p-value**	**% of total variation**
(1) P	11564	2.8	0.001*	10.6
(2) HDS	11245	2.8	0.001*	10.3
(3) Exca H^+	9193	2.2	0.002*	8.43
(4) Hydroregime	4808	1.1	0.35	4.41
(5) Exca Ca^{++}	4703	1.1	0.33	4.31
(6) Silt	4050	0.9	0.54	3.71
(7) Ca water soluble	4849	1.1	0.29	4.44
Best Solutions				
Variable Selections	**Adj R^2**	**RSS**	**R^2**	**% of total variation**
1-4,7	0.157	73560	0.3257	32.57
1-4,6,7	0.153	70225	0.35628	35.63
1-3,7	0.153	77620	0.28849	28.85

1-4	0.150	77900	0.28592	28.59

A plot of the DistLM separation of the different supralittoral relevés is presented below (Figure 7.7) with Pearson Rank correlation vector overlays of the variables that contributed to the distribution pattern (*$r>0.02$)*. In the dbRDA plot based on the multivariate relationship of environmental variables to species cover, increasing P (0.84) and increasing human impact (HDS:0.52) best explain separation from left to right between Worst and Reference relevés along the primary or x-axis (dbRDA 1); collectively explaining 11.6% of total variation.

Increasing HDS (0.7) and decreasing exchange capacity as expressed by exchangeable hydrogen cations (-0.63) best explain the separation between relevés from bottom to top along the y-axis (db RDA 2); explaining 9.3% of total variation. These two axes explain 64% of the model fitted variation and 20.9% of the total observed variation in the combined environmental and vegetation assemblage data sets.

The influence of the qualitative human disturbance impacts (HDS) and eutrophication as a result of elevated soil phosphorus are the most apparent sources of difference between Reference and Worst relevés.

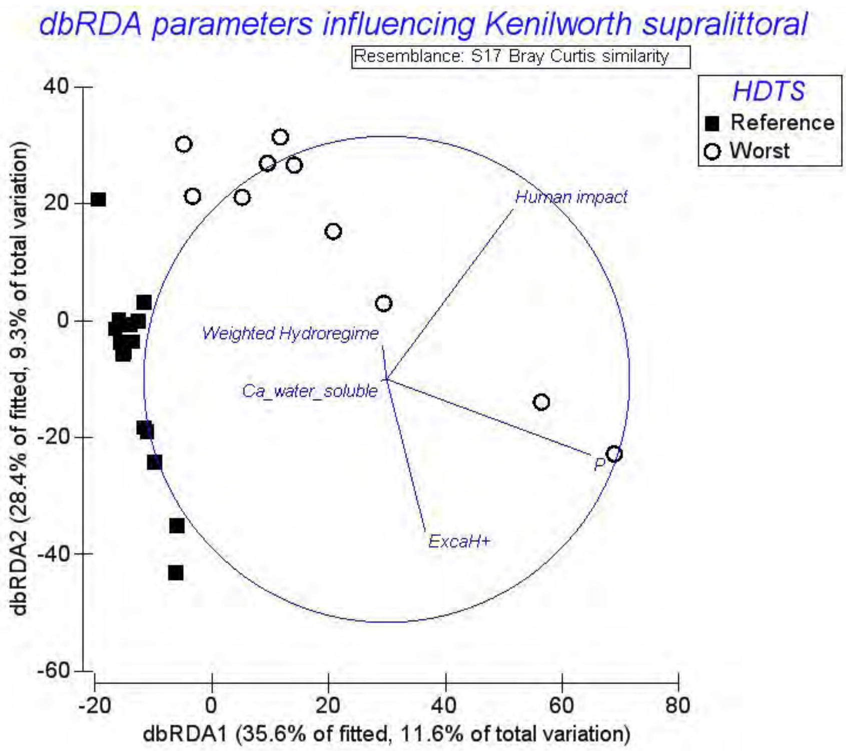


Figure 7.7 Constrained ordination by the most influential environmental variables in determining different vegetation communities in Reference and Worst disturbed supralittoral-Kenilworth relevés determined with dbRDA. Vectors are Pearson correlations (*r>0.02)*.

7.2.5.2. Discriminatory species in Kenilworth supralittoral relevés

Species with discriminatory cover between Reference and Worst relevés were sought using SIMPER. The SIMPER analysis revealed 16 species with discriminatory potential between the Reference (n=16) and Worst (n=10) Kenilworth supralittoral relevés (Figure 7.8). The average and typical cover of these species in each disturbance category is represented in the graph, the length of the bar being equivalent to the cover value.

As is apparent from Figure 7.8, the mega-graminoid *Pennisetum macrourum*, the graminoids *Pentaschistis pallida, Juncus capensis* and *Restio quinquefarius*, the shrubs *Berzelia abrotanoides*, *Psoralea pinnata* and *Rhus laevigata var. laevigata*, the herb *Plecostachys serpilifolia*, the fern *Histiopteris incisa* and the sedge *Chrysitrix capensis* all occurred with greater average and typical abundance in the Reference than in the Worst supralittoral relevés at Kenilworth.

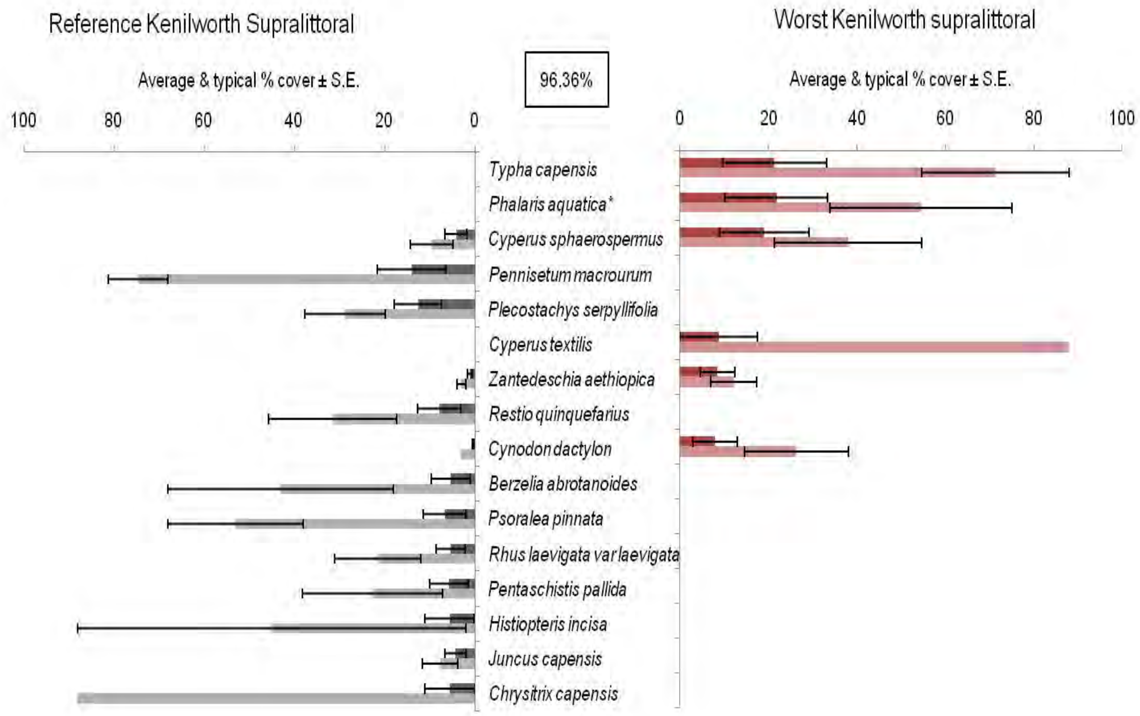


Figure 7.8: Average (dark bars) and typical (light bars) species cover (± standard error) in the Reference and Worst vegetation relevés in the supralittoral hydrological habitat of the Kenilworth locality. Species without error bars have standard error of zero (often meaning they were present only in a single sample). The dissimilarity percentage between the species assemblages of the different disturbance categories is presented in the rectangle at the top of the graph.

The mega-graminoid *Typha capensis*, the indigenous lawn grass *Cynodon dactylon*, the alien tussock grass *Phalaris aquatica**, the sedges *Cyperus sphaerospermus* and *Cyperus textilis*, and the arum lily *Zantedeschia aethiopica* all occurred with greater average and typical cover in the Worst than in the Reference relevés.

Chrysitrix capensis and *Cyperus textilis* were present only in single relevés and are therefore potentially of low fidelity. They do, however, represent typical stands of supralittoral vegetation at Kenilworth. The clear separation between Reference and Worst relevés in the ordination (Fig 7.6) and dbRDA plots (Fig 7.7) suggests that the species displayed in Figure 7.8 are relatively good discriminators between Reference and Worst environmental conditions in the supralittoral habitat of the Kenilworth locality.

7.2.5.3. Diversity differences between Reference and Worst relevés

A total of 80 species were recorded in the Kenilworth supralittoral Reference and Worst relevés. Measures of diversity of functional groups that occurred with significantly different value in Reference and Worst supralittoral-Kenilworth habitat are reported in Table 7.17.

- In the Reference relevés, 64 taxa were recorded with an average cover of 14% per species; the Worst relevés held only 25 taxa with an average cover of 22% per species. The greater mean number of taxa per relevé in the Reference compared to the Worst category wetlands suggests potential for metric development.
- Greater mean number and cover of aliens were recorded in Worst than in Reference relevés. In total, 10 alien species were recorded in Worst and 6 in Reference relevés; typically fewer than 2 aliens were recorded in any Reference sample. More than 2 alien species were recorded in only 5 out of the 11 relevés representing Worst disturbance. Although the magnitude of difference in the number of alien species is too small to justify the development of a metric at this stage, the large difference in cover of alien taxa does suggest that this may turn out to be a useful metric for phyto-assessment purposes.
- A greater richness of FW and OW taxa occurred in Reference than Worst relevés, suggesting a potential metric.
- Greater mean number and cover of woody taxa were recorded at Reference than at Worst relevés. Within the Reference relevés, 9 indigenous woody taxa were recorded; whilst in Worst relevés, only 2 woody taxa were recorded; both of which were alien taxa.
  - ➢ The greater number of indigenous woody taxa in Reference than Worst relevés suggests a comparison upon which metrics could be developed.
  - ➢ The large cover difference of woody taxa between disturbance categories (as averaged per relevés) also suggests a useful metric for phyto-assessment purposes. When measured as an average value per disturbance category the woody cover difference is >13% *vs.* <1% in Reference *vs.* Disturbed. This therefore suggests the potential for a metric of greater woody cover averaged per wetland rather than per relevés.

Table 7.17: Diversity differences between Reference and Worst Kenilworth supralittoral relevés. Values in disturbance categories represent the average per sample (±S.E.).

Diversity variable	Taxa type	Reference	Worst	t-test	p-value
Number	All taxa	8.3 (1.2)	4.8 (0.7)	2.2	0.04
Number	aliens	0.3 (0.1)	2 (0.5)	3.3	0.005
cover	aliens	0.6 (0.3)	23 (9)	3.1	0.001

richness*	FW and OW ***	1.3 (0.2)	0.7 (0.1)	2.1	0.04
number	woody	1 (0.2)	0.3 (0.2)	2.6	0.02
cover	woody	14 (4)	0.5 (0.3)	2.5	0.02
number	Sclerophyllous shrubs	0.5 (0.2)	0	2.6	0.015
number	Leafless graminoids	0.67 (0.2)	0	2.3	0.03
cover	graminoid	56 (9)	82 (5)	2.2	0.04

*Margalef's species richness relative to total species cover

***Species with Facultative to Obligate affinity for wetland habitat (Reed 1988)

- Sclerophyllous shrubs typical of fynbos were absent from the Worst relevés whilst 7 species were recorded in Reference (and 4 in Moderate) relevés.
- Four species of *Restionaceae* (leafless graminoids) were recorded in Reference relevés, whilst only one species was recorded in Moderate and none in Worst relevés.
- A greater mean cover of graminoid taxa was recorded in Worst than in Reference relevés because a significantly greater cover of alien graminoid taxa occurred in the Worst than Reference relevés (t=2.7, p=0.001). Averaged over all relevés in each category, the mean cover of alien graminoid taxa was considerably smaller in Reference (< 1%) than in Worst (18%) relevés. Thus, the mean cover of alien graminoid taxa per wetland is a potentially useful metric for the Kenilworth supralittoral habitat.
- No difference in the indigenous graminoid cover was apparent between the categories of disturbance. A metric based on alien graminoid taxa should not be used in conjunction with the cover of all alien taxa as this would lead to double counting.

7.2.6. Kenilworth littoral assemblages

In the 11 Kenilworth wetlands, 8 Reference, 13 Moderate and 7 Worst littoral relevés were assessed. PERMANOVA suggested that the Reference *vs*. Worst and Moderate *vs.* Worst Kenilworth littoral vegetation relevés were distinctly different (Section 7.1, Table 7.4). ANOSIM suggests that Reference and Moderate category relevés are not very different from each other (R=-0.001, p<0.4), as evidenced by small and negative *R* values. All of the wetlands at Kenilworth, including those categorized as Reference, are considered to have been disturbed to some extent. The Moderate and Reference littoral samples (relevés) were therefore combined into a single unit suggesting vegetation with a “Mild” disturbance category. Ordination revealed distinct separation between the Mild and Worst disturbed relevés (Figure 7.9).

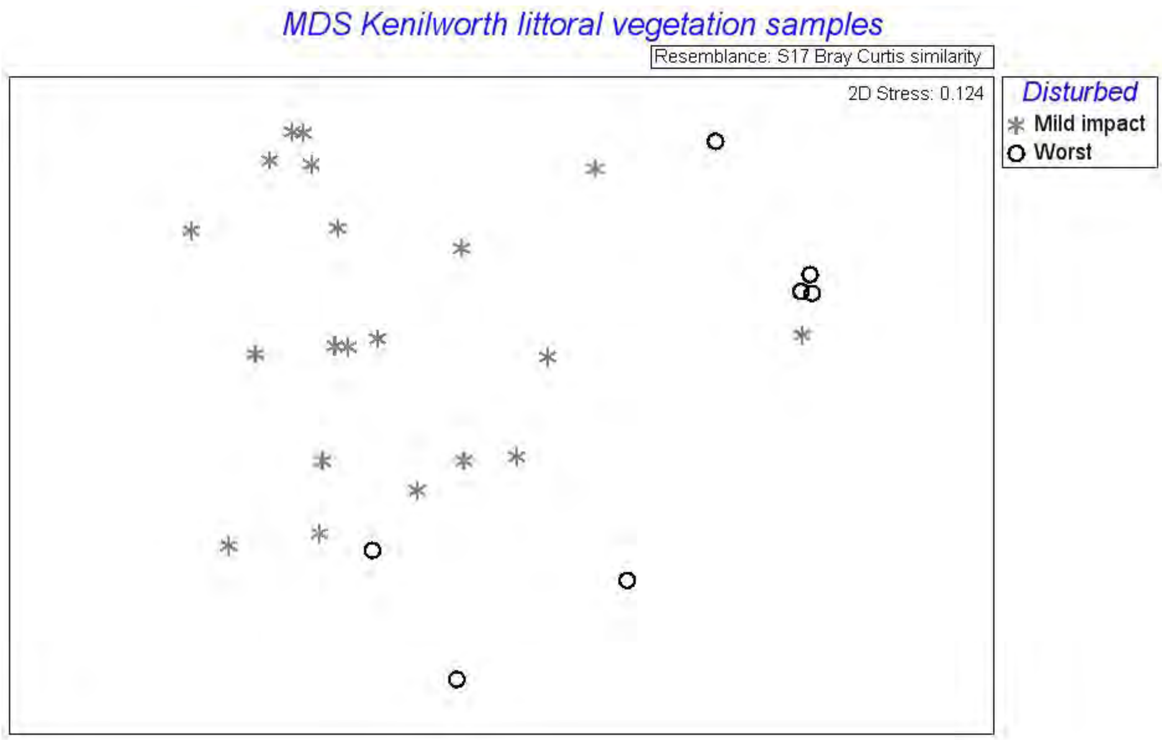


Figure 7.9: Multi-Dimensional Scaling ordination of the littoral Kenilworth vegetation relevés as categorized as Mild and Worst disturbed.

Examination of the environmental variables was then performed to search for any obvious outliers and for any natural or anthropogenic causes of differences between Mild and Worst samples. Water variables, as measured per wetland, were not measured in wetland Ken20. Soil parameters were not recorded for three samples (Ken5_6, Ken1_2_2 and Ken1_2_3). Sample Ken04_6 is an outlier in terms of its environmental characteristics in much the same way as Ken04_5 was in the supralittoral samples described in Section 7.2.4. The Ken04_6 sample was therefore excluded from further analyses. After excluding samples for which no soil and no water variables were measured, and the environmental outlier Ken04_6, only four Worst relevés remain.

The limited number of Worst relevés (Mild = 20 *vs.* Worst = 4) suggests limited potential to derive meaningful metrics for purposes of phyto-assessment. Within the remaining 20 Mild and four Worst relevés, eight environmental parameters exhibited significantly different values between disturbance categories as presented in Table 7.18. None of these variables were collinear.

The mega-graminoid *Typha capensis*, the indigenous lawn grass *Cynodon dactylon*, the alien tussock grass *Phalaris aquatica**, the sedges *Cyperus sphaerospermus* and *Cyperus* *textilis*, and the arum lily *Zantedeschia aethiopica* all occurred with greater average and typical cover in the Worst than in the Reference relevés.

Table 7.18: Environmental variables that proved with univariate t-tests to occur with different average value (± S.E.) in the Mild and Worst littoral samples (relevés) of the Kenilworth wetlands.

Parameter	Unit	Mild (n=20)	Worst (n=4)	t-test	p-value
inundation depth	cm	22 (3)	30 (3.7)	2.8	0.005
Wetland Size	score	1.6 (0.13)	2.6 (0.3)	3.03	0.006
Silt	%	1.05 (0.2)	3.6 (1.5)	2.7	0.01
Resistance	ohms	1956 (237)	3708 (744)	2.7	0.02
P	$mg.kg^{-1}$	7.3 (1.3)	530 (105)	8.7	0.001
CEC	score	2.8 (0.2)	2 (1.2)	2.3	0.03
K water soluble	$mg.kg^{-1}$	6.96 (0.7)	3.9 (0.3)	2.1	0.04
Ca water soluble	$mg.kg^{-1}$	14.4 (1.8)	25.2 (6.5)	2.8	0.01

The high soil P levels, (which were suggestive of eutrophic conditions), was only due to the results of the soil sample analysis (see Chapter 5). The change of disturbance category from Reference to Mild did not alter the HDS scores. Therefore, , for the littoral Kenilworth samples, the average HDS of all Worst samples was relatively low in comparison to the average for all other Mild samples in this study.

Anthropogenic influence on differential environmental condition in Mild and Worst samples is apparent without the use of DistLM. There was a very high concentration of soil phosphorus recorded in the Worst samples (530 $mg.kg^{-1}$) relative to that recorded on average in the Mild samples (7 $mg.kg^{-1}$). In four out of the five Worst samples in which P was measured, its concentration suggested anthropogenic enrichment. Cation exchange capacity of the Worst samples is lower than of the Mild suggesting that Mild samples are more fertile, but this difference is small and not necessarily unnatural.

7.2.6.1. Kenilworth littoral DistLM Results

A DistLM was run to ascertain which parameters were important in differentiating between the Mild and Worst samples. The DistLM consistently selected the combination of water-soluble calcium, soil phosphorus, potential annual inundation depth and resistance as parameters that best explain the distribution of species between Mild and Worst vegetation samples. Whilst only water-soluble calcium appears to have an influence in terms of its independent influence on distribution (see Marginal Tests Table 7.19).

The dbRDA suggested that all four of these parameters and water soluble potassium (K) influence observed vegetation distribution, but the apparent negative environmental impact

Table 7.19: Test statistics for DistLM based on "best" selection procedure and the Adjusted R^2 selection criterion for the average vegetation assemblage in "Mild" and "Worst" disturbed Kenilworth littoral vegetation relevés. SS = Sum of Squares, RSS = Residual Sum of Squares, R^2 = RSS/SS. Significance at p<0.05 marked *.

MARGINAL TESTS:				
Variable	**SS(trace)**	**Pseudo-F**	**p-value**	**% of total variation**
(1) Ca water soluble	7244	2.1	0.03*	8.64
(2) P	5875	1.7	0.1	7.01
(3) potential depth	3938	1.1	0.3	4.7
(4) Wetland Size	3827	1.1	0.4	4.57
(5) % Silt	4917	1.4	0.2	5.87
(6) Resistance	5494	1.5	0.1	6.55
(7) CEC	3529	1	0.5	4.2
(8) K water soluble	3167	0.9	0.5	3.78
Best Solutions				
Variable Selections	**Adj R^2**	**RSS**	**R^2**	**% of total variation**
1,2,3,5,6,8	0.10271	55589	0.33678	33.68
1,2,3,5,6	0.10206	58902	0.29726	29.73
1-6	0.10158	55659	0.33595	33.6

of eutrophic soil P is unequivocal (Figure 7.10). The independent Pearson correlations of each of these variables with species distributions is greater than 40% (*r>0.4).*

Increasing P (0.5), and water soluble Ca (0.6) and decreasing water depth (-0.4) best explain separation along the x-axis or primary dbRDA axis. This axis explains 11.6% of total observed variability. Water soluble Ca (0.4), resistance (0.3), and P (-0.7) best explain separation between Mild and Worst samples along the second dbRDA axis. This second axis explains 7.4% of the total observed variability. Together these axes explain 19% of total observed and 56.4% of model fitted variation. Eutrophic soil phosphorus concentration in Worst samples appears to be the parameter explaining most of the separation between Mild and Worst vegetation samples. Thus, human disturbance is an important influence in separating Mild from Worst vegetation samples. Species with discriminatory cover between

Mild and Worst disturbed relevés, were therefore sought using SIMPER. This process is described below.

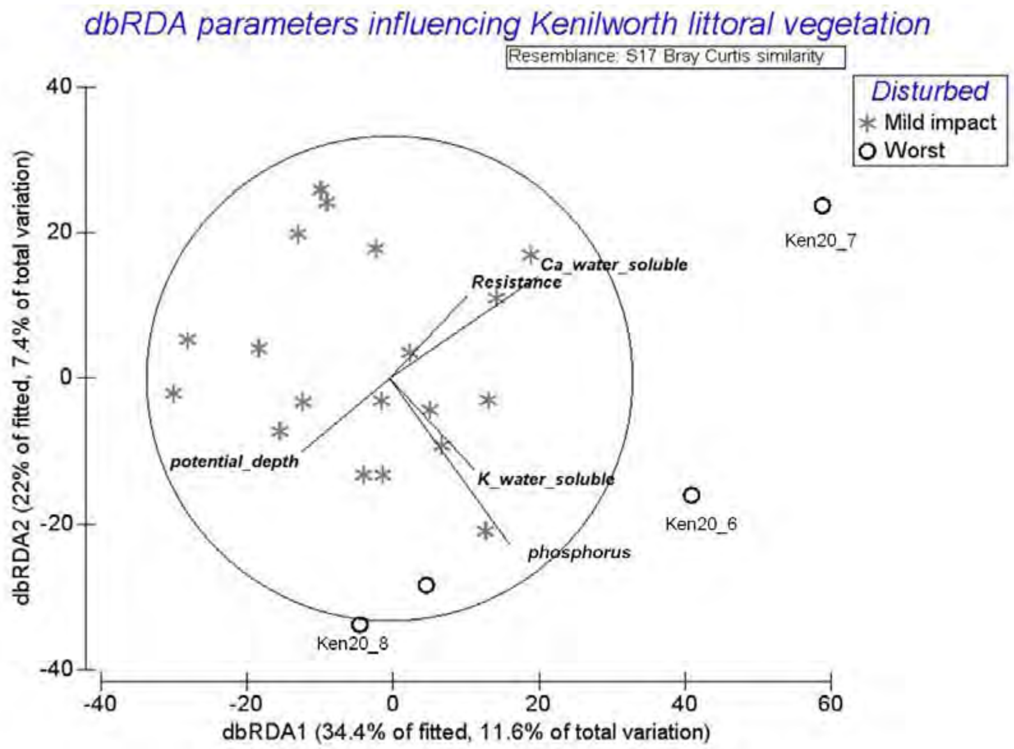


Figure 7.10: Constrained ordination by the most influential environmental variables determining different vegetation communities in Mild and Worst disturbed littoral-Kenilworth relevés determined with dbRDA. Vectors are Pearson correlations (*r>0.04)*.

7.2.6.2. Discriminatory species in Mild and Worst Kenilworth littoral relevés

Average and typical coverage of species that discriminate between the Mild and Worst disturbed relevés are displayed in Figure 7.11. Relevés from both disturbance categories were dominated by graminoid taxa. The Mild relevés were dominated particularly by the mat-forming sedge *Isolepis rubicunda.* Other species with greater typical and average coverage in the Mild relevés were the lawn grasses *Stenotaphrum secundatum* and *Cynodon dactylon,* the rhizomatous sedge *Fuirena hirsuta*, the rush *Juncus oxycarpus* and the restio *Elegia tectorum.* The Worst relevés were mostly dominated by monospecific stands of the mega-graminoid *Typha capensis* or by the sedge *Ficinia indica* and standing dead material of the same species. *F. indica* was only represented, in living form, in Ken20_7; with dead material of this species in Ken20_8. The sedge *Cyperus* *sphaerospermus* had greater typical coverage in Worst relevés but greater average cover in Mild relevés.

The environmental outlier Ken04_6 was included in the determination of discriminatory species using the SIMPER analysis. The inclusion of this sample had little influence on the species (or their values) chosen as discriminatory species. However, the sedge *Cyperus textilis* occurred only in this saline-sodic environmental outlier.

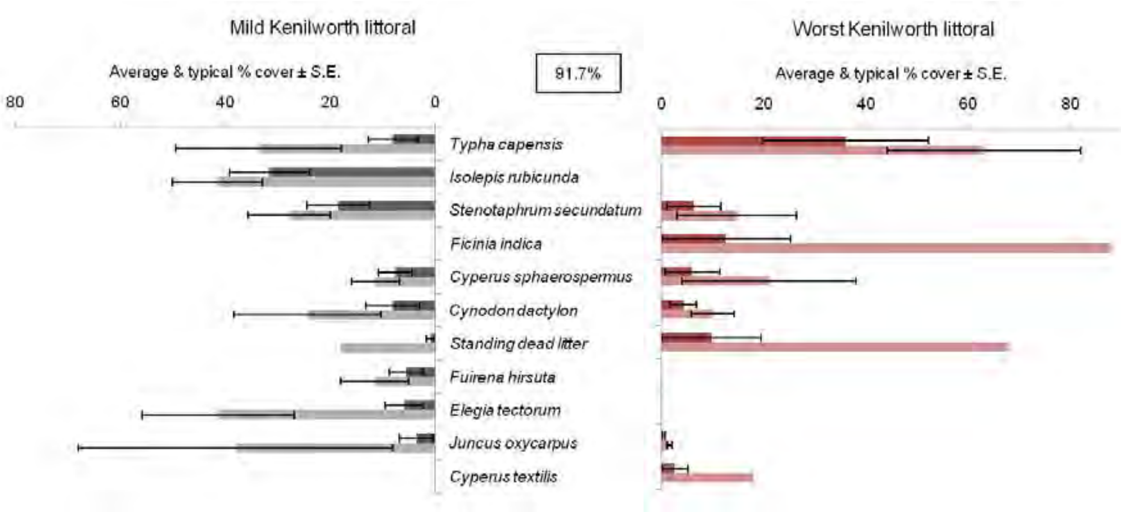


Figure 7.11: Average (dark bars) and typical (light bars) species cover (± standard error) in the Mild and Worst disturbed vegetation relevés in the littoral hydrological habitat of the Kenilworth locality. Species without error bars have standard error of zero (often meaning they were present only in a single sample). The dissimilarity percentage between the species assemblages of the different disturbance categories is presented in the rectangle at the top of the graph.

7.2.6.3. Diversity differences between Mild and Worst relevés

In total 45 species were recorded in the Kenilworth littoral habitat. Measures of species diversity differences between disturbance categories within a number of functional taxa groupings are presented in Table 7.20.

Table 7.20: Diversity differences between Mild and Worst Kenilworth littoral vegetation relevés. Values in disturbance categories represent the average per sample (±S.E.).

Diversity variable	Taxa type	Mild	Worst	t-test	p-value
number	All species	6 (0.8)	3 (0.7)	2.1	0.05
cover (unstandardized)	All species	107 (4)	80 (9)	2.9	0.01

richness*	All species	1.1 (0.2)	0.5 (0.16)	2.1	0.04
number	FW and OW††	5.3 (0.7)	2.3 (0.7)	2.4	0.03
richness*	FW and OW	1 (0.16)	0.3 (0.2)	2.4	0.03
diversity ***	FW and OW	1 (0.15)	0.3 (0.2)	2.3	0.03
evenness**	FW and OW	0.5 (0.07)	0.2 (0.1)	2.1	0.04

*Margalef's species richness relative to total species cover

**Simpsons evenness: largest value when all species have the same cover/abundance

***Shannon Wiener diversity ($\log_e$)

††Species with Facultative to Obligate affinity for the wetland habitat (Reed 1999)

- There was a greater number, cover and richness of species of all taxa in the Mild than the Worst disturbed relevés. The mean cover values for all taxa were calculated from data that was not as yet standardized by the total sample cover values. This cover data does not therefore represent a proportion but rather the total cover per sample; and as species can occur in many layers and with different heights there is often more than 100% cover in relevés that are well populated. A total of 40 taxa were recorded in the Mild relevés with mean cover of 18% per species, whilst only 12 taxa were recorded in Worst relevés and those had an average of 25% cover.
- A greater number, richness, evenness and diversity of FW/OW taxa were found in Mild than Worst relevés. As described in section 6.4, a determination of diversity based on evenness is a comparison of the cover/abundance of each species in a dataset, relative to the cover/abundance of every other species present. Greater evenness in the FW/OW taxa in the Mild relevés, suggests that they held more of these taxa with similarly low cover per taxon than the Worst relevés which were dominated by fewer FW/OW taxa with relatively greater cover per taxon. Whilst 34 FW/OW taxa were recorded in the Mild relevés with an average cover of 18%, only 9 were recorded in Worst relevés with an average cover of 30%. Development of richness, evenness or diversity metrics are potentially possible but a metric based on total numbers of FW/OW taxa is intuitively much simpler to apply.

7.2.7. Lotus supralittoral assemblages

In the eleven Lotus wetlands, 26 Reference and Moderate combined and 14 Worst supralittoral vegetation relevés were recorded. The supralittoral relevés from Lotus wetlands were dominated by Cape Lowland Freshwater vegetation, from sandy depressions with uniform soil depth. PERMANOVA suggested that the Reference *vs.* Worst and Reference *vs.* Moderate Lotus supralittoral relevés were distinctly different (Table7.4). Ordination of these relevés revealed limited distinction between the different categories of disturbance, with particularly limited difference between Moderate and Worst relevés (Figure 7.12). Relevés Lot5_6 and 10_7 were outliers from the rest of the data set. Lot05_6 was dominated by *Cyperus textilis* (88% median cover) whilst 10_7 was dominated by *Cliffortia strobilifera* (88% median cover). Relevés Lot5_6 and Lot10_7 were removed from the data set to enable the ordination presented in Figure 7.12.

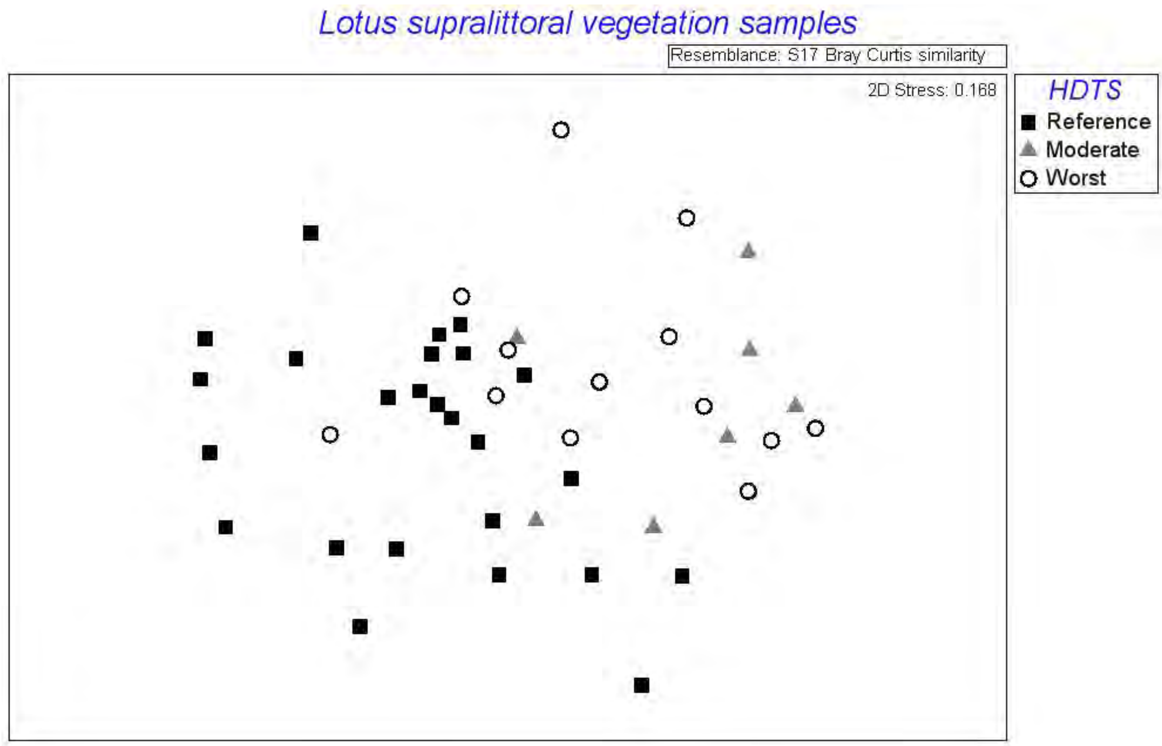


Figure 7.12: Multi-Dimensional Scaling ordination of the supralittoral Lotus vegetation relevés.

The Worst and Moderate vegetation relevés show insignificant differences when analyzed with ANOSIM (R=0.058, p<0.3), or PERMANOVA (t-test=1.2, p=0.08). An ANOSIM of the environmental differences between all three disturbance categories also revealed no significant difference between the Moderate and Worst relevés (R=0.14, p<0.1). A PERMANOVA did reveal environmental difference between these Moderate *vs.* Worst relevés but it was an order of magnitude less (0.01 *vs.* 0.001) than the difference between Reference *vs.* Worst and Reference *vs.* Moderate relevés. The Moderate and Worst relevés were therefore combined into a single category called “Disturbed”. Another approach is to simply exclude the seven Moderately disturbed relevés. However, the fact that their vegetation is not statistically different from the Worst relevés suggests that the Moderate and

Worst relevés could be used to create metrics that reflect the differences between Disturbed and Reference conditions.

Table 7.21: Collinear environmental parameters in the Lotus supralittoral data set.

	Soil pH	**Turbidity**	**SRP**	P	K	**Exca Na**	Exca Ca
Soil Redox	**-0.98**						
Turbidity	-0.05						
SRP	0.004	**0.96**					
P	-0.01	**0.95**	**0.97**				
K	-0.04	0.7	0.8	0.8			
Exca Na	-0.2	0.03	0.05	0.2	0.6		
Exca K	-0.03	0.7	0.8	0.8	**0.99**	0.6	
Exca Ca	0.5	0.1	0.2	0.2	0.2	-0.1	
T-Value	0.4	0.1	0.2	0.3	0.4	0.1	**0.96**
Na water soluble	-0.3	0.01	0.01	0.2	0.5	**0.98**	-0.2
TIN	0.06	**0.96**	**0.99**	**0.98**	0.8	0.05	0.3

Exca stands for exchangeable cations

A number of the environmental variables measured in the Lotus relevés were collinear (Table 7.21). Of these, soil P and soil K, water soluble Na, soil pH and exchangeable calcium cations were retained for analysis.

A large number of the environmental variables proved, with univariate (t-tests), to have significantly different average value between Reference and Disturbed relevés (Table 7.22).

Table 7.22: Environmental variables that proved with univariate t-tests to occur with different average value (± S.E.) in the Reference and Disturbed supralittoral relevés of the Lotus wetlands.

Parameter	Units	Reference (n=26)	Disturbed (n=20)	t-test	p-value
Water pH	pH	8.1 (0.1)	7.6 (0.1)	4.9	0.001
Dissolved Oxygen	$mg.l^{-1}$	8 (0.1)	5 (0.4)	7.7	0.001
% Clay	%	0.5 (0)	0.8 (0.2)	2.2	0.01
Bulk Density	$kg.L^{-1}$	1.5 (0)	1.4 (0)	2.0	0.05
Resistance	ohms	822 (103)	346 (56)	3.7	0.002

P	mg.kg^{-1}	3 (0.3)	11 (4)	2.3	0.001
K	mg.kg^{-1}	27 (3)	72 (14)	3.5	0.001
exchangeable cations Mg^{++}	cmol(+).kg^{-1}	1.2 (0.1)	1.8 (0.3)	2.2	0.04
N %	%	0.09 (0.01)	0.14 (0.02)	2.7	0.004
C %	%	0.9 (0.1)	1.5 (0.1)	3.2	0.006
CEC	score	3 (0.2)	4.7 (0.4)	3.9	0.001
Na water soluble	mg.kg^{-1}	1058 (169)	2524 (453)	3.3	0.001
K water soluble	mg.kg^{-1}	15 (2)	38 (8)	3.4	0.001
Ca water soluble	mg.kg^{-1}	68 (4)	113 (15)	3.2	0.003
Mg water soluble	mg.kg^{-1}	21 (3)	45 (9)	2.9	0.003
Altitude	m	7 (0.1)	9 (0.7)	2.5	0.001
HDS	score	44 (4)	113 (3)	13.8	0.001
Evapotranspiration	mm	113 (0.1)	112 (0)	6.6	0.001

The three horizontal partitions represent a separation of water. soil and altitude + HDS+ climate parameters.

The pH of the water ranged from neutral to mildly alkaline for all relevés. Low average resistance and high average water-soluble Na content suggest that the Disturbed relevés were generally saline-sodic. Eight of the 26 Reference relevés were also saline-sodic but predominantly lower water soluble sodium content and higher resistance in the remaining Reference samples meant that most of the Reference relevés were not saline-sodic. Of all of the environmental variables in Table 7.22, human disturbance (HDS) had the greatest t-value, suggesting the greatest impact of all environmental variables with p-value less than 0.001. The qualitatively determined human disturbance impacts on environmental condition that make up the HDS predominantly showed that the Disturbed relevés were more impacted than the Reference relevés. Only the hydrological impact difference was not significant between Reference and Disturbed relevés (Table 7.23).

Table 7.23: Qualitatively assessed human disturbance impact gradients in Reference and Disturbed supralittoral relevés at Lotus wetlands. Gradients that proved with univariate t-tests to occur with significantly different average value (± S.E.) in each disturbance category are marked with *.

Parameter	**Reference**	**Disturbed**	**t-test**	**p-value**
Hydrological impacts	-0.8 (1.5) Drier	2.3 (3.7) Wetter	0.8	0.4
Water Quality impacts	16 (0.8) Better	54 (3) Worse	12.5	0.001*
Physical Disturbance	17 (2) Less	42 (0.8) More	10.1	0.001*
Buffer Width	5 (0.9) Broad	11 (0.5) Narrow	5.3	0.001*

HDS	44 (4.3)	123 (3) Worse	14.125	0.001*

This suggestion of an anthropogenic, rather than a natural cause of difference between relevés of each disturbance category, led to the use of the environmental variables in Table 7.22 in a redundancy analysis to determine the combination of environmental variables most responsible for the difference between the vegetation of Reference and Disturbed relevés.

7.2.7.1. Lotus supralittoral DistLM Results

The DistLM suggested that ten variables each independently explained a considerable and significant ($p<0.05$) percentage of the variation between Reference and Disturbed vegetation relevés as displayed by the results of the Marginal Tests in Table 7.24.

These ten variables were HDS, five soil parameters (water-soluble sodium, resistance, water-soluble calcium, percentage nitrogen and percentage carbon); two water column parameters (dissolved oxygen and pH) and finally altitude and evapotranspiration. The DistLM consistently selected a combination of the first nine of these significantly different environmental variables to best explain the vegetation differences between Reference and Disturbed relevés. Evapotranspiration was the tenth and excluded variable. The percentage of clay in the sediments, the concentrations of soil phosphorus and exchangeable magnesium cations; and the collective cation exchange capacity were also consistently chosen as variables that influenced the difference in vegetation between Reference and Disturbed relevés. Constrained ordination of the vegetation relevés using the chosen linear combination of environmental variables revealed the variables with greatest individual influence on differences between Reference and Disturbed vegetation. This constrained ordination is displayed in Figure 7.13.

Table 7.24: Test statistics for DistLM based on "best" selection procedure and the Adjusted R^2 selection criterion for the average vegetation assemblage in Reference and Disturbed Lotus supralittoral vegetation relevés. SS = Sum of Squares, RSS = Residual Sum of Squares, R^2 = RSS/SS. Significance at $p<0.05$ marked *.

MARGINAL TESTS:				
Variable	**SS(trace)**	**Pseudo-F**	**p-value**	**% of total variation**
(1) Altitude	8034.5	2.0	0.01*	5.15
(2) Water pH	10419	2.6	0.002*	6.68
(3) Dissolved Oxygen	8494.7	2.1	0.01*	5.45

(4) N %	7438.5	1.8	0.02*	4.77
(5) C %	9340	2.3	0.006*	5.99
(6) Na water soluble	6153.2	1.5	0.05*	3.95
(7) Ca water soluble	9890.3	2.4	0.003*	6.34
(8) HDS	10187	2.5	0.005*	6.53
(9) Evapotranspiration	7641	1.9	0.02*	4.90
(10) Resistance	6762.9	1.6	0.04*	4.34
(11) CEC	6462.4	1.6	0.08	4.14
(12) K water soluble	6075.6	1.5	0.06	3.90
(13) Clay %	3638	0.9	0.7	2.33
(14) Bulk Density	4603.5	1.1	0.3	2.95
(15) P	5407.4	1.3	0.2	3.47
(16) K	5097.8	1.2	0.3	3.27
(17) Exca Mg	3711	0.9	0.6	2.38
(18) Mg water soluble	5406.5	1.3	0.1	3.47
Best Solutions				
Variable Selections	**Adj R^2**	**RSS**	**R^2**	**% of total variation**
1-8,10,11,13,15,17	0.22469	75166	0.51805	51.81
1,3-8,10,11,13,15,17	0.22084	78824	0.4946	49.46
1-3,5-8,10,11,13,15,17	0.22081	78827	0.49458	49.46
1,3-11,13,15,17	0.22025	75597	0.51529	51.53

The vectors shown are the Pearson correlations, independently calculated for each variable (Figure 7.13). Of these parameters, higher resistance and dissolved oxygen content, and lower water-soluble Ca and HDS, distinguish Reference from Disturbed relevés. Altitude is in the same plane as the split of Reference from Disturbed relevés and has no effect on separating them.

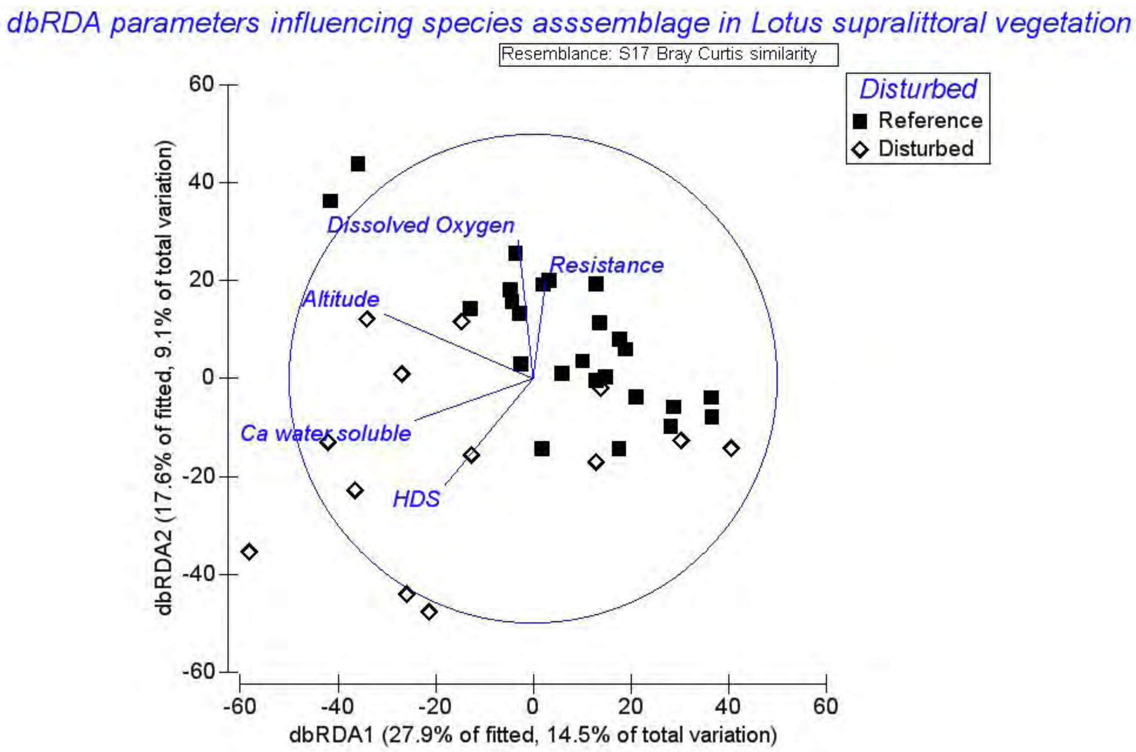


Figure 7.13: Constrained ordination by the most influential environmental variables determining different vegetation communities in Reference and Disturbed supralittoral Lotus relevés determined with dbRDA. Vectors are Pearson correlations (*r>0.04)*.

The multivariate dbRDA selected decreasing altitude (-0.6) and water-soluble Ca (-0.49) as variables best explaining change from left to right along the x-axis. This axis explains 14.5% of total variability between relevés. Decreasing HDS (-0.44), increasing dissolved oxygen in the water column (0.57), and increasing resistance in the soil (0.4) best explain separation from bottom to top along the y-axis. This axis explains 9.1% of total variability. In combination these axes explain 45.5% of fitted model variation.

Essentially, human disturbance impacts (HDS), dissolved oxygen and resistance levels were important in distinguishing Reference from Disturbed relevés and suggest an anthropogenic cause for difference.

7.2.7.2. Discriminatory species in Reference and Disturbed Lotus supralittoral relevés

Species discriminating between the disturbance categories were sought using SIMPER. The Reference relevés were dominated by graminoids, with some shrub cover. Species with greater average and typical cover in the Reference relevés were the sedges *Carpha glomerata, Ficinia nodosa* and *Fuirena hirsuta*, the bunch grasses *Imperata cylindrica* and *Ehrharta calycina*; the restio *Elegia tectorum*; and the shrubs *Metalasia muricata* and the alien *Acacia saligna*. The rush *Juncus kraussii* and the herb *Plecostachys serpilifolia* both occurred with greater typical cover in Disturbed than Reference relevés as a result of their cover in a single Disturbed relevé. These two species were, however, found in both Reference and Disturbed relevés with similar cover values and their discriminatory potential is therefore questionable. The rush *Juncus capensis,* and tussocky sedge *Carex aethiopica,* each occurred with a median cover of 68%, but only in single Reference relevés.

The Disturbed relevés were also dominated by graminoid taxa. The alien lawn grasses *Paspalum vaginatum** and *Sporobolus virginicus**, along with the large tussock-sedge *Schoenus nigricans*, the trailing herb *Centella asiatica*, and the succulent herb *Triglochin bulbosa* all occurred with greater average and typical cover in the Disturbed than Reference relevés. The mat-forming sedge *Isolepis rubicunda*, the alien grasses *Pennisetum clandestinum** and *Digitaria debilis**, the mega-graminoid rush *Typha capensis* and the shrub *Cliffortia ferruginea* all occurred with greater average and typical cover in Disturbed than in Reference relevés as a result of 68 to 88% cover dominance, each in a single sample.

A discussion of the metric potential of species with 68 to 88% median cover in single relevés (suggestive of low fidelity) within a data set is provided in Section 7.3 and Chapter 8.7.6. The top two and bottom three species in Figure 7.14 are examples of species with high cover in single relevés in the data set that have therefore potentially low fidelity.

The Disturbed relevés are saline-sodic, as were a third of the Reference relevés. This salinity gradient is a source of potentially natural difference that may be confounding the search for potential indicator species or metrics of difference between disturbance categories. *Triglochin bulbosa*, *Juncus kraussii* and the alien grass *Paspalum vaginatum** which occurred with greater cover in the saline-Disturbed than fresh-Reference relevés, are all known to be important taxa of brackish conditions in Cape Lowland Freshwater habitats (Mucina *et al.* 2006a).

7.2.7.3. *Diversity differences between Reference and Disturbed relevés*

Vegetation diversity differences between the disturbance categories were examined as a source of further potential metrics for phyto-assessment. A total of 73 species were recorded in the Lotus supralittoral relevés. Excluding the outliers Lot5_6 and 10_7, removed species *Cyperus textilis* and *Cliffortia strobilifera*. Diversity variables were determined from the standardized sample cover values in each functional group; significant variability in diversity measures is depicted in Table 7.25.

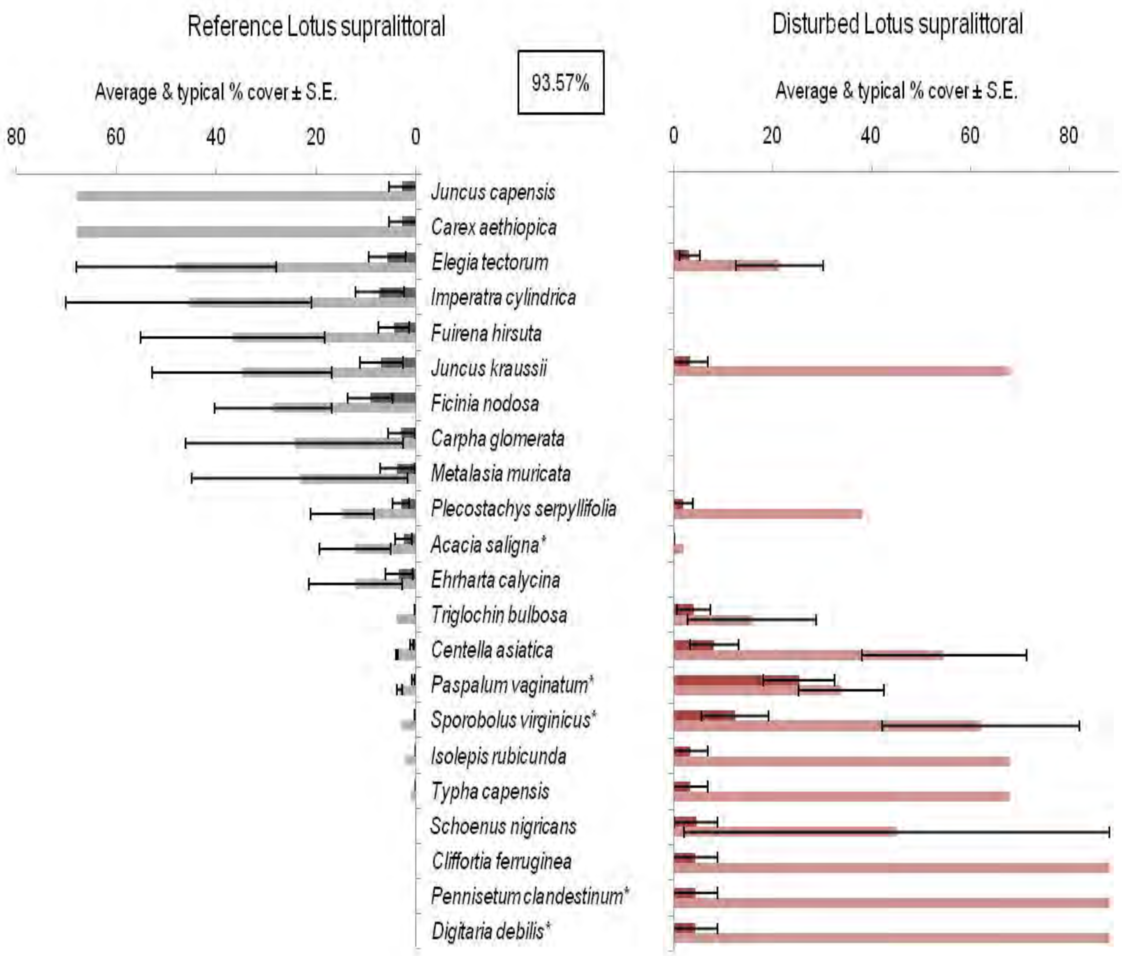


Figure 7.14: Average and typical species cover (± standard error) in the Reference and Disturbed vegetation relevés in the supralittoral hydrological habitat of the Lotus locality. Species without error bars have standard error of zero (often meaning they were present only in a single sample). The dissimilarity percentage between the species assemblages of the different disturbance categories is presented in the rectangle at the top of the graph.

Table 7.25: Diversity differences between Reference and Disturbed Lotus supralittoral vegetation relevés. Values in disturbance categories represent the average per sample (±S.E.).

Diversity variable	Taxa type	Reference	Disturbed	t-test	p-value
number	All taxa	6.5 (0.5)	5 (0.6)	2.5	0.02
cover	alien	5 (2)	38 (9)	3.9	0.001
number	Woody	1.2 (0.2)	0.2 (0.09)	4.2	0.001
cover	Woody	19 (6)	4 (2)	2.3	0.02
diversity***	Woody	0.3 (0.07)	0	3.4	0.004

***Shannon-Wiener diversity ($\log_e$)

- A total of 57 taxa with an average cover of 17.5% were recorded in the Reference category whilst only 37 taxa with an average cover of 26% were recorded in the Disturbed category. The greater number of taxa in Reference than Disturbed categories suggests a potential metric. When assessed per relevé rather than averaged over all relevés representative of a category, there was not a large enough margin of difference between categories for either number or cover to be of use as a metric.
- A lower mean cover of alien vegetation was recorded per Reference than per Disturbed relevés. The mean alien cover per disturbance category was similarly lower in Reference than Disturbed relevés (<5% *vs.* >23% respectively). Both mean cover per relevé and mean cover per category suggest potentially useful metrics. The average value per disturbance category suggests the potential to establish a metric based on percentage cover for the supralittoral zone in Lotus wetlands.
- A greater mean number, mean cover and mean diversity of woody taxa were recorded in Reference than in Disturbed relevés.
    - Nine indigenous woody taxa were recorded in Reference *vs.* only two in Disturbed relevés; including aliens, these numbers increased to 11 *vs.* 3. The overall number of woody taxa per disturbance category (rather than the average value per relevé displayed in Table 7.25) suggests a potentially useful metric that can be based on the number of woody taxa in the supralittoral habitat per wetland.
    - Whilst the mean cover per relevé of all woody taxa was greater in Reference than in Disturbed relevés (Table 7.25), the mean woody cover per disturbance category was lower in Reference (19% indigenous or 16% including aliens) than Worst (28% indigenous or 22% including aliens). The mean woody cover per relevé suggests a potentially useful metric as the cover difference bridges more than two levels of the Braun-Blanquet scale (Table 3.6), and is thus suggestive of a difference that would be easily discernable to multiple users of the scale.

7.2.8. Lotus littoral assemblages

The littoral relevés from Lotus wetlands were dominated by Cape Lowland Freshwater vegetation from sandy depressions of uniform soil depth. Results of a PERMANOVA analysis suggest that the Reference (n=19) *vs.* Worst (n=20) and Worst *vs.* Moderate (n=5) Lotus littoral relevés were distinctly different but that the Reference *vs.* Moderate relevés were not different (Table 7.4). An ANOSIM also confirmed that the Reference and Moderate relevés were not significantly distinct from one another (R=0.099, p>0.8). Dispersion differences between the Reference and Worst littoral relevés (Table 7.5 of Section 7.1.2) seem inconsequential, given the large location difference apparent between Reference and Worst relevés in unconstrained ordination. The PERMANOVA and ANOSIM results do therefore accurately reflect that vegetation communities in Reference and Worst relevés are significantly different and do not simply have different ranges of cover values.

Ordination of these relevés revealed limited separation or distinction between the different categories of disturbance, with particularly limited difference between Reference and Moderate relevés (ordination not shown). The Reference and Moderate relevés were therefore combined into a category considered to be "Mildly" disturbed and re-ordinated as displayed in Figure 7.15.

Relevé Lot14_1 was 100% covered (88% median cover) by the herb *Berula erecta* and was an outlier in the Worst disturbed category that needed to be removed before MDS ordination could be performed. After the removal of outlier Lot14_1, the Reference relevé Lot10_1, which, was 100% covered by the macro-alga *Chara ecklonii*, was also evident as an outlier (Figure 7.15).

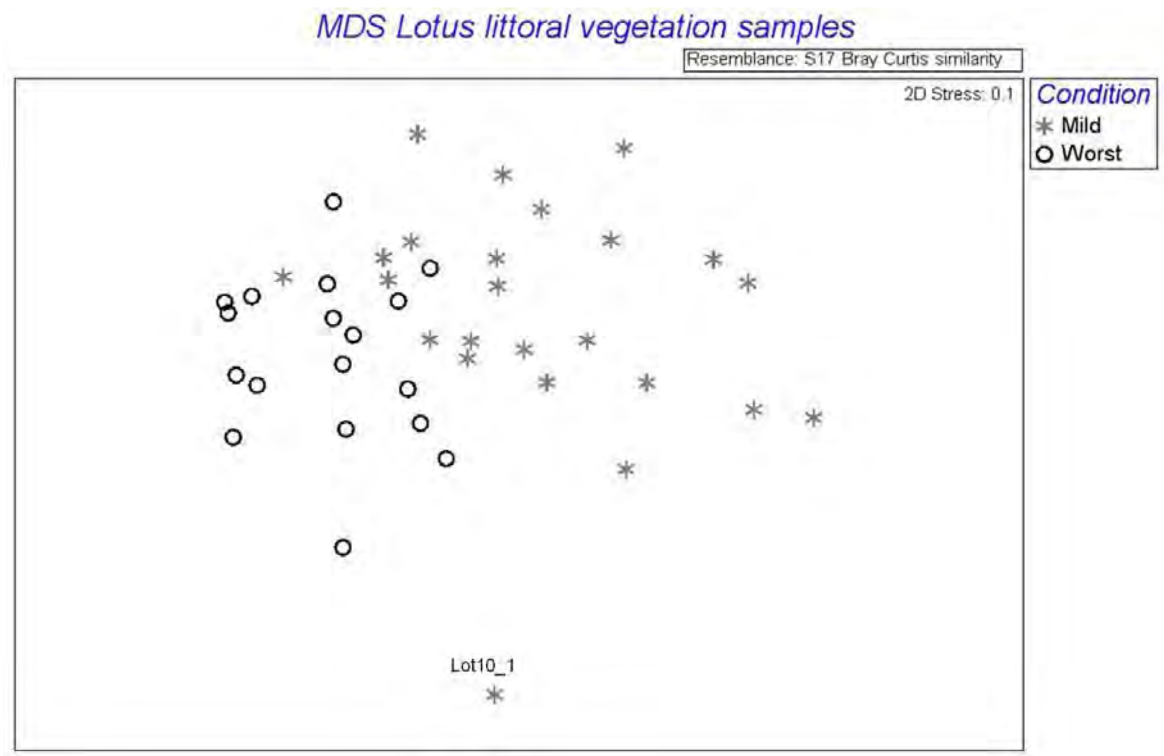


Figure 7.15: Multi-Dimensional Scaling ordination of the Lotus littoral relevés after Reference and Moderate relevés were combined as Mildly disturbed.

Examination of the environmental variables revealed low resistance and high water soluble sodium content in most relevés, suggesting saline-sodic conditions. PERMANOVA results suggest that the environmental conditions of Mild and Worst relevés are significantly different from each other (t=3.4, p=0.001).

A number of collinear variables existed amongst the parameters measured in the Lotus littoral relevés as displayed in Table 7.26. Turbidity and TIN in the water column; as well as exchangeable cations sodium and potassium, percentage of sand from the soil variables, and altitude, were all removed from the dataset as they were each represented by another variable.

Table 7.26: Collinear environmental parameters in the Lotus littoral data set.

	Altitude	Turbidity	SRP	% Silt	% Sand	K	Exca Na
Turbidity	**0.96**						
SRP	**0.98**	**0.99**					
% Silt	0.7	0.8	0.7				
% Sand	-0.7	-0.8	-0.8	**-0.95**			
K	0.8	0.8	0.8	0.8	-0.8		
Exca Na	0.0	0.2	0.1	0.4	-0.3	0.5	
Exca K	0.8	0.8	0.8	0.8	-0.8	**0.99**	0.5
Na water soluble	-0.01	0.2	0.08	0.3	-0.3	0.5	**0.99**
TIN	**0.96**	**0.98**	**0.99**	0.7	-0.7	0.8	0.1

A total of 15 environmental variables had significantly different average value in the Mild relative to the Worst disturbed relevés as determined using *a posteriori* PERMANOVA t-tests. These differences and t-tests are displayed in Table 7.27.

The different gradients of qualitatively determined human disturbance impacts on environmental condition that make up the HDS all suggested that the Worst relevés were more impacted than the Mild relevés. This was determined with t-tests as displayed in Table 7.28.

It is therefore apparent that differences between Mild and Worst relevés appear, at least in part, to be due to anthropogenic rather than natural differences. Linear modelling and redundancy analysis were therefore employed to determine which parameters were most responsible for the environmental difference as described in the next section.

Table 7.27: Environmental variables that proved with univariate t-tests to occur with significantly different average value (± S.E.) ($p<0.05$) in the Mild and Worst littoral relevés of the Lotus wetlands.

Parameter	Unit	Mild	Worst	t-test	p-value
dissolved oxygen	$mg.L^{-1}$	7.1 (0.3)	5.9 (0.4)	2.3	0.03
SRP	$\mu g.L^{-1}$	8 (1)	319 (110)	2.7	0.001
Silt %	%	1.5 (0.2)	3.3 (0.4)	4.4	0.001
Bulk Density	$kg.L^{-1}$	1.5 (0.03)	1.3 (0.04)	2.9	0.003
ph Soil KCl	pH	8.2 (0.1)	7.5 (0.1)	4.3	0.001
resistance	ohms	416 (50)	251 (50)	2.3	0.02
P	$mg.kg^{-1}$	3.3 (0.4)	16.5 (5.8)	2.2	0.008
K	$mg.kg^{-1}$	46.7 (6.8)	104 (20)	2.6	0.009
N	%	0.09 (0.01)	0.14 (0.01)	2.9	0.01
C	%	0.9 (0.1)	1.6 (0.1)	3.5	0.001
Na water soluble	$mg.kg^{-1}$	1987 (295)	4255 (748)	2.8	0.003
K water soluble	$mg.kg^{-1}$	27 (4.4)	51 (9)	2.4	0.009
Ca water soluble	$mg.kg^{-1}$	96 (10)	190 (23)	3.7	0.001
HDS	score	64 (7)	131 (3)	8.5	0.001
evapotranspiration	mm	112.5 (0.1)	112 (0)	4.6	0.001

Table 7.28: Qualitatively assessed gradients of human disturbance in Mild and Worst littoral relevés from Lotus wetlands. Gradients that proved with univariate t-tests to occur with significantly different average value (± S.E.) in each disturbance category are marked *

Gradient	Mild	Worst	t-test	p-value
Hydrological impact	-5 (2)	14 (3) Wetter	5.7	0.001*
Water Quality Impact	20 (2)	61 (3) Worse	12.6	0.001*
Physical Disturbance	21 (3)	41 (2) More	5.5	0.001*
Buffer Width	6 (1)	10 (0.5) Narrower	3.8	0.001*
HDS	55 (7)	131 (3) Worse	9.4	0.001*

7.2.8.1. Lotus littoral DistLM Results

Distance linear modelling confirmed that all 15 variables prove to have significantly different value between Mild and Worst relevés as apparent from Marginal Tests displayed in Table 7.29.

Table 7.29: Test statistics for DistLM based on "best" selection procedure and the Adjusted R^2 selection criterion for the average vegetation assemblage in Mild and Worst disturbed Lotus littoral vegetation relevés. SS = Sum of Squares, RSS = Residual Sum of Squares, R^2 = RSS/SS. All parameters have significant influence at p<0.05.

MARGINAL TESTS:				
Variable	**SS(trace)**	**Pseudo-F**	**p-value**	**% of total variation**
(1) Dissolved Oxygen	12606	3.1	0.003	7.92
(2) SRP	13984	3.5	0.001	8.79
(3) % Silt	13010	3.2	0.001	8.17
(4) Bulk Density	7346	1.7	0.04	4.61
(5) pH (KCl) soil	9497	2.3	0.008	5.97
(6) Resistance	10968	2.7	0.005	6.89
(7) P	12193	2.99	0.002	7.66
(8) K	11598	2.8	0.005	7.29
(9) N %	7556	1.8	0.04	4.75
(10) C %	8542	2.04	0.02	5.37
(11) Na water soluble	10205	2.5	0.003	6.41
(12) K water soluble	9991	2.4	0.006	6.28
(13) Ca water soluble	9110	2.2	0.006	5.72
(14) HDS	15835	3.98	0.001	9.95
(15) Evapotranspiration	14504	3.6	0.001	9.11
Best Solutions				
Variable Selections	**Adj R^2**	**RSS**	**R^2**	**% of total variation**
1,2,5,6,9-11,14	0.26127	92164	0.42099	42.1
1-3,5-7,9-11	0.25761	89427	0.43819	43.82
1-3,5,6,9-11	0.25701	92696	0.41765	41.77
2,3,5,6,9-11,14	0.25382	93093	0.41516	41.52

Using the adjusted R^2 variable selection criterion, higher dissolved oxygen in the water column, along with higher pH, higher resistance, lower percentages of carbon and nitrogen, and lower water soluble sodium in the sediment seemed consistently to reflect differences in species assemblage between Mild and Worst-disturbed vegetation relevés. Lower SRP in the water column of Mild than Worst relevés was included in the top three best solutions. Lower silt percentage in the Mild *vs.* Worst relevés was included in the 2nd, 3rd and 4th best solutions. Lower soil phosphorus in Mild *vs.* Worst relevés was included only in the 2nd solution. And lower HDS in Mild *vs.* Worst relevés was included in the 1st and 4th best solutions. A modification of Akaike's (1973) "An Information Criterion" (AIC_c) adapted for use in situations where the number of biotic relevés is low relative to the number of environmental variables (Anderson *et al.* 2008) was used to determine a more parsimonious solution. The AIC_c variable selection criterion selected HDS, SRP, soil P and water soluble Na as best explaining the difference between Mild and Worst relevés. Constrained ordination of the vegetation relevés using the adjusted R^2 choice of environmental variables revealed the variables with greatest individual influence on vegetation difference between Mild and Worst vegetation. This ordination is displayed in Figure 7.16. The AIC_c derived constrained ordination (not shown) included only the vectors of SRP, HDS and water soluble sodium and created a similar arrangement of vegetation relevés.

Considering the lengths of independent vectors in Figure 7.16, a higher HDS and content of water-soluble Na in the Worst than Mild relevés, and particularly high SRP concentrations in the four Lot06 relevés, prove to be the most important variables separating relevés of these disturbance categories. Collectively, resistance (0.4), carbon content of the soil (0.4), SRP (-0.5), and HDS (-0.36) best explain separation along the x-axis. Along the y-axis, HDS (0.58), water soluble sodium (0.49) and SRP (-0.59) best explain the separation. The first

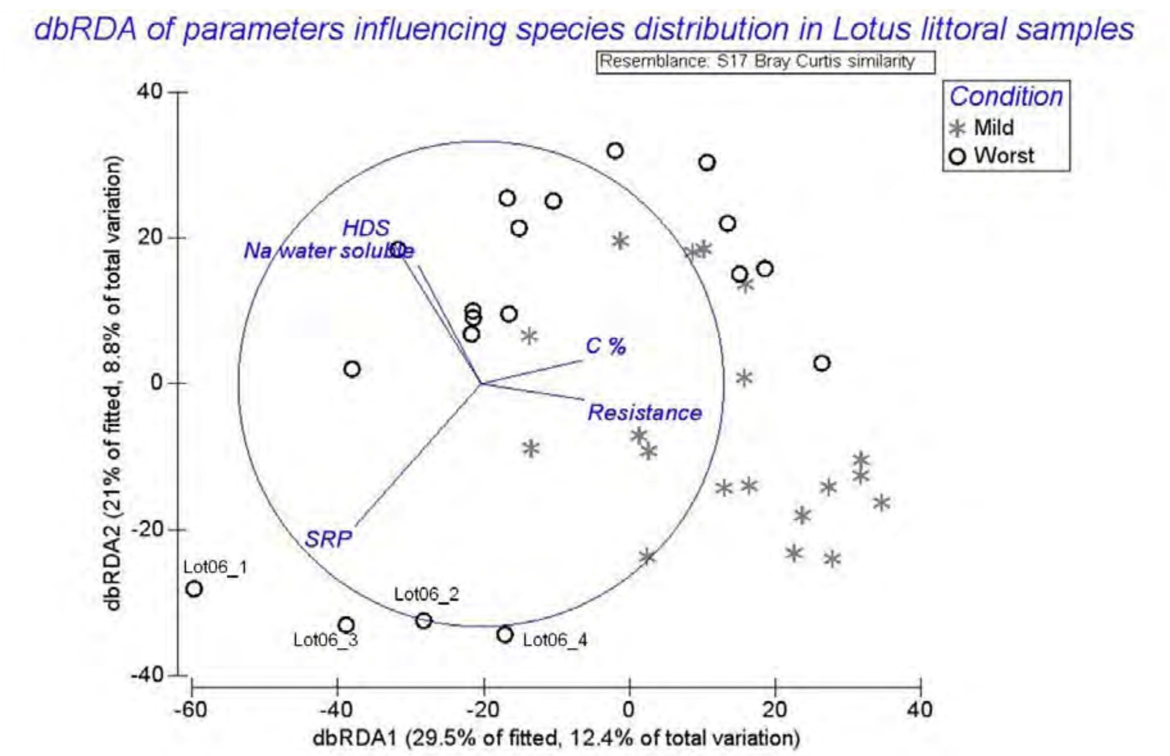


Figure 7.16: Constrained ordination by the most influential environmental variables determining different vegetation communities in Mild and Worst littoral-Lotus relevés determined with dbRDA. Vectors are Pearson correlations (*r>0.04)*.

two axes collectively explain 21.2% of the total variation between Mild and Worst relevés and 50.5% of the fitted variation is described.

Human disturbance impacts, including SRP and all of those impacts qualitatively included in the HDS (Table 7.28) have a considerable influence on species distribution, relative to all variables assessed. A salinity difference is also suggested by lower resistance and higher water-soluble Na content in the Worst than in the Mild relevés, suggestive of saline-sodic conditions in Worst samples. Species with discriminatory cover between the disturbance categories were therefore sought using SIMPER.

Removal of the Moderate relevés facilitates clearer separation between Reference and Worst relevés in the constrained ordination. On using this approach, however, there is no change in the variables chosen to best distinguish between the categories.

7.2.8.2. Discriminatory species in Lotus littoral relevés

As in the supralittoral relevés, the less disturbed (Mild) littoral relevés were predominantly dominated by graminoids, with some shrub cover (Figure 7.17). Species with greater average and typical cover in the Mildly disturbed littoral relevés were the sedges *Isolepis rubicunda,* the restio *Elegia tectorum,* the rush *Juncus kraussii,* the mega-graminoid *Cladium mariscus,* the relatively miniature sedge *Isolepis cernua,* the shrub *Cliffortia strobilifera,* and the sedges *Schoenus nigricans* (low fidelity) and *Ficinia nodosa*. Alien grasses *Paspalum vaginatum** and *Paspalum distichum*,* the rhizomatous sedge *Bolboschoenus maritimus,* the mega-graminoid *Typha capensis,* and the herbs *Sarcocornia sp.* and *Persicaria decipiens* (low fidelity) all occurred with greater average and typical cover in the Worst disturbed relevés.

Species distribution between Mild and Worst relevés does not reflect differences in salinity in Worst and Mild relevés. *Sarcocornia natalensis*, *Bolboschoenus maritimus*, *Juncus kraussii* and the alien grass *Paspalum vaginatum** are all known to be important taxa of brackish habitat in lowland wetlands dominated by Cape Lowland Freshwater vegetation (Mucina et al.2006a). In this littoral Lotus habitat, more *Paspalum vaginatum* and Sarcocornia natalensis* were recorded in the Mild relevés but more *Bolboschoenus maritimus* and *Juncus kraussii* were recorded in the brackish and Worst disturbed relevés.

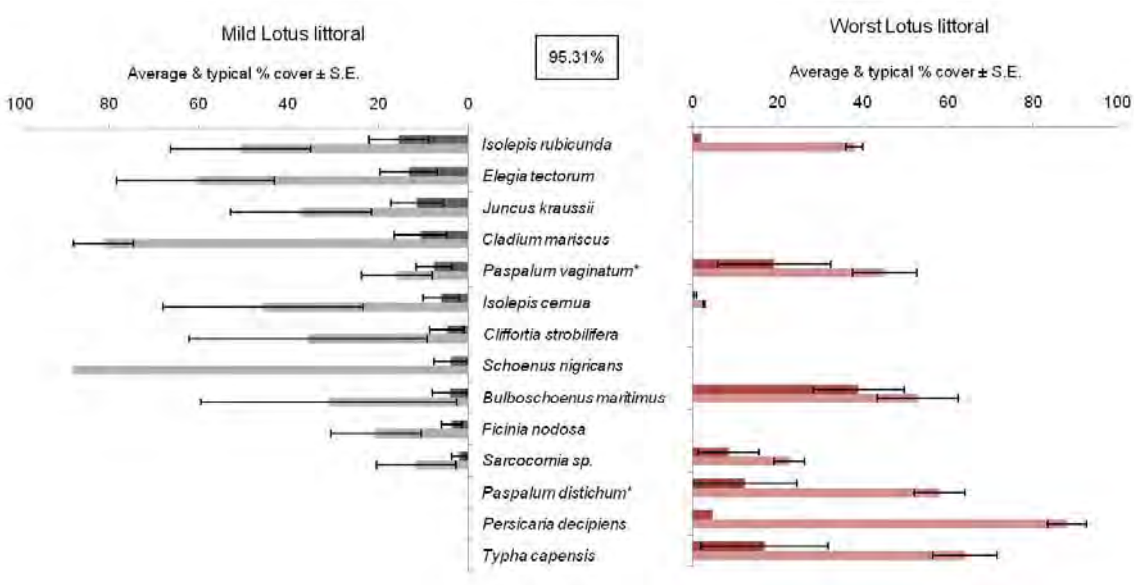


Figure 7.17: Average (dark bars) and typical (light bars) species cover (± standard error) in the Mild and Worst vegetation relevés in the littoral hydrological habitat of the Lotus locality. Species without error bars have standard error of zero (often meaning they were present only in a single sample). The dissimilarity percentage between the species assemblages of the different disturbance categories is presented in the rectangle at the top of the graph.

Removal of Moderate relevés and recalculation of the similarity percentage removes *Persicaria decipiens* and *Schoenus nigricans* (both of which had low fidelity in the Mild relevés) from the species that discriminate between Reference and Worst categories.

7.2.8.3. Diversity differences between Lotus River Mild and Worst relevés

Potential phyto-assessment metrics were sought from differences in diversity in Mild and Worst disturbed relevés by examining all species, as well as functional, structural and life-history related species groups. A total of 36 species were recorded in the Lotus littoral relevés. Significant diversity differences between the Mild and Worst category of disturbance for various functional groups are presented in Table 7.30.

- A greater mean number and mean richness of all taxa were recorded per relevé in the Mild than in the Worst disturbance categories. Per disturbance category a greater total number of taxa was again recorded in Mild (30) than Worst (16). A greater mean cover of all taxa was recorded to occur in Worst (42%) than Mild (28%) relevés; this was however, not significantly different between categories.
- No leafless graminoids were recorded in the Worst relevés whilst an average 16% cover, derived from two taxa occurred in Mild relevés (seven Reference and a single Moderate

sample). The Cyperaceae *Ficinia nodosa* and Restionaceae *Elegia tectorum* were responsible for this difference between Mild and Worst relevés. The cover difference suggests potential for metric purposes; the difference in number of taxa does not suggest reliability.

- Taxa with facultative to obligate affinity for wetland habitat (*sensu* Reed 1988) made up the vast majority (33 of 36) of all species in these littoral relevés. Not surprisingly these species, also had a greater mean number and taxon richness in Mild than in Worst relevés. Per disturbance category there was also a greater mean number of these taxa in Mild (28) than in Worst (15) relevés; suggesting that greater numbers of these taxa are likely to be found in wetlands from the Mild than Worst disturbed categories. Per disturbance category there was lower mean cover of FW and OW taxa in Mild (>28%) than Worst (42%) wetlands; which, in combination with the greater number of species in Mild than Worst categories (or wetlands) explains the greater richness (number of species standardized against the total cover occupied by each species) observed in the Mild category of relevés or wetlands.
- A lower mean cover of alien taxa was recorded per Mild than per Worst relevé (Table 7.30), suggesting a potential metric.. This was echoed by a lower mean cover of alien vegetation per disturbance category in Mild (<14%) than Worst (>42%); again suggesting a potentially useful metric of greater mean alien cover in Worst than Mild wetlands.

Table 7.30: Diversity difference between Mild and Worst Lotus littoral vegetation relevés. Values in disturbance categories represent the average per sample (±S.E.).

Diversity variable	**Taxa type**	**Mild**	**Worst**	**t-test**	**p-value**
number	All taxa	4 (0.4)	3 (0.3)	2.2	0.04
richness*	All taxa	0.6 (0.1)	0.4 (0.1)	2.2	0.03
number	Leafless graminoids	0.37 (0.1)	0	2.9	0.007
cover	Leafless graminoids	16 (6)	0	2.3	0.03
cover	aliens	8 (4)	27 (8)	2.3	0.02
number	FW and OW ††	3.6 (0.4)	2.7 (0.3)	2.1	0.04
richness*	FW and OW	0.6 (0.1)	0.4 (0.1)	2.2	0.05

†† Species with Facultative Wetland to Obligate Wetland habitat affinity (*sensu* Reed 1988)

*Margalef's species richness relative to total species cover

Removing the Moderate relevés and recalculating diversity differences served to decrease the representation of alien cover in the Reference relevés, increasing the magnitude of difference in this potential metric. No other changes in the results were obtained on removal of the Moderate relevés.

Margalef's species richness of all taxa and FW and OW taxa per sample do not suggest useful metrics for phyto-assessment purposes; they do however have the potential for use in long term monitoring projects in which comparison will be made over time or between relevés.

7.2.9. General observations

Identifying whether it is anthropogenic disturbance or natural variation that is responsible for the observed differences in vegetation communities is a critical aspect of this study., This was achieved by both examination of each variable using univariate analyses (t-tests) and in combination by multivariate analyses (DistLM and dbRDA). The DistLM procedure was generally successful in distinguishing anthropogenic and natural differences between the vegetation relevés. Driftsands littoral and Lotus supralittoral and littoral relevés had differential saline-sodic conditions between Reference and Worst disturbance categories, typically with greater salinities in more disturbed relevés. Whether this was the result of anthropogenic influence is not clear from the recorded data. Eutrophic phosphorus concentrations, both in the soil and water-column, appear to be of anthropogenic origin in the littoral relevés at Lotus and Kenilworth, and in the supralittoral Kenilworth habitat.

Elevated dissolved oxygen content in the water column seems to be an important variable in distinguishing less-, from more-disturbed relevés of Lotus supralittoral and littoral vegetation. Decreased dissolved oxygen in the water column (and associated increased release of toxic gases) can be caused by the high oxygen demand of bacterial decomposition of organic matter (Walmsley and Butty 1980). In situations of anthropogenically driven increased organic matter such as caused by algal blooms due to elevated phosphorus levels this is further indication of anthropogenic disturbance.

In general a clear difference between less- and more-disturbed relevés was indicated by a few of the measured environmental parameters, along with the land-use impacts qualitatively assessed by the HDS. No single overall environmental parameter was an essential discriminatory variable between disturbance categories in all cases assessed. Soil and water column phosphorus, dissolved oxygen, and HDS were frequently useful to decipher differences between disturbance categories. Soil particle size, water soluble sodium content, resistance and soil or water pH levels are all important variables in the determination of natural differences between relevés within any locality

In the separate hydrological habitat units of localities assessed above, the species and diversity differences that were shown to exist between the HDS categories of vegetation were accepted as being the result of human disturbance of environmental condition rather than natural variability The use of such differences for the development of phyto-assessment metrics is considered justified. Conclusions about the Western Coastal Slope vegetation based on the DistLM and PERMANOVA analyses are presented in the following section.

7.3. Discussion of characteristic species differences

There is no general pattern of species association with different categories or conditions of disturbance that would facilitate determination of phyto-assessment metrics across the whole of the Western Coastal Slope. The only broad or general patterns that were apparent were that:

1. vegetation was considerably different at each locality assessed within the Western Coastal Slope (Table 7.1 in Section 7.1.1); and
2. the vegetation of the littoral and supralittoral habitats differs considerably across the whole Western Coastal Slope (Table 7.3 in Section 7.1.1).

A number of species metrics and functional diversity metrics were common to more than one locality as will be discussed below (Sections 7.3.1 and 7.4). The inherent natural variability known to exist as a result of climatic and soil (and related geological) differences over the Fynbos Biome creates different dryland vegetation types (Rebelo et al. 2006) and from the present study can clearly be seen to determine differences in wetland vegetation between sub-regions and even between localities within sub-regions. From the differences apparent between the Driftsands and Lotus wetlands, both of which are within the dryland Cape Flats Strandveld vegetation type, it is evident that considerable natural variability exists even within dryland vegetation units and this variability also drives differences in wetland vegetation. Examination of differences between disturbance categories is therefore essential within hydrological habitat units within each locality or area that is shown to hold a homogenous community of wetland vegetation. A brief overview of the few species that appear to occur with characteristic discriminatory cover in different disturbance categories across more than one habitat-locality within the Western Coastal Slope is presented in the next section along with species that show a more localized characteristic response to disturbance. The following section (7.4) describes diversity differences with general patterns at the sub-region scale.

7.3.1 Characteristic differences in species cover

Of the 365 species recorded in the present study, only four occur with consistently characteristic difference in cover between disturbance categories in more than one habitat-locality dataset. A synopsis of the results for these four species, including their potential as phyto-assessment metrics, is presented in Table 7.31 and discussed below. And in Section 7.3.1.1other methods of determining metrics from individual taxa are explored. All the cover values discussed in this section are based on median values representative of the different categories of cover in the Braun-Blanquet scale in Table 3.6 and again at the bottom of Table 7.36.

Table 7.31: Species that showed characteristic and differential association with degrees of disturbance in the Western Coastal Slopes. The percentages given are the amount of area that a species covered per sample, or per wetland

Species	Reference	Disturbed	Where in Western Coastal Slopes	Where in South Africa[1]
Typha capensis	<10% cover/sample or /wetland	≥18% cover/sample or /wetland	Cape Flats and Overberg wetlands	All provinces – limited distribution in Northern Cape
Cyperus textilis	Absent	18-88% cover/sample (not per wetland)	Cape Flats and West Coast	Western and Eastern Cape, and KwaZulu-Natal
*Paspalum vaginatum**	<5%	≥18% cover/wetland or sample	Lotus wetlands	Western and Eastern Cape, KwaZulu-Natal, Free State and Gauteng
Juncus capensis	1 to 10 specimens; up to 8% cover/sample	Absent	Fynbos associated wetlands	Western and Eastern and Northern Cape (limited distribution in latter province)

Plants of South Africa – online checklist (POSA) (www.posa.sanbi.org)

7.3.1.1. Typha capensis

Typha capensis occurred with greater average and typical cover in six of the seven Worst or more disturbed sample sets that were shown to hold significantly different vegetation assemblages from the Reference or less disturbed sample sets (Table 7.4). An apparent exception to this pattern existed within Driftsands supralittoral vegetation, however, where *T. capensis* was represented by 8% median cover in two Reference relevés and was absent in the Worst relevés. However, the low cover value in these two Reference relevés is not incongruous with the fact that generally, greater cover of *T. capensis* is found in the more disturbed relevés in a given locality. By its consistent association with greater cover in more disturbed than in less disturbed conditions, *Typha capensis* suggests a potentially consistent metric for wetlands within the Western Coastal Slope.

In the Western Cape this species is known to increase coverage in areas where the season of saturation is extended (Hall 1992). The results of the present study also suggest that in situations with sufficient water to support *T. capensis* growth, this species increases in cover as a result of other disturbances including elevated physical disturbance (all sites) as well as elevated water-column TIN (Hermanus) and SRP (Lotus) concentrations, and due to elevated soil P concentration (Kenilworth). In the Western Coastal Slope data set, *Typha capensis* with 18% or more typical cover indicated a Moderate to Worst category of environmental condition. Typical coverage of up to 8% was apparent in Reference relevés. This suggests that any sample with greater than 18% cover indicates a potentially disturbed condition. Average cover per wetland values of up to 8% (2±1%) were observed in Reference wetlands whilst in Worst disturbed wetlands up to 38% cover was observed.

Typha capensis probably has indicator potential outside of the Cape Coastal lowlands. There is much literature available on the *Typhaceae*, and it a genus that is known to be tolerant of poor water quality, being capable of growing in conditions of high nutrient loadings. It and may even store heavy metals. The work of Hall (1993) suggests that the presence of *Typha* indicates extended hydroperiod and newly saturated sediments. *Typha capensis* is a tall (2 m) erect graminoid (the bulrush) connected underground by robust rhizomes. The plant is easily recognizable throughout the year and often dominant in marshes, backwaters, lagoons and pools and along water courses, growing from sea-level to ± 1950 m throughout Southern and Central Africa (Cook 2004).

7.3.1.2. Cyperus textilis

Cyperus textilis occurred with greater typical cover in the Worst relevés of Kenilworths' supralittoral (88% median) and littoral (18% median) hydrological habitats, based on the presence in a single sample in each habitat. This species was also represented in a sample where it occurred with 88% median cover that was an outlier from the Worst relevés in the Lotus supralittoral vegetation; and hence, would equally have been considered a characteristic species of Worst disturbed relevés. This species was not considered discriminatory in other localities assessed.

The targeted sampling approach means that only vegetation of stands that were characteristic of the ecosystem were assessed; hence, the presence of this species in only single relevés in each habitat-locality where it was found does not exclude its use as a potential indicator species. *Cyperus textilis* also occurred with 68 and 38% median cover in two moderate relevés and with 18% cover in a single Worst sample at Verlorevlei. There was also a sample anomalous to the general pattern that suggested greater *Cyperus* textilis cover. This was at Waskraalvlei in the Overberg, where *Cyperus textilis* was occurred with 88% median cover in a single Reference sample. In all relevés where this rhizomatous species occurred it created a dense stand to the exclusion of all other taxa. The soil pH level in all *Cyperus textilis* containing relevés ranged from 4.6 to 7 whilst salinity ranged from fresh to brackish. The lack of Reference relevés on the West Coast and Disturbed relevés in the Overberg restrict determination of whether this species is consistently useful as an indicator of disturbance/environmental conditions.

Cyperus textilis Thunberg is a robust and leafless sedge (Cyperaceae) with a creeping and woody rhizome that typically allows the plant to dominate the area in which it establishes (Cook 2004). *Cyperus textilis* is found along the coast in wet places and in still or flowing shallow waters of the Western and Eastern Cape as well as in KwaZulu-Natal. *Cyperus textilis* is fairly easily recognizable if not confused with the introduced alien *Cyperus involucratus* Rottbøll [*C. alternifolius* Linnaeus subsp. *flabelliformis (Rottbøll) K*ükenthal] which, has a sharply angled culm (stem) below the inflorescence rather than the rounded culm of *C. textilis.* A further difference between the two species is smooth rays *vs.* serrated rays (*C. involucratus*).

7.3.1.3. Paspalum vaginatum

The C4 and alien grass *Paspalum vaginatum** occurred with greater average and typical cover in Disturbed than in the Reference Lotus supralittoral and littoral hydrological habitat within the Cape Flats sub-region. This grass was represented in many Lotus relevés in each habitat and each disturbance category as is apparent in Table 7.32.

Table 7.32: *Paspalum vaginatum** representation in vegetation relevés in Lotus wetlands.

Habitat	# Reference relevés	# Disturbed relevés
Lotus-supralittoral	4 of 25	15 of 20
Lotus-littoral	11 of 23	8 of 19

Median cover of 12.5-25% or more of *Paspalum vaginatum** is considered to be a reliable indicator of disturbance within the Lotus wetlands of the Cape Flats. *Paspalum vaginatum** was recorded, albeit with less than 5% cover, in the Verlorevlei wetlands on the West Coast; where again it occurred with marginally greater cover in Worst than Moderate wetland vegetation relevés.

*Paspalum vaginatum** is not confined to wetlands but is often found within wetlands near the coast or in brackish conditions but also in inland fresh water wetlands. Whilst the grass typically occupies the supralittoral margins of wetlands (Goldblatt and Manning 2000), it may also occur within the littoral zone where the stems (culms) may float on the water surface (Cook 2004). The species is distributed in the tropics and sub-tropics of both global hemispheres and within South Africa it is known to occur in the Western and Eastern Cape and in KwaZulu-Natal (Cook 2004). *Paspalum vaginatum** is relatively easily distinguishable from *Cynodon dactylon* by using the inflorescence and the presence in the leaf sheath of a membranous ligule *vs.* a fringe of hairs.

7.3.1.4 Juncus capensis

Juncus capensis was found only in wetlands surrounded by Fynbos vegetation. *Juncus capensis* occurred with greater average and typical cover in Reference and Moderate than Worst supralittoral Kenilworth relevés, being present in half of the Reference relevés. It also occurred in a single Reference supralittoral Lotus sample. *Juncus capensis* was found in Reference and Moderate relevés with an average cover of 3±1% and with typical cover of 8±3% in Kenilworth and 24±22% in Lotus relevés. Average cover of 3% actually represents abundances of <10 specimens per sample (Braun Blanquet scale, Table 7.36). *Juncus capensis* was not found in Worst disturbed Kenilworth and Lotus supralittoral relevés. In fact this species only occurred in two worst disturbed relevés (again with low abundance of less than 10 specimens) at Hermanus wetlands out of the 24 Worst Fynbos relevés assessed.

Generally *Juncus capensis* occurs with greater cover/abundance in Reference than Worst disturbed wetlands surrounded by Fynbos in the Western Coastal Slopes wetland region. In combination with other metrics this species may provide a potentially useful metric for phyto-assessment in wetlands surrounded by dryland vegetation of the Fynbos type.

Juncus capensis is a polymorphic but typically multi-stemmed graminoid with a bunched or tufted growth form that occurs in supralittoral to terrestrial conditions (Cook 2004). The plant is endemic to the Western, Eastern and Northern Cape provinces of South Africa.

7.3.2. Metrics relating to Sensitive and Tolerant species

The identification of taxa that are particularly sensitive to, or tolerant of, disturbance has been used in the development of metrics for phyto-assessment purposes (e.g. Adamus *et al.* 2001, US EPA 2002c). Species that are sensitive to disturbance will not occur (or may occur, but only with reduced cover/abundance) where disturbance has impacted an ecosystem. Tolerant taxa may occur under all environmental conditions. They are, however, expected to be more abundant in impacted than in minimally impacted wetlands. A brief description of the development of these concepts of tolerance and sensitivity from the current dataset is provided below, along with a discussion of the potential for metrics using these species.

7.3.2.1. Sensitive species

A species that is recorded in at least two wetlands with reference environmental condition and only a single impaired wetland, has been considered sufficient evidence of sensitivity to human disturbance (see Gernes and Helgen 2002 and/or Section 2.7.3.1.ii in this volume). Gernes and Helgen (2002) considered that no species that is known to be invasive or an alien species should be classified as sensitive even if (by chance) they fulfil the previous condition. The nature of invasive species is that they invade minimally impacted ecosystems and as such must be considered an early sign of impact, yet they also have a natural environment within which they originated/evolved. Not all invasive and/or alien species will be tolerant of disturbance events or impacts and it would perhaps be more accurate to classify species into sensitive/tolerant/alien/invasive categories. For the purpose of the present study, however, only sensitive and tolerant species were identified.

A number of species were recorded in single Reference relevés only and thus, according to the above criteria, do not qualify as sensitive species. The sedge *Chrysitrix capensis*, and fern *Histiopteris incisa* each of which occurred in a single Kenilworth Reference sample and shrub *Berzelia abrotanoides* that occurred in a Reference sample at Kenilworth and the less disturbed relevés of Hermanus, are examples of such species. Further sampling needs to be done to determine whether these three species are consistently associated only with Reference or less disturbed conditions and are thus sensitive species. Whilst *Chrysitrix capensis* is relatively uncommon, *Histiopteris incisa* and *Berzelia abrotanoides* are fairly common within Fynbos associated vegetation.

In the present study nine, of the 375 species encountered in individual vegetation relevés were recorded in reference wetlands only, with no record in disturbed wetlands (Table 7.33).

A further two species were recorded from two or more Reference wetlands and only a single impaired wetland (Table 7.34).

Cliffortia strobilifera is one of the species that was reported by wetland ecologists to be a general indicator of disturbance in wetlands, typically believed to occur with increased abundance in eutrophic conditions. Certainly in wetlands that this author has surveyed, this species has been observed in association with canalisation of river beds (Ratels River, Agulhas Plain) and surrounding storm-water dams. On the other hand, in four Reference vegetation relevés from the Lotus River wetlands *C. strobilifera* was encountered with median cover varying from 1% to 88%. In two of these relevés the plant covered 88% of the surface in wetlands that had only limited human disturbance impacts. In the Verlorevlei wetlands, *C. strobilifera* occurred in two Moderately disturbed relevés with 2% and 88% median cover. Dominant or large cover of this species does not therefore always indicate an impacted or environmentally degraded condition.

A number of the sensitive species listed in Table 7.33 7.34 are from plant functional groups that are characteristic of the Fynbos Biome, namely; restios and sclerophyllous shrubs and that are known to have limited distribution (Linder *et al.* 1992). Aquatic herbs such as *Potamogeton* and *Aponogeton* have submerged leaves and are considered (Adamus *et al.* 2001) to be sensitive to turbidity (shading in the water column caused by suspended dissolved particles). A number of the species in the above two tables (Tables 7.33 and 7.34) were recorded in only two reference wetlands as well as in some instances in a single impaired wetland. Many more observations should be recorded before any definitive list can be derived of sensitive indicator species.

Table 7.33: Sensitive species as determined from preferential occurrence in only reference wetlands.

Sub-region	Locality	Associated upland vegetation type	Species	Growth Form*	# of Reference wetlands
Cape Flats	Lotus	Cape Flats Strandveld	*Albuca fragrans*	Geophytic herb	2
			Cladium mariscus	Megagraminoid	3
			Metalasia muricata	Low shrub	2
Cape Flats and Overberg	Lotus, Hermanus, Ratelsvlei	Cape Flats Strandveld, Overberg Sandstone Fynbos, Agulhas Limestone Fynbos	*Hippia frutescens*	Low shrub	4
Cape Flats and Overberg	Kenilworth and Ratelsvlei	Cape Flats Fynbos, Overberg Sandstone Fynbos	*Ischyrolepis paludosa (Pillans) H.P.Linder*	Graminoid	2
Overberg	Ratelsvlei and Waskraalvlei on Agulhas Plain	Overberg Sandstone Fynbos, Agulhas Limestone Fynbos, Central Ruens Shale Renosterveld	*Potamogeton pusillus*	Aquatic herb	3
Overberg	Ratels Vlei and Waskraalvlei on Agulhas Plain	Overberg Sandstone fynbos, Central Ruens Shale Renosterveld	*Agrostis cf. bergiana*	Graminoid	2
Overberg	Agulhas Plain	Elim Ferricrete Fynbos	*Aponogeton fugax*	Aquatic herb	2
Overberg	Agulhas Plain	Elim Ferricrete Fynbos	*Spiloxene aquatica*	Geophytic herb	2

*Growth forms described by Mucina and Rutherford 2006.

Table 7.34: Sensitive species as determined from preferential occurrence in two or more Reference wetlands and only one impaired wetland.

Sub-region	**Locality**	**Associated upland vegetation**	**Species**	**Growth Form***	**# of Reference Wetlands**	**# of Moderate wetlands**
Cape Flats, Overberg	Driftsands, Lotus, Ratels Vlei	Cape Flats Strandveld, Overberg Sandstone Fynbos	*Potamogeton pectinatus*	Aquatic herb	3	1
West Coast, Cape Flats	Verlorevlei, Lotus	Leipoldtville Sandstone Fynbos, Cape Flats Strandveld	*Cliffortia strobilifera*	Low shrub	2	1

7.3.2.2. Tolerant taxa

Five of the 375 species recorded in individual vegetation relevés occur in more than one Worst wetland, as well as in some cases in a Moderate wetland, but not in Reference wetlands (Table 7.35). All of the alien invasive species are considered as tolerant taxa; since non-indigenous or alien taxa are generally considered tolerant of disturbance (e.g. Wilcox 1995). A total of 73 alien plant taxa were recorded in the dataset collected for this study. However, many alien species were also recorded in Reference relevés and the presence of alien taxa is thus considered one of the first signs of impairment and degradation of a wetland ecosystem. Alien invasion is not only an indicator of, but also one of the aspects of, ‘disturbance’, through competition for space and light and other resources and causing shading and eutrophication (Witkowski and Mitchell 1987). As shown in the diversity analyses in Section 7.2, and discussed in Section 7.4.1 below, the average cover of alien taxa in Disturbed relevés/wetlands was often greater than that in Reference relevés/wetlands. This therefore would corroborate the findings in the USA (Wilcox 1995, Adamus *et al.* 2001, US EPA 2002c), that alien or non-indigenous taxa should be considered tolerant of disturbance. The alien and C4 grasses *Pennisetum clandestinum*, Paspalum vaginatum*, P. distichum*, Lolium perenne** and *Phalaris aquatica** all occurred in many Worst wetlands (and some Moderates) as well as in a few Reference wetlands. The cover of these alien grasses in Reference wetlands was typically less than 5% per sample. These grasses are often associated with physically disturbed conditions in the Cape and extensive cover by these taxa can be considered indicative of disturbance.

Indigenous species that were tolerant, as determined by presence in only Worst or Moderate wetlands, are presented in Table 7.35. *Berula erecta* and *Persicaria decipiens* occurred with 88% median cover in a number of relevés considered to be in Worst or Moderate condition. The short grass species *Polypogon strictus* was only recorded in the Darling locality of the West Coast sub-region; in which, it occurred with greater coverage in Worst than Moderate relevés. No Reference wetlands were assessed on the West Coast. Other than in a single Worst sample where *P. strictus* occurred with 68% cover, it was recorded with less than 5% cover and with an abundance of between 1 to 1000 individuals.

Table 7.35: Tolerant species as determined from preferential occurrence in only Worst and Moderate wetlands.

Sub-region	**Locality**	**Associated upland vegetation**	**Species**	**Growth Form***	**Worst**	**Moderate**
Cape Flats	Driftsands and Lotus	Cape Flats Strandveld	*Berula erecta*	Herb	2	0
West Coast, Cape Flats, Overberg	Lotus, Kenilworth, Hermanus, Verlorevlei	Cape Flats Strandveld, Cape Flats Fynbos; Overberg Sandstone Fynbos and Leipoldtville Sandstone Fynbos	*Persicaria decipiens*	Aquatic Herb	4	1
West Coast	Darling	Swartland Granite Renosterveld	*Polypogon strictus*	Graminoid	2	1
Cape Flats	Driftsands	Cape Flats Strandveld	*Tolypella cf. nidifica var. glomerata*	Macroalga	2	0

The macro-alga *Tolypella cf. nidifica var glomerata,* a "stonewort", occurred in four Worst disturbed littoral relevés at Driftsands on the Cape Flats and in no others. The qualitative determination of disturbance (HDS) on these relevés suggested predominantly water quality, physical and water-reducing impacts. These sandy relevés were slightly saline with low levels of TIN, SRP, soil P, conductivity and turbidity. Despite a relatively high HDS score based on human land use that was thought to negatively impact water quality, quantitative measurements of the aforementioned variables suggesting that impacts were predominantly physical in nature and probably related to a shortened hydroperiod. The anticipated high levels of water quality impacts did not occur. Whilst the classification of these relevés as Worst according to the present HDS was not incorrect, the breadth of the impacts assessed by this scoring system meant, for instance, that wetlands with only changed hydroperiod but that were otherwise unimpacted could still be classed as Worst disturbed. Further investigation into the resilience of stoneworts in South African wetlands should be undertaken before *T. nidifica* is considered to be tolerant of disturbance.

Berula erecta (Hudson) Coville [*Sium thunbergii* A.P. de Candolle] is recorded by different sources as occurring in the Western and Eastern Cape (Cook 2004) as well as in Gauteng (POSA 2009) and is nearly cosmopolitan in its global distribution (Cook 2004 and Goldblatt and Manning 2000). *Polypogon strictus* Nees is known to occur from many locations in wet habitats along the coast of the Western and Eastern Cape (POSA 2009). *Persicaria decipiens* (R. Brown) K.L. Wilson *(Polygonum salicifolium* Broussonet ex Willdenow) is an almost cosmopolitan species in temperate to tropical climate, existing almost throughout Africa (Cook 2004). *Tolypella nidifica* (O. Müller) Leonhardi has widespread global distribution, being found locally in the Western Cape of South Africa. The wide distribution of these species does perhaps suggest tolerance to a wide range of environmental conditions, but it must also be considered that species have a natural habitat in which their presence denotes potentially minimally impacted (Reference) conditions). Hence many more observations should be made before any of the indigenous taxa in Table 7.35 can be considered unequivocal indicators of, or tolerant of, disturbance.

7.4. Discussion of characteristic diversity differences

The presence of certain combinations of taxa, such as obligate wetland species, aliens and woody taxa, suggested some general patterns of characteristic association with different categories of disturbance or environmental conditions. It is important to note that there were no patterns of functional diversity that were apparent across the entire study region. A number of patterns did however exist over more than one of the individual habitat-locality combinations within the Western Coastal Slope, as will be described below for four taxa groupings.

7.4.1. All taxa

A greater number of taxa were recorded in the less- rather than in the more-disturbed supralittoral habitat of wetlands at Hermanus, Kenilworth and Lotus wetlands, and in the littoral habitat at Kenilworth and Lotus wetlands. The reverse trend was observed to occur for “mean cover” of all taxa in all of these habitat-locality combinations. These aforementioned observed patterns were based at the hydrological zone scale in which total number and mean cover per habitat zone are determined. At this hydrological scale of measurement, the common trend in the habitat-localities that proved to have significantly different sets of vegetation in the HDTS categories, was that there were generally more taxa in less disturbed than in more disturbed conditions.

At the sample scale of measurement, it is only in the Lotus littoral habitat that a greater mean number and richness of all taxa were recorded in less disturbed than more disturbed wetlands. It is not possible to generalize further about the number, or any other measureable diversity parameter (richness, evenness, dominance, or cover) of the combination of all taxa found in relevés from different disturbance categories. It is therefore necessary to search for diversity differences within sub-groups of taxa such as can be created from functional groups, growth forms and life-history attributes such as perenniality and affiliation to wetland habitat. Aliens, woody species and taxa with obligate and facultative association to the wetland habitat did, however, show some general patterns as are discussed below.

7.4.2. Alien taxa

On the Cape Flats, a greater mean cover of alien vegetation was recorded in the more (Worst or Disturbed) than in the less (Reference or Mild) disturbed Lotus supralittoral and littoral habitats as well as in Kenilworth supralittoral habitat. In these habitats, median cover of 38%, or higher, as averaged from all relevés within a habitat zone of a wetland, appears to indicate a negatively impacted environmental condition. Values of less than 38%, generally suggest only Mild levels of impact or disturbance.

When values were averaged for each -relevé, a greater cover of alien taxa was recorded in the more than in less disturbed relevés in the supralittoral of the Lotus and Kenilworth wetlands and in the littoral of the Lotus wetlands,. The Driftsands littoral habitat again represented an anomalous situation, however, with more alien vegetation cover recorded in Reference than the Worst relevés; although the difference was minimal. Overall a greater mean cover of alien vegetation was encountered in Worst (23±7) than in Reference (4±2) relevés. A greater cover of alien vegetation may therefore be expected to occur in Worst disturbed than Reference relevés on the Cape Flats. The same conclusion cannot be drawn from the datasets collected on the West Coast and South Coast (Overberg); partly due to insufficient comparative wetlands sampled in each of these sub-regions.

7.4.3. Woody taxa

More woody taxa (averaged per sample) were apparent in the less (Reference or Moderate) than more disturbed (Worst or Disturbed) supralittoral vegetation relevés of the Hermanus, Kenilworth and Lotus wetlands. The interaction of fire exclusion, grazing and harvesting are known drivers of woody vegetation in wetlands (Clark and Wilson 2001, Middleton 2002). Examination of the number of woody species typically[2] encountered per sample revealed a less clear picture. In general the numbers being compared per sample (usually 2 *vs.* 1) are too low to facilitate any generalization about the number of woody taxa characteristic of different disturbance categories. However, as averaged for all relevés from a habitat zone of a wetland, a greater number of woody taxa indicated a less disturbed condition in the supralittoral habitat of Hermanus, Kenilworth and Lotus. The number of woody taxa that is representative of Reference and Worst disturbance appears to vary. In the Cape Flats supralittoral habitat, nine indigenous woody taxa were recorded in Reference *vs.* two or less taxa in Disturbed conditions. At Hermanus 16 indigenous woody taxa were recorded in Moderate relevés whilst only four were recorded in Worst relevés. Generally, it would appear that an order of magnitude more woody taxa indicate a Reference condition. More than seven and fewer than three woody indigenous taxa may be useful guidelines for Reference *vs.* Disturbed conditions.

A greater Shannon-Wiener diversity of woody taxa was recorded in less disturbed than in more disturbed supralittoral relevés in the Hermanus and Lotus wetlands. This suggests that a greater heterogeneity, or greater number of different woody taxa with greater proportional cover, occur in the less disturbed than in more disturbed relevés. Greater levels of disturbance apparently reduce the number of woody taxa that are able to survive and therefore also reduce their proportional cover. Greater number and cover of woody taxa were also recorded in the Kenilworth supralittoral relevés, although diversity was not significantly different. The difference in diversity of woody taxa between less and more disturbed wetlands has some potential for metric development.

Measured as the average value per sample or as the average value per habitat, the cover of woody vegetation is considerably greater in less disturbed than in more disturbed supralittoral habitats for both Kenilworth and Lotus wetlands. Differences are large (18% *vs.* <5% cover), being in the order of more than two levels of the Braun-Blanquet cover scale

[2]Typical: as derived from the average value from only those samples in which these species are recorded rather than the average value from all samples with in a disturbance category within a wetland or habitat zone.

(2b *vs.* 2m: Table 3.6 or bottom of Table 7.36), and warrant investigation for metric development.

The paucity of cover, diversity and number of woody taxa in the more disturbed wetlands may result from physical disturbance, including vegetation harvesting.

Sclerophyllous shrubs are a sub-set of the woody taxa. A greater average number of these shrub taxa occurred in the less disturbed *vs.* more disturbed supralittoral vegetation samples of Fynbos-associated localities Hermanus and Kenilworth. The average magnitude of this difference is insignificant however, being on average one specimen *vs.* none.

7.4.4. Facultative and obligate wetland taxa

A greater number (richness and diversity) of facultative wetland (FW) and obligate wetland (OW) taxa appear to be characteristic of less disturbed situations. A greater number of facultative- and obligate-wetland taxa were recorded in the less- (Reference or Mild) than Worst-disturbed vegetation of the littoral habitat in both Lotus and Kenilworth wetlands. The number of FW and OW species typically encountered in a sample is low (Ref: 5±0.9 *vs.* Worst 3±0.6); but there is a consistent pattern. In the Kenilworth littoral habitat, the total number of FW and OW taxa was more than three times that recorded for Mild or Worst relevés; suggesting an intuitively simple metric to apply in the field. Kenilworth littoral habitats with 15 or more of these taxa *vs.* those with fewer could perhaps be considered a starting guideline for a metric for separating Reference from Worst conditions. Such a metric would however require strict comparison of wetlands of similar hydroperiodicity and depth or volume – a guideline that was not adhered to in the present study and that should inform further studies of this nature.

Whilst fewer FW and OW taxa may suggest that the more impacted wetlands/relevés were drier, the measurement of hydrological impacts on these wetlands suggested, that the Worst wetlands actually suffered less overall water loss than Mild wetlands. These Worst relevés were, however, on the whole shallower (16±3 cm deep) than the Reference (14±2 cm) and Moderate (21±3 cm) relevés as determined by maximum annual inundation depth. Thus a natural difference in water depth may account for the greater number of FW and OW species in Reference and Moderate than in Worst relevés although the differences are neither large nor significant. It is also possible that plants with dryland affinity are more able to invade wetlands that are exposed to greater physical disturbance by human land-use and related activities. The Worst-disturbed wetlands in Lotus and Kenilworth were considerably more physically impacted than were the Moderate and Reference wetlands.

Margalef's species richness is based on the number of different taxa standardized against the total cover each species occupies; therefore, species with little cover add proportionately less to the index. Greater species richness of the FW and OW taxa was recorded in less disturbed, than in more disturbed, relevés in littoral vegetation at Lotus and Kenilworth and in the Kenilworth supralittoral vegetation. For richness per sample, the range of value differences (0.6-1.3 for Reference *vs.* 0.3-0.7 for Worst) between less and more disturbed relevés in these three habitats-localities is too great for richness per sample to be a consistent metric.

7.4.5. Other groups of taxa

Graminoid taxa, which constitute a considerable proportion of all taxa in wetlands, did not show any general pattern other than covering more area in more disturbed relevés of the Kenilworth supralittoral habitat. The graminoid taxa responsible for this greater cover were, however, all alien species; and occurred with an average cover of 18% in Worst *vs.* 1% in Reference habitats. Cover of alien graminoids also successfully distinguished Reference from Worst relevés in the littoral habitat of Driftsands and Lotus wetlands and the supralittoral habitat of Lotus wetlands. Alien graminoid cover is therefore a potentially useful metric for phyto-assessment in the Cape Flats wetlands. Leafless graminoids occurred in greater average cover in Lotus littoral relevés. The number of these leafless graminoids was not consistent across different locality-habitat combinations.

Annual taxa showed a significantly greater number, cover and diversity in the Worst than in the Moderately disturbed supralittoral habitat of seeps at Hermanus. For this Hermanus habitat, the greater cover of annual taxa in Worst than Moderate conditions suggests a potential metric as measured as an average value per habitat rather than an average value per sample. A similar numerical result was apparent in the Driftsands supralittoral habitat between Reference and Moderate categories of disturbance but the relationship was not significant.

7.4.6. Potential diversity metrics

A few overarching principles for the development of metrics can therefore be derived from diversity differences:

- Greater cover of alien taxa (Reference 4% *vs.* Worst 23%) indicated greater levels of disturbance in the Cape Flats wetlands. No pattern was apparent for the number of different species (richness) of alien plants in Cape Flats wetlands, however.
- Greater cover (Reference 18% *vs.* Worst 3%) of woody taxa indicates less disturbed supralittoral wetland habitat in both Hermanus and Cape Flats wetlands.
- Greater numbers (5±0.9 *vs.* 3±0.6) of taxa with facultative to obligate affiliation to wetland habitat can be expected to occur in less, than in more, disturbed relevés from Cape Flats wetland environments. A total count of 15 of these taxa per wetland appears to represent an approximate cut off, with more than this number representing good conditions and less than this representing a disturbed condition.
- Greater cover/abundance of annual taxa in Hermanus supralittoral seeps indicated greater levels of disturbance, with less than 2% cover (less than 11 individual annual plants) indicating less disturbed and more than 5% cover indicating more disturbed conditions. A similar result was found in the Driftsands supralittoral vegetation with less than 3% cover (less than 100 individual annual plants) in undisturbed habitat relative to greater than 5% cover in disturbed conditions.

It is possible that these 'general' trends are more correlated with the type of disturbance rather than with the magnitude of disturbance. Some types of disturbance (for example a high nutrient loading) will result in mono-dominance of a single species, whereas other impacts (for example overgrazing) may result in the creation of gaps, thus opening space for many ruderal, short-lived species. This is an important aspect that requires research before successful phyto-assessment tools can be developed.

7.5. Potential metrics for an Index of Biological Integrity

Potential metrics derived from the above results are collated in Table 7.36. The sensitive and tolerant taxa discussed in Section 7.3.2 are not included in these metrics, since further research is needed before these species can be definitively categorized as sensitive to, or tolerant of, disturbance.

All of these metrics are derived at least in part from the data collected from wetlands of the Cape Flats. None of these metrics have been tested against another data set comprised of similar wetland habitat, due to the small size of the localities that were deemed comparable for phyto-assessment development purposes in the present study (Chapter 4).

Table 7.36: Potential metrics for an Index of Biological Integrity for Cape Lowland Freshwater dominated wetlands from the Western Coastal Slopes with particular emphasis on the wetlands of the Cape Flats. The Braun-Blanquet scale is attached at the bottom of this table for clarity in the field application of these metrics.

Species/attribute	**Reference**	***Vs.***	**Disturbed**	**Where applicable in Western Coastal Slopes**
Typha capensis	<12.5% cover/sample or /wetland	*vs.*	≥12.5% cover/sample or /wetland	Cape Flats and Hermanus wetlands
Cyperus textilis	Not present	*vs.*	12.5-100% cover/sample	Cape Flats and West Coast
Juncus capensis	10 specimens and/or 5-12.5% cover/sample	*vs.*	Absent	Fynbos-associated wetlands (Cape Flats and Hermanus)
*Paspalum vaginatum**	None or <5%	*vs.*	≥12.5% cover/wetland or /sample	Lotus wetlands (Cape Flats)
Alien taxa per hydrological habitat zone	<25% cover	*vs.*	≥25% cover	Cape Flats
Alien taxa cover per sample	<5%	*vs.*	≥12.5%	Cape Flats
Alien graminoid taxa cover per sample	<5%	*vs.*	12.5-25% or more	Cape Flats littoral and supralittoral habitat
Number of Woody-indigenous taxa per hydrological zone	>7 taxa	*vs.*	<3 taxa	Cape Flats and Hermanus
Woody indigenous cover per sample	12.5-25%	*vs.*	<5%	Cape Flats and Hermanus
Number of obligate to facultative wetland taxa	>15/wetland	*vs.*	<15/wetland	Cape Flats
Annual taxa cover per sample	<5%	*vs.*	≥5%	Driftsands (Cape Flats) and Hermanus

Braun Blanquet cover scale for field sampling after Table 3.6 in Chapter 3 of this volume:

"r":	(or 1)	1-2 specimens (ex)	2m:	(or 4)	> 100 ex, < 5%	3:	(or 7)	25-50%
"+":	(or 2)	3-10 ex	2a:	(or 5)	5-12.5%	4:	(or 8)	50-75%
"1":	(or 3)	11-100 ex	2b:	(or 6)	12.5-25%	5:	(or 9)	75-100%

The combination of all the above metrics into an Index of Biological Integrity (IBI) to assess the environmental condition of inland depressional wetlands dominated by Cape Lowland Freshwater vegetation on the Cape Flats is feasible as there will be more metrics that work for a given location and they are likely to outweigh those that do not. The decision as to which of these metrics to use should only be taken once the data are collated, as one or more of these metrics may not be determinable from field data. A combination of species-specific, functional group (life history and growth form) and community-based (species richness) metrics, as determined by cover/abundance and community biodiversity measurement are included in Table 7.36 thereby fulfilling some of the criteria for optimal metric choice (Section 2.7.3 of this volume). These metrics have all been chosen as a result of empirical comparison of disturbed relative to less disturbed wetlands and all make ecological sense, yet none has been tested or shown to change in a predictable way with disturbance. For instance, and of particular concern amongst these metrics, is the predictability of the response of *Cyperus textilis* to the degree of disturbance.

The use of only one of the four alien taxa metrics (including *Paspalum vaginatum**) and one of the two indigenous woody-taxa metrics would prevent double-counting (collinearity) as these metrics are essentially measuring the response of the same functional groups of species. This would reduce the number of metrics to seven if all of the species required for species-specific metrics were present in a wetland being assessed, which is an unlikely eventuality as *Juncus capensis* was found only in wetlands surrounded by Fynbos and *Paspalum vaginatum** was found only in Strandveld-associated wetlands.

Seven metrics is the minimum number recommended by the US EPA (1998c) as providing sufficient information to create a reliable IBI (see details in Section 2.7.2.1 this Volume). Assuming a maximum of seven metrics, giving each of the metrics a binary score of 1 *vs.* 0 depending on whether the Reference or Disturbed condition is indicated by the metric would return a summed score of between 0 and 7 for any wetland assessed. Wetlands scoring five or more on this scale would be considered to be in good condition. Wetlands scoring three or less would be considered disturbed and those scoring four would be considered moderately disturbed.

Application of this IBI to wetlands dominated by saline, “Vernal Pool” or “Alluvial” vegetation (*sensu* Mucina *et al.* 2006a) would not be expected to work as these wetlands hold different vegetation from the Cape Lowland Freshwater wetlands from which the metrics for this IBI were derived.

7.6. The benefits of recording data at smaller spatial scales

In a phyto-assessment index, such as the above IBI, the application of metrics based on average value per habitat or wetland average data are possibly more intuitive than metrics based on averages per sample. It is also quicker to assess the average cover values of species per hydrological zone than to assess multiple homogenous vegetation relevés from which to extract data to populate a phyto-assessment index based on habitat scale averages. However, the development of phyto-assessment metrics requires the examination of the individual relevés in order to be able to determine average values per habitat that make ecological sense for any ecosystem.

The sample-scale analysis in this present chapter provided consistently greater ability to differentiate between the vegetation assemblages of different disturbance categories than the wetland-average scale provided in Chapter 6. Four localities were revealed to have considerably different vegetation between wetlands of Reference and Worst environmental condition when using sample scale data, whilst only a single locality revealed a difference when using the wetland average data. In part, this is perhaps due splitting the relevés into supralittoral and littoral hydrological habitat groupings. Hydrological habitat was a major discriminator between vegetation community composition.

Investigation at the sample scale facilitated comparison of the similarity of relevés of each disturbance category within a given hydrological habitat at any given locality. In this way any disturbance categories that proved to have no significant vegetation differences, such as the vegetation of Lotus Moderate and Worst categories, could be amalgamated into a single Disturbed category. Unconstrained ordination of the wetland average data was considerably less revealing of the separation between disturbance categories than unconstrained ordinations using sample datasets. Amalgamation into Mild (Reference + Moderate) or Disturbed (Moderate + Worst) increased the number of relevés being compared, enhancing the ability to determine (using DistLM) the predominant environmental parameters influencing vegetation distribution. Examination of only the Reference and Worst relevés in these cases did not increase the clarity of separation between disturbance categories in constrained or unconstrained ordination attempts. Furthermore, outcomes of DistLM analyses when searching in un-combined categories did not enhance the determination of which environmental variables were most responsible for the difference between vegetation communities or species distributions. At this early stage of developing environmental assessment tools in South Africa, it is perhaps best to attempt only to distinguish between minimally impaired and more impaired habitats.

The potential for the wetland average scale investigation (Chapter 6) to determine difference between categories of disturbance would probably have been enhanced by an *a priori* separation into hydrological habitats and thus separate analysis of each habitat-locality combination. The greater interrogation potential that the sample-scale analyses provided suggested there would be no point in re-examining hydrological zone scale data sets. It is easier to separate into hydrological and other naturally occurring sample sets (e.g. soil textures), with the vegetation sample data set, as each sample was categorised as supralittoral or littoral and has other associated sample scale environmental data. The recording of both environmental and vegetation data at the individual sample scale facilitates greater interrogation potential of natural variability than the wetland scale.

This splitting of relevés into naturally different groupings, such as hydrological habitats, removed some of the obviously apparent natural variability, thereby focusing on any anthropogenic or other environmental influences that were indicated by the environmental parameters measured at each sample. This detailed scale of recording data is therefore imperative at this early stage of development of phyto-assessment methods. Some natural variability may still have existed within the Driftsands and Lotus datasets particularly as a result of salinity differences in that some brackish conditions were apparent. The existence of other causes of natural variability was not apparent from the 54 environmental variables that were measured in the present study.

8. CONCLUSIONS AND RECOMMENDATIONS

In this study, the potential of using wetland plants to assist in the determination of wetland environmental condition was researched. The ability to assess the condition of wetlands will assist in the application of the National Water Act (1998), as well as in a wider range of activities, such as resource deployment in conservation planning and management. Wetlands of the Cape coastal lowlands were studied as a heterogeneous microcosm and will provide a useful template to an increased understanding of the macrocosm of South Africa's wetlands. The present research has revealed a number of critical findings concerning the phytogeography of wetland taxa, as well as reasons for the natural variability of macrophyte distribution within wetlands (hydrologically driven) and within regions (driven by HGM type, climate and geological differences). This information may, in future, be used to inform the development of a national framework sampling strategy, and a metric development protocol to be used as a guide in the future development of metrics and indices for regions of South Africa that prove to hold naturally homogenous, and thus comparable, units of wetland vegetation.

This chapter contains a discussion of both these findings and the application of phytoassessment metrics in order to determine the environmental condition of wetlands. Suggestions are made as to potential avenues of future research that may increase our understanding of the phytosociology of wetland plant communities and the impacts of disturbance on them, and thereby inform our efforts to develop accurate phytoassessment tools.

8.1. Wetlands are not azonal

If the entire vegetation complement, within what is defined to be wetland, is used to describe the wetland plant community, then contrary to the hypothesis of Mucina *et al.* (2006a), these communities are not azonal (*sensu* zonobiomes: Section 2.9.1). In other words, the same climatic and geological drivers that determine the distribution of zonal (dryland) vegetation also influence the distribution or biogeography of wetland species. This was made apparent by the analyses in Chapter 4 that revealed considerable species turnover or gamma diversity in the Cape Lowland Freshwater vegetation across the Western Coastal Slopes (Section 4.2), with significant differences in vegetation (Section 4.3, 4.4) and environmental parameters (Section 4.5) between every geographical locality assessed.

For instance, it is especially apparent in the supralittoral vegetation, relative to neighbouring dryland plants, that increased water availability in wetlands alters the microclimate of the ground in which wetland plants are rooted. Ambient temperature and rainfall, however, affect the amount of water available and this has a “zonal” influence on both dryland and wetland plants. In wetlands in which the influence of upwelling groundwater is significant, the zonal influence of ambient rainfall may be less important in determining the microclimate of the wetland habitat. Similarly, under natural conditions, water availability and landform affects nutrient concentration as a result of influx, leaching and evaporative concentration of salts, but the availability of these salts is predominantly determined by the underlying geology, which, has a zonal influence on vegetation. The littoral vegetation complement of wetlands with seasonal to permanent inundation, does however, contain some (sub)cosmopolitan species as a result of the azonal influence of the hydroregime on increased water availability. In the present study, the inclusion of all plants within what is classified as wetland habitat, (i.e. including the ephemerally and seasonally saturated margins of wetlands) is considered part of the reason that wetlands from different localities exhibited such considerable species turnover and significant difference. However, a comparison of only the littoral vegetation from different localities also revealed significantly different wetland communities at each location (Section 7.1.1).

That wetland vegetation is not azonal, but determined by the same factors of climate and geology that determine the distribution of dryland vegetation, is a key finding of the present study. The outcome of this finding is that the wetland vegetation within the Western Coastal Slope wetland region is not indicative of a single phytogeographical region as was postulated by Cowan (1995). Equally, the Cape Lowland Freshwater (CLF) vegetation type is not uniform throughout the Cape coastal lowlands as was suggested by Mucina *et al.* 2006a. The fact that every locality that was assessed exhibited significantly different wetland plant communities precluded the possibility of comparison between localities or sub-regions and necessitated the search for potential metrics for phyto-assessment within each separate locality.

8.2. The importance of hydrological zonation within wetlands

An important consideration that became apparent during initial sampling attempts using the vegetation sampling protocol of the US EPA (2002c) was that the wetlands of the CapeCoastal Lowlands are stratified into hydrologically determined concentric zones. The influence of such zonation on habitat availability is considerable and separates vegetation communities into significantly different littoral and supralittoral groups (Section 7.1.1). This suggested that the single large plot sampling methods recommended by the US EPA (2002c) would not reflect the heterogeneity of habitats found in the wetlands of the Fynbos Biome and that numerous plots would be required to do so. The Braun Blanquet vegetation sampling protocol was subsequently adopted and successfully facilitated the sampling of homogenous vegetation stands from each of the supralittoral, littoral and aquatic hydrological zones.

Assessments of vegetation type or condition are influenced by the hydrological zones in which samples are taken. The development of wetland vegetation type classification or phyto-assessment metrics, and the outcome of phyto-assessments, must therefore take into consideration both the influence of hydrological zonation and the full complement of habitats within what is defined to be the wetland.

8.3. Phyto-geography of distinct wetland vegetation units

International approaches to wetland phyto-assessment suggest that metrics need to be developed independently for regions that have different vegetation communities (Section 2.7.1). Uncertainty about the distribution of regions containing comparable wetland vegetation communities in South Africa, and the suggested use of Cowan's (1995) wetland regions in the classification of wetland vegetation types (Mucina *et al.* 2006a), led to an investigation of the homogeneity of wetland vegetation within one of these (essentially untested) wetland regions.

All effort was focused within the Western Coastal Slope region, rather than spreading the sampling effort over a number of regions. This was because the more regions that were included, the greater would be the natural differences in terms of, inter-alia, climatic and soil-type drivers of species distribution and the less clear the resulting trends. Within the Western Coastal Slope wetland region (Cowan 1995) inland, isolated freshwater wetlands were sampled in a number of localities, within three sub-regions, to determine whether the wetlands held comparable plant communities. Should all of the wetlands have proved comparable, the Western Coastal Slope would have been considered a single phytogeographical region and would have sufficed for the purposes of the development and application of phytoassessment metrics. The outcome was however that, even within the Western Coastal Slope region, different wetland plant communities existed, and the spatial scale of these differences was relatively small, occurring within 10's of kilometres, as a result of geological substrate and climatic differences.

It was found that the wetlands of the Cape Flats have plant communities significantly different from those to their south-east on the wetter Agulhas Plain, and from those on the drier West Coast, north of the Cape Flats. Within the Cape Flats, the most comprehensively sampled of these above three sub-regions of the Western Coastal Slope, lowland freshwater wetlands of the same HGM type have significantly different vegetation if, for instance, they are from acidic or alkaline substrates; and wetlands from different localities for both of which the dryland vegetation type is the same (suggesting similar environmental and particularly geological conditions), can still have significantly different wetland vegetation (for details see Section 4.4.2.ii and discussed further in 8.3.1 below).

The differences between wetland vegetation at each locality within the Western Coastal Slope region suggests that none of the currently defined wetland regions (Cowan 1995), ecoregions (Kleynhans *et al.* 2005), bioregions (Brown *et al.* 1996 or Rebelo *et al.* 2006) or even terrestrial vegetation units (Rebelo *et al.* 2006) provide distinct and homogenous units of Cape Lowland Freshwater vegetation. None of these means of delineating geographical areas therefore provides a definitive unit suitable for attempting the development of metrics for phyto-assessment purposes.

8.3.1. The use of terrestrial vegetation for identifying wetland vegetation units

Homogenous communities of wetland vegetation appear to exist within each locality of the Western Coastal Slope region tested in the present study. Each locality was associated with a particular terrestrial or dryland vegetation type (*sensu* Rebelo *et al.* 2006). These dryland plant community types could potentially provide a surrogate for delineating homogenous units of wetland vegetation. Yet, even within dryland vegetation units in which more than one locality was assessed, differences were apparent between the wetland plant communities sampled. The Driftsands (Kuils River floodplain) and Lotus River floodplain wetlands are both situated within Cape Flats Strandveld dryland vegetation and yet still proved to hold significantly different wetland vegetation communities. These vegetation differences between the two localities are likely to be a consequence of natural ambient climatic and geological environmental differences. Significant differences between the wetland plant communities of these two localities both from within the same dryland vegetation type reduced the ability to identify phyto-assessment metrics that would be applicable in both areas. Within the above two Cape Flats Strandveld associated localities, very few characteristic indicators of Reference and Disturbed conditions were common to wetlands of both locations. The presence of *Schoenus nigricans* and the genus *Carex* were characteristically indicative of a Reference condition in both localities, whilst a high percentage of *Typha capensis* was indicative of the Worst condition. A high percentage cover of *T. capensis* was however considered characteristic of the Worst condition in most of the localities that had sufficient comparable samples facilitating determination of metrics. Alternative indicators of the Worst condition that occurred in both of these locations are *Berula erecta* and *Pennisetum clandestinum**, both "Tolerant Taxa". Only *P. clandestinum** was considered discriminatory between disturbance categories and then only within the supralittoral habitat in Lotus wetlands. Between other localities, however, apart from *Typha capensis, Cyperus textilis and Juncus capensis* no other taxa were found to be characteristically indicative of different disturbance categories between a number of different localities (Section 7.3.1). More indicator species were therefore shared between the *Cape Flats Strandveld* associated wetlands, than between wetlands of any other localities in the Western Coastal Slope. Dryland vegetation type does therefore provide a phytogeographical unit of land with greater potentially comparable wetland plant taxa and potential phytoassessment metrics than any other land unit assessed.

Part of the distinct differences shown to exist between Lotus and Driftsands wetlands discussed above, may have been due to the wetlands in each of these localities acting as a source of propagules for other local wetlands, thereby entrenching local similarity. The localities assessed in this study were focused in areas with large wetlands or conglomerates of many wetlands. Distances between vegetation communities of the same type are known to influence similarity, partially due to propagule dispersal ability, but also due to environmental differences (*sensu* Cody 1975 and 1983). Fragmentation, as a result of increased distance between wetlands, is known to impact on community composition and adaptability to human alteration (Adamus *et al.* 2001). Assessment of other wetlands, between the Driftsands and Lotus foci, but still within *Cape Flats Strandveld* vegetation, may reveal a continuum of similar wetland vegetation that was not apparent due to the restricted focus of the present study.

8.3.2. Biogeographical regions beyond the Fynbos lowlands

The Western Coastal Slope is part of the Cape Floral Region. In terms of dryland vegetation, the *Capensis* and *Drakensbergensis* Floral regions are recognized areas of high floral endemism and diversity. Given the diversity shown to exist within the wetland plant communities of the Western Coastal Slope in this present study, it is safe to assume that the

Capensis and *Drakensbergensis* Floral regions are both likely to contain many areas with naturally distinct wetland vegetation communities. Within the highlands of Mpumalanga, initial investigations also suggest that considerable natural environmental heterogeneity appears to exist between isolated depressional wetlands in the Chrissies Meer area (Martin Ferreira, Pers. com. PhD candidate, University of Johannesburg). Regions that have greater spatial homogeneity of the environmental parameters that drive species distribution, than in the Western Coastal Slope, will probably require less subdivision into areas of distinct vegetation. The homogeneity of the environmental parameters within the western Free State suggests considerable homogeneity in wetland vegetation (Nacelle Collins, Pers. com. PhD candidate, University of Free State).

8.3.3. Plant stress tolerances and indicator species

In all of the Cape Flats localities in which wetlands were assessed in the present study a number of samples were considered to be saline-sodic and species apparently indicative of brak or saline conditions were often associated with these samples. Whether these saline-sodic conditions were natural or caused by anthropogenic stressors was not determinable from the sampled data. The presence of certain species that are apparently indicative of saline or brak conditions is a good example of the potentially tenuous situation of relying upon single indicator species to classify environmental conditions before such species have been categorically determined as intolerant of other conditions.

Species of *Sarcocornia, Salicornia, Limonium* and a host of other genera are recorded as important taxa of the Cape Inland Saltpan vegetation unit by Mucina *et al.* (2006a) and a concentration of such taxa should be considered indicative of saline conditions. The presence of any one of these species alone, does not, however, necessarily indicate the salinity of that system. Accurate determination of the salinity tolerance of a wide range of species would assist our ability to determine whether the presence of any single species categorically indicates a position on the salinity scale. Not all of the taxa found within the saline vegetation unit are truly halophytic taxa – those that are adapted to saline conditions and similarly some halophytes and other species that tolerate saline conditions are to be found in freshwater environments. The *Sarcocornia* species (cf. *natalensis*) found in the present study at Driftsands, Lotus River, Berg River and Darling wetlands is a halophytic example of this fact and *Phragmites australis, Sporobolus virginicus* and *Senecio halimifolius* are other species commonly found within the Cape Lowland Freshwater wetlands assessed in the present study as well as within Cape Inland Saltpans (Mucina *et al.* 2006a). The presence of these species may be considered to indicate brak conditions within a more typically freshwater context. With our currently limited baseline autecological knowledge, it is therefore important to assess the whole community of vegetation in order to be able to accurately categorize a wetland vegetation type, rather than simply to look for species that indicate saline, brak or fresh conditions. Similarly lack of baseline data on tolerance for nutrient, light, and other stressors that alter the competitive ability of plants to survive, limits our ability to determine accurate indicator species. The development of species characteristically associated with different degrees of disturbance as performed in the present study thus provides an interim means of being able to assess wetland condition without the baseline knowledge required for determining indicator species.

8.4. Spatial applicability of metrics

Significant differences in vegetation community between the localities assessed within the Western Coastal Slope reduced the likelihood of determining metrics with broad geographical application. For instance, the fact that the discriminatory species and diversity differences between disturbance categories in the hydrological habitats of the Lotus wetlands are not the same as those in the Driftsands wetlands, suggests that metrics from either of these *Cape Flats Strandveld* localities may not even work in both. Thus considerable difference between the vegetation communities of wetlands of different areas restricts the potential that metrics developed and tested for one geographical region will work in other areas.

8.4.1. Validation of metrics

The targeted (vs. random) site choice used in the WHI study is considered by some researchers to reduce the inferential power of this type of data set (see Fore 2003); suggesting that putative metrics may not be applicable in any other wetlands or wetland vegetation stands within the same area (See Section 2.11.3 in this volume). A targeted sampling approach, however, is used extensively for phyto-assessment metric development across North America (US EPA 2002a and c); where a pragmatic approach to testing metrics is adopted. Testing is performed by checking, within wetlands not used in the development of the metrics, whether the metrics prove to be robust indicators of difference between vegetation samples from *a priori* determined Reference and Disturbed categories of human disturbance. This should be done in wetlands of a similar HGM and vegetation type to those used in the development of the metrics. A suggested approach is to split the sampling data during phyto-assessment development and develop metrics from one half and test them on the other half of the data set. This was not done in the current study due to insufficient comparative samples as a result of the extreme heterogeneity of the flora of wetlands within the Western Coastal Slope region sampled.

8.5. Phyto-assessment in other regions of South Africa

In South Africa, phyto-assessment has potentially broader applicability than the use of invertebrates for inferring environmental condition, since such methods are restricted to habitat that is inundated to at least ten centimetres in depth (Bird 2010). The arid to sub-arid climate of much of South Africa results in many ephemeral, seasonal and even perennial wetlands that are, at their wettest, only briefly saturated. Hence, phyto-assessment has nation-wide potential, provided reliable region-wide metrics can be developed for homogenous regions of wetland vegetation. A framework for determining wetlands with comparable habitat along with protocols for vegetation sampling and phyto-assessment development protocol appropriate for any region of South Africa are presented in Chapter 9 of this volume.

8.6. Best season for metric development and for phyto-assessment

Species identification, comparability of nutrient concentration, and practicality of sampling techniques all influence the season during which sampling should be undertaken for metric development purposes. In the present study it was, however, realized that once initial species identification and metric development is complete for an area, the length of time in a year (the season) in which the species required for metrics are identifiable, will affect the period (number of seasons) in which the metric can be used for phyto-assessment purposes. This length of time is referred to as “the index period” (US EPA 2002c).

8.6.1. Season of metric development

Preliminary WHI field sampling trials, performed in July to September, provided useful guidance for the development of a field sampling protocol for seasonally inundated palustrine wetlands in the winter rainfall region of South Africa. During these winter and early spring sampling attempts, the lack of fully developed floral material in many of the abundant graminoid species hindered identification of lesser known/obscure taxa. Many species cannot be identified with full confidence if flowers are absent. Some of the sedges cannot be identified to species level unless their seeds are developed. These factors suggested that sampling later in the year would be advantageous and would assist with phytosociological and autecological interpretation as well as with metric development for phyto-assessment. Sampling for biogeographical analysis and metric development purposes was thus conducted during late spring and early summer of 2007 (September-December). This later season corresponded more or less with the peak flowering maturity of the wetland plant community as a whole, missing only ephemeral geophytic taxa which had already set seed. For metric development purposes in wetlands within the winter rainfall region, this late spring to early summer sampling season appears most appropriate.

8.6.2. Index period

In North America many species used as metrics are only identifiable for a restricted season, thereby limiting the index period. Many of the species used for metrics in the present study (Table 7.36) are potentially identifiable for an extended season, and in some cases are identifiable all year round. On the other hand, almost half of the taxa identified in this study as sensitive to disturbance (Section 7.3.2) are geophytes and would thus not be useful as metrics in the dry-season as these taxa are typically only apparent above ground in the wet season, and only flower in spring. The other sensitive taxa and tolerant taxa that were discriminatory between disturbance categories can, however, be identified all year round. Species diversity measures such as the total cover of alien and woody taxa are reliant on species that, for the most part, can be identified all year, or almost all year, in the case of some annual alien taxa. Essentially for anyone who is familiar with Cape species and the aliens that invade this area it is quite possible that the index period could be considerably longer than the length of the season most favourable for metric development. The annual taxa, some of the alien grasses and obligate wetland taxa may however pose an identification problem that would restrict the index period to between August to February for Cape Flats wetlands. The limited index period or sampling season for most other biotic based wetland assessment tools suggest that vegetation metrics that facilitate an extended season or year round assessment would be most beneficial.

8.6.3. Comparability of nutrient concentration between seasons

During metric development, inter-wetland comparability of nutrient concentrations is important due to the need to accurately rank wetlands according to nutrient load. Water column nutrient concentration is seasonally variable as a result of dilution by seasonal rainfall or groundwater levels (Malan and Day 2005c). Similarly, soil nutrient levels for soluble inorganic phosphorus and nitrogen are both affected by fluctuation of temperature and redox conditions or saturation (Mitchell *et al.* 1984 and 1987). Comparison of water nutrient concentration from the time of greatest inundation depth, coinciding in the Western Cape with early spring, to levels in early summer when wetlands are often drying out would be likely to reveal different concentrations within the same wetland. In the present study, water nutrient samples were all collected in winter, during the season of maximum inundation (Bird 2010). If soil and water nutrient concentrations are to be used to rank nutrient load, it is important to compare samples taken in the same season. It is therefore recommended, that as far as possible, sampling should be limited within a year to a single season with stable hydrology. This suggests a possible source of error in the ranking of wetlands using soil nutrient data in the present study, in which an extended sampling season from September when wetlands were still inundated through to when they were mostly dry in December was employed. Comparisons were predominantly made within localities, however, within which, soil samples were all taken and dried within a few days of each other, thus, within localities, errors due to temporal variation in nutrient levels are considered to be minimal.

8.6.4. Practicality of sampling in different seasons

The depth of flooding of the wetlands in July, August and September made it impossible to estimate the amount of ground covered by lawn-like or short vegetation when it was inundated to any depth precluding visibility. When the ground-surface is visible, estimates of cover can be made. This suggests that accurate vegetation sampling of seasonally inundated wetlands would best be conducted outside of the season of peak inundation. This affects both the season of metric development, and potentially, the index period if inundated species are discriminatory between disturbance categories and prove to be important species used in developed metrics. *Paspalum vaginatum**, a lawn-forming invasive alien grass species, the cover of which was chosen as a potential metric, was one of the species that drew attention to the issue of visibility of inundated surfaces. This species typically creates a mono-specific mat of grass and indicates disturbed conditions when covering more than 12% of a sample or a wetland. *Paspalum vaginatum** typically occurs in the supralittoral or marginal vegetation around the inundated zone of wetlands but is tolerant of seasonal inundation. Inundation in the index period would not unduly impair the ability to determine the percentage cover of this species since it is in the supralittoral zone that it most dominant.

8.6.5. Duration of phyto-assessment development process

The phyto-assessment development processes of field sampling, species identification, data analysis, metric determination, and index development and validation could probably be streamlined to take a single researcher four months for any area of homogenous vegetation.

8.7. Sampling Design: What worked versus what didn't work?

8.7.1. Human Disturbance Score

Measures of human disturbance need to be derived independently from biotic data in order to avoid simply choosing aspects of disturbance or measures of biology that match our expectations (Fore 2003). For the current study, the use of certain aspects, such as the extent of monospecific indigenous or alien vegetation coverage within a target wetland were thus considered inappropriate for the assessment of the amount of human disturbance. The extent of monospecific stands, of alien vegetation and "expert" judgement of habitat heterogeneity, were initially included in the measurement of HDS, however, they were subsequently removed to avoid circular reasoning or researcher expectation or bias.

In the present study, allied to the measure of soil and water chemistry, the HDS proved reliable in its ability to assess the impacts and disturbances affecting wetlands and their immediate surrounds as a result of human land use and associated activities. The inclusion of two additional factors could add to the accuracy of the HDS:

i. The roughness of the buffer zone of dryland vegetation around each wetland would have been useful as an indication of the ability to reduce influx of sediments and associated nutrient load. Whilst all wetlands in the present study were in relatively flat land forms, in sloped landscapes, the slope of the buffer zone may also be an important consideration. Correlations between buffer zone vegetation roughness, slope and sediment influx have yet to be determined for South African wetlands.
ii. Landscape and vegetation can both be structurally impacted by physical disturbance events or land use. Separately scoring each of these would be useful in HDS determination. Only landscape impacts were scored in the present study. Impacts to vegetation should only be assessed in the 100 and 500 meter radius surrounding wetland, as scoring those impacts within the wetland may be considered scoring the same impacts twice (i.e. if a physical disturbance were measured in the landscape and then again measured in its impact on the wetland vegetation).

As an alternative to the HDS, it would be possible to utilize the WIHI (Rountree *et al.* 2007) or WET-Health (Macfarlane *et al.* 2008) to rank environmental condition within a set of wetlands. However both of these tools use vegetation and impacts on vegetation as indicators of disturbance, thereby reducing their independence from the biological target for phyto-assessment development purposes. Aspects of both of these tools were incorporated in the development of the HDS. Both assessment methods (especially WET-Health) are time-intensive, and it would have been impractical to use them in the present project. Re-iteration of the HDS with improvements based on experience gleaned from the present study and any future sampling seasons will enhance the accuracy with which this tool is able to rank and categorize degrees of cumulative human disturbance.

8.7.2. Avoid excessive natural environmental variability

Homogenous vegetation in Reference wetlands within a geographical region is suggestive of naturally uniform determining environmental conditions or limited natural variability. Homogenous vegetation communities can, however, also exist as a result of environmental disturbance that causes conversion from a more naturally heterogeneous habitat and set of vegetation to uniformly disturbed communities with mono-specific domination for instance by *Typha capensis* or *Pistia stratoides.* The identification of natural reference conditions as opposed to uniformly disturbed and subsequently regenerated communities is probably impossible to determine without historical records.

In the Western Coastal Slope, many different HGM and vegetation type habitat combinations were sampled thereby including considerable natural variability besides the many gradients of recorded disturbance impacts. Each sub-region that was assessed proved to hold significantly different communities of vegetation and within each sub-region the vegetation of every locality was also significantly different. Natural variability within some of these locality data sets (Darling, Verlorevlei and Agulhas Plain) as a result of multiple geological substrates within a single locality (e.g. granite and shale and aeolian sands at Darling) reduced the comparability between wetlands or samples from within each locality. For the wetlands of the Berg River, Darling, and Verlorevlei on the West Coast and the Agulhas Plain in the Overberg, too great a range of natural variability in environmental and vegetation diversity was encompassed within each locality to determine any significant differences between disturbance categories. For instance, separation of naturally different vegetation units was possible within the Agulhas Plain in which, significant differences were evident between vegetation of the Ratels Vlei, Waskraalvlei and Melkbospan localities; making these localities non-comparable for the purposes of phyto-assessment development. Within the

West Coast and Agulhas Plain localities in the dataset of the present study, a lack of detectable difference in vegetation between disturbance categories does not necessarily mean that disturbance was not impacting on these wetlands or changing their vegetation; but rather that too great a range of natural variability was incorporated within these localities.

In the present study, within localities in which Reference wetlands and Worst (or Disturbed) wetlands from the same substrate were compared, significantly different vegetation communities were detectable between samples of each disturbance category such as at Hermanus, and at each of the Cape Flats localities. As discussed in Section 8.3.1, a means of limiting natural variability within a set of wetlands to be studied for a particular area is therefore to only compare wetlands within similar geological substrates.

8.7.2.1. Samples from littoral habitat

As for all samples from different wetlands, it is necessary to ensure that littoral-habitat samples from different wetlands are comparable for the purposes of phyto-assessment development. The potential depth of annual inundation should, therefore, be taken into consideration when choosing sample sites. The depth of annual inundation can determine the species and resultant vegetation community that can survive in a given location. For instance, *Typha capensis* will typically not be found in habitat that inundates to more than two metres for any length of time, whilst aquatic taxa are more likely to dominate samples at such depths. If, as occurred in the Driftsands littoral sample set (Section 7.2.5), all wetlands from a given disturbance category are recorded from greater average depths than those from other categories, then the samples of each of these categories are no longer comparable as they represent different habitat conditions for plant species. Sampling a range of depths, or focusing on a given depth, in each wetland is therefore important.

8.7.3. How many samples are sufficient?

On the Cape Flats and at Hermanus where differences were discernable between disturbance categories within hydrological zones within each locality, an average of 20(±2) less disturbed (Reference or Mild) and 14(±3) Disturbed or Worst samples were compared. In localities in which no significant difference between disturbance categories was discernable, too few comparable vegetation stands were sampled. For instance, putting aside natural variability shown to exist at Verlorevlei (Section 7.2.1), in the Verlorevlei-supralittoral habitat, only 2 worst and 19 Moderate samples were collected. In the Verlorevlei-littoral habitat, only 4 Worst and 12 Moderate samples were collected. Even if natural variability had not been incorporated, insufficient samples from Worst disturbed wetlands were collated to facilitate accurate comparison between Worst and Moderate samples. From this work it appears that a minimum of 20 Reference and 15 Disturbed samples from comparable habitat should be used for metric development purposes. For metric testing, an approximately equal number of comparable samples are also required.

8.7.4. Nutrient analyses

In the wetlands in which they were measured, water column nutrient concentrations, assessed as an average value per wetland, were useful for determining the disturbance category each wetland was assigned to. Soil nutrient data was assessed per vegetation sample rather than as an average value per wetland. The multiple soil samples per wetland revealed considerable intra-wetland variability in nutrient concentration (Section 5.3.1). This revealed that more accurate environmental requirements (autecology) of a given taxa can only be determined if nutrient data are measured where a vegetation sample is taken, rather than using a value from a single sample, or even the average of a number of pooled samples per wetland. Investigation suggests that intra-wetland variation in water column nutrient concentrations is also apparent when multiple samples are tested per wetland (Ractliffe, G. Pers. com. University of Cape Town). The homogenizing influence of water on these water soluble nutrients is, however, likely to reduce the extent of intra-wetland variability.

Measurements of total nitrogen in the soil samples of the present study were not useful as indicators of impacts or the availability of this nutrient for plants. In the water column, total inorganic nitrogen showed hypertrophic concentration in highly impacted wetlands such as Lot06 (Section 5.2.2). These high concentrations may also have been revealed by a 1:2 water extraction of inorganic N from soils (Kotze, R. Pers. com., Bemlab cc) The measurement of a 1:2 water extraction of inorganic N from soils is thus recommended in situations where surface waters are un-available for testing in the process of developing phyto-assessment metrics.

Within the many possible soil parameters it is possible to measure, the concentrations of water soluble/plant available N, P and the water soluble cations proved most useful for differentiating between vegetation samples from different disturbance categories (Section 7.2). Soil pH and particle size were important, in the present study, for the differentiation of comparable samples. In some instances, pH and particle size analysis, may be important for differentiating between categories of disturbance; for instance in situations of elevated silt percentage due to erosion or elevated pH due to additions of lime.

Both water and soil chemistry was used in the determination of comparable wetlands and to search for evidence of the impacts of human disturbance using DistLM. The lack of a comprehensive set of soil and water chemistry measures for all wetlands of the present study makes it difficult to determine which is more important or informative about the impact of human disturbance and the habitat requirements of any given species and whether both are essential for the determination of human impact and phytosociological information. The measurement of the soil data per sample made this dataset far more informative about the environmental requirements of plants in the present study. Measurement of water chemistry data per vegetation sample would increase our ability to determine whether both soil and water chemistry data are required for phytosociological work.

8.7.5. Should species demographics be sampled per wetland or vegetation stand?

The weighted-average cover values determined per species per wetland were not successful in terms of determining difference between Reference and Worst disturbed wetlands (Chapter 6). The measurement of cover/abundance derived per sample within a homogenous vegetation stand was far more informative than the weighted-average developed from these samples. Sampling characteristic and homogenous vegetation stands following the Braun Blanquet protocol, facilitated the determination of an estimate of the cover and abundance of plants that occupied Reference or Disturbed samples and could be averaged per hydrological zone, per wetland or per locality (Chapter 7). Averaged cover values for a given species in a given hydrological zone, considerably underestimated the typical cover of said species as found in sampled vegetation stands and yet overestimated the total found in the whole wetland. For instance using an average from all samples within a given hydrological zone, *Typha capensis* may occupy only 30% of a wetlands littoral zone, yet within stands in which it is found it will often occupy a median cover of 88%; and it may have a total wetland cover of only 10%. This was the reasoning behind the adoption of weighted-averaging in Chapter 6, but as will be argued in Section 8.7.6 the approach adopted was not appropriate for purposes of the present study. The way in which cover/abundance is sampled has important implications for the way in which metrics are scored for phyto-assessment purposes (for details see Section 8.7.7).

The species inventory list with associated cover estimates for the whole wetland represents a simple and rapid means of obtaining approximate wetland cover values. This process is faster than the process of sampling characteristic and homogenous stands of vegetation. If recorded per hydrological habitat rather than per wetland such data may provide a rapid assessment technique for wetland vegetation. In terms of its holistic overview of the plant community, this species inventory and wetland cover estimation technique is similar to the sampling method used in VEGRAI (Kleynhans *et al.* 2007). The accuracy of estimation over an entire wetland or even hydrological zone is likely to reduce with increasing wetland size. In the present study, estimation of wetland cover values for the important species listed per wetland was done only for a few wetlands. These data were not intended to be used for comparative purposes and this approach was used only as an experiment to determine feasibility of using Braun Blanquet cover codes at the wetland scale. The lower values of the scale, representing abundance when cover is less than 5%, do not necessarily make sense to use when estimating abundance in an entire wetland and median cover class values are perhaps more appropriate (Table 3.6). Further investigation is required to determine whether average cover values per hydrological zone derived using this species inventory sampling method would facilitate the determination of differences between Reference and Worst wetlands.

8.7.6. The importance of fidelity in the development of phyto-assessment metrics

Do the average and typical cover of species that occur in more than one sample within any locality present more accurate comparative information than a species only represented by a single sample? Intuitively, the typical cover of a species that occurs in only a single sample, but over a large area, suggests considerable importance. Such a species, however, is considered to have low "fidelity", being neither a constant companion "*occurring in 60% of samples from a community*", or a dominant species "*a constant companion with 25% mean cover*" (Westhoff and Van der Maarel 1978). In the Braun Blanquet protocol, however, a stand of vegetation is only sampled if it is considered representative of the plant communities present at a site. Stands with 100% cover (88% median cover) by a single species (mono-dominance), often represent an impacted state such as when invaded by an alien species or by a very successful competitor for resources such as by *Typha capensis* or *Cyperus textilis*. Essentially such samples are indicative of conditions that are favourable for such species. *Typha* stands, being relatively homogenous are typically only sampled once in a vegetation survey of a wetland. To state that "*Typha* is not dominant in a wetland where it occupies 30% of the land surface with a median cover of 88%" simply because it is only represented by one sample, is to ignore the fact that the species probably has the greatest total spatial cover of any species within the wetland.

An estimate of the actual cover that a surveyed homogenous stand represents in a wetland, or a zone thereof, would assist with the determination of the total cover that a species represents. Similarly, an estimate of the total cover of mono-dominant species within a wetland or its hydrological zones would be a useful step in the development of metrics for phyto-assessment purposes. The concept of weighted-average used to determine the total cover of a species per wetland in the present study (Section 3.8.5.2), was based on the proportional wetland area (percentage) occupied by the hydrological zone that a sample was found within rather than the proportional area of the homogeneous stand of vegetation that it represented. Estimation of the total cover that a species represents would facilitate greater accuracy in determination of metrics.

8.7.7. Application of metrics in phyto-assessment

In a phyto-assessment, species and diversity metrics must be used at the same scale from which they were derived in the development phase. For instance, species or diversity metrics developed from an average value per hydrological zone must be scored in a phyto-assessment using average cover per hydrological zone. Other scales of measurement, such as an estimate of the typical cover per sample from the vegetation stands in which a species is characteristic, or the approximate cover of the species within the whole wetland, would result in inaccurate scores. The metrics outlined in Table 7.36 are all qualified by an area, be it a sample, hydrological zone or wetland average, within which they represent different values for reference relative to disturbed conditions.

Three possible scales of measurement have been investigated in this study:

a) weighted-average values per wetland or hydrological zone,
b) the averages of cover/abundance values determined per characteristic vegetation stand, per hydrological zone or per wetland; and
c) the overall approximate inventory of cover per species per hydrological zone or wetland.

Perhaps the simplest and quickest of these techniques, that of approximate inventory of species cover per hydrological zone, is the most user friendly for application of phyto-assessments.

8.7.8. Species identification and quality control

During this study well over 500 species were identified within the confines of what is classified as being wetland habitat (*sensu* DWAF 2003). Numerous field errors, as well as pre-existing herbarium and even database errors were corrected using the collected specimen numbering system or field code (Section 3.5.8.1 and Appendix 4). The use of a sample numbering system is therefore strongly recommended.

The rigorous specimen collection protocol employed in the present study facilitated expert confirmation of taxa for which field identification was uncertain. A quality control process is therefore proposed in order to ensure identification standards are maintained. A percentage of the specimens identified in the field should be collected for herbarium verification. A recommendation made by the US EPA (2002c) is to randomize this selection process in order to remove possible researcher bias[3].

Species found very rarely in the development dataset of the present study were not useful for species based metrics. Species found in only one wetland or sample (termed "singletons") are, in many bioassessment programmes, simply discarded as they do not generally provide any comparative potential. Metrics created from plant growth forms and/or plant functional groups, such as woody or graminoid taxa may utilize singletons, but in such cases species identity is not required. In the present study, because so little is known about South African wetland vegetation, singletons were identified to species level in order to expand the baseline data on wetland species inventory and distribution. It is important to be aware that, considerable time can be expended on the identification of singletons to species level, and that their species identity may not be of direct use for metric development purposes.

8.8. Future recommendations

From the evidence and information provided by the present study it is clear that phyto-assessment does have considerable potential for the determination of the environmental

[3] Use a random sample of 10% or 10 specimens of total number of species identified in the field. Collect species using a random number to determine plots in which to collect a quality control (QC) specimen and a random number using total number of species found in the selected plot to choose which species is to be taken for verification. The percentage accuracy of identification = (number of species correctly identified in field/total number confirmed)*100].

condition of wetlands in South Africa. A number of suggestions are given in the following sections that could assist with the endeavour to utilize already collected data about our wetland vegetation to address phytosociological understanding and phyto-assessment needs. The creation of a user-friendly manual on the development and application of phyto-assessment metrics could assist in the endeavour of putting this research into practice. As mentioned in Section 8.5, a framework for determining comparable wetlands for phyto-assessment purposes and the protocol for developing metrics are presented in Chapter 9 and the field sheets for determination of the human disturbance score and for field sampling of vegetation are also appended to this present volume.

8.8.1. National wetland plant databases

Reviews of the literature suggested that there is no clarity as to which plant taxa indicate a given habitat or environmental condition and there is a lack of base-line information that could inform our ecological understanding of biotic and abiotic interaction in wetlands. There is limited knowledge of what biota, environmental parameters, anthropogenic disturbances or stressors and ecological conditions exist in our wetlands and a concomitant lack of understanding of the resultant ecological interactions that occur. For instance in South African wetlands, there is limited existing baseline information on:

- Plant autecological information, phyto-sociology, and community successional development;
- phyto-geography of comparable and distinct units of vegetation.

Ecological interpretation of such baseline data is currently in its infancy in this country and this current state of knowledge for South African wetland vegetation suggests the need for collation to form a national database of existing data and baseline research.

The ‘important species’ listed for the dryland vegetation types of the Fynbos Biome in the Vegetation Atlas of Mucina and Rutherford (2006) includes associated wetland taxa and soil types from which the natural range of nutrient requirements for certain wetland plants could be derived. This would require an exhaustive cross referencing review of the studies used to compile the vegetation types and their associated environmental data. If sufficiently useful environmental data was collected in these studies, then the resultant data base that such a review could provide would prove invaluable in the separation of taxa into groups based on biogeography, nutrient requirement, soil types, climate, etc. Such data could be used in a meta-analysis to determine the phytosociological nature of wetland flora.

At the outset of the present study, a presentation and mini-workshop was conducted at the National Wetlands Indaba in 2007 in order to determine what wetland ecologists and consultants thought that wetland plant species were able to indicate with regards to the environmental condition of a wetland.

The following were suggested:

a) On the South African Highveld, *Imperata cylindrica* indicates water with low levels of total dissolved solids (TDS) (Allan Batchelor pers. comm.); and
b) *Cliffortia strobilifera* occurs with increased dominance under conditions of nutrient enrichment.

This workshop elicited no further informative responses and it was apparent that very little ecological interpretation of wetland field data has been done and currently there is only a very poor understanding of what determines the intricacies of wetland species plant distribution and response to disturbance.

Given these recognized limitations of existing literature, wetland plant lists, and the workshop outcome, it is apparent that considerable inventory type information on wetland plant taxa will be required in order to determine ecological indicators that can be applied locally or nationally. A vast amount of information would be gained by a phytosociological study describing species distribution and association with controlling environmental and anthropogenic variables. A WRC study is currently underway to provide this baseline data and will be informative about species that can be expected to occur under reference conditions for given habitat (WRC K5/1980 Sieben, E. pers. com. 2010). The data collated in the present study will add to this baseline dataset. Until our understanding of South African wetland autecology and phyto-sociology is much improved, phyto-assessment indices may only be able to differentiate between the environmental condition of wetland vegetation communities at the broadest levels of disturbance. This was found to be the case in the Cape Lowland Freshwater vegetation of the Western Coastal Slope during the present study.

8.8.2. Census of taxa indicative of disturbance

There are many dryland plants in South Africa, that can be classified as ‘facultative’ or ‘facultative dry’ species, that establish successfully in drained wetlands. Some of these are indigenous but there are also many non-indigenous dryland taxa. Accurate lists of what species constitute the various categories of obligate-wetland to dryland plants, in each region[4] of South Africa could assist in wetland phyto-assessment. Changes in species composition and proportions of cover/abundance would facilitate determination of hydrological status change over time. The list of taxa affiliated with South African wetlands, goes part of the way in providing this information (Rene Glen pers. com. for detail see Appendix 5 of this volume). Within this national list the number of observations by which species were designated as obligate or facultative wetland taxa was, however, not included and hence the accuracy of these designations is unknown. A wetland vegetation database (as proposed above in Section 8.9.1) should assist with this endeavour.

South African publications on alien and invasive plants in wetlands, including aquatic taxa, are relatively comprehensive in the number of taxa that are included (e.g. Henderson 2001, Henderson and Cilliers 2002, Gerber *et al.* 2004). Lists of weedy species (ruderals), both indigenous and alien, that are invasive and negatively impact wetlands could be compiled to assist in determining species indicative of a negatively impacted state (see Appendix 6 in the present volume). For instance, the Australian "wattles" *Acacia saligna** and *A. cyclops** are known to elevate the phosphorus content of the litter layer causing negative impact for indigenous vegetation beyond the more immediately apparent shading impact that these small trees have on wetland vegetation (Witkowski and Mitchell 1987: See Section 5.2.1 this volume).

8.8.3. Potential improvement of the dataset from the present study

The data collated in the present study was useful to determine metrics for those localities in which only a single geological substrate was included, namely Kenilworth, Lotus River and Kuils River wetlands on the Cape Flats and Hermanus in the Overberg sub-region (Section 8.7.2).

Samples from the Kuils River locality as represented by wetlands from Driftsands and Mfuleni were least useful amongst these localities with single substrates in terms of determining metrics. At Kuils River the number of reference samples was low relative to disturbed samples in comparison to other more successful localities. The addition of information from more reference samples of comparable wetlands at Kuils River is

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[4] This present WHI project contributed to the development of a list of wetland associated taxa in the wetlands of the Winter Rainfall Region of South Africa (Appendix 5 in this volume). A list of facultative to facultative dryland plants that are weedy and invasive was contributed to the WET-Health tool (Macfarlane *et al.* 2008: see appendix 6 in the present volume).

considered likely to provide sufficient information to facilitate development of metrics for this locality.

The 16 Moderate and 10 Disturbed samples from three Hermanus hillslope seep wetlands assessed in the present study provided sufficient information to develop five possible metrics for this area (See table 7.36). Broader sampling in Hermanus wetlands surrounded by the terrestrial/dryland *Overberg Sandstone Fynbos* zonal vegetation unit would equally facilitate development and testing of metrics for an IBI applicable to such wetlands. Focusing sampling within depressional rather than seep wetlands within the Hermanus area may also show that metrics for supralittoral vegetation from depressional wetlands are equally applicable to hillslope seeps.

In any of the other localities in which multiple geological substrates were sampled in the present study (Darling, Berg River, Verlorevlei, Agulhas Plain) further sampling – concentrated within one or all of the substrates of these localities would assist with the determination of metrics for that area. A total of 40 samples from Reference and 30 samples from Disturbed wetlands from any single geological substrate of these localities would provide sufficient data to facilitate the determination and testing of metrics for phyto-assessment (for detail see Section 8.7.3).

The currently developed metrics for the Cape Flats and Hermanus wetlands (Table 7.36) provide a guideline to the type of metrics based on species, community diversity and functional groups that have emerged from Cape Lowland Freshwater vegetation wetlands. This may guide development of metrics for any further localities assessed in this manner. Further metrics may also emerge as a result of a larger sample size that increases the certainty with which species are characteristically associated with reference or disturbed conditions and can therefore be categorized as tolerant of or sensitive to disturbance (*sensu* Section 7.3.2).

8.8.4. Indicators of functional change that result from nutrient enrichment

Research in the USA suggests that nitrogen (N) and phosphorus (P) are the primary nutrients limiting productivity in wetlands. In ecosystems where these nutrients are limiting, increased availability of N and P generally results in functional changes in wetlands such as increased storage of these nutrients in the tissues of wetland plants and a resultant increase in net primary productivity (NPP) (e.g. Shaver *et al.* 1998). Changes in nutrient uptake and NPP alter the extent **of** storage, and release of carbon (C), N, and P; thereby affecting ecosystem process such as wetland energy and nutrient cycling, accumulation of soil organic matter and organic carbon export. Such changes can compromise wetland environmental condition by altering niche/habitat characteristics that in turn affect wetland vegetation community composition and associated faunal assemblages.

‘Functional indicators’ of nutrient enrichment or eutrophication include leaf N and P content and metrics of NPP in the form of biomass production and stem height. Whilst stem density also reflects increased biomass it can also reflect other factors such as vigorous clonal growth (e.g. in *Cyperus textilis*) and thus is not a reliable indicator of nutrient enrichment. Leaf nutrient contents respond most rapidly to nutrient enrichment followed by the response of stem height and increased above ground biomass (U.S.EPA. 2002). A potential avenue for further development of metrics of environmental condition specifically relating to nutrient enrichment of wetlands would therefore be to explore the N:P ratio in leaf material, biomass production and changes in stem heights of species/genera/functional groups of taxa that have proved elsewhere to be useful for the determination of functional change.

8.8.5. Designation of plant functional groups

Taxa from the present study were separated into functional groups (as explained below) in an attempt to group species that respond similarly to human alteration of the wetland environment and that could thus potentially provide functional units of taxa within which to search for metrics of disturbance. If all species in a functional group show persistent, unidirectional change to disturbance, then their combination into a functional group metric might provide a stronger metric of disturbance than each of its component individuals. It is necessary to check the response of individuals before looking for averages from functional groups (Karr and Dudley 1981).

Two different approaches were used to form functional groupings of taxa in the present study:

1. Broad groupings of taxa were formed in the present study based on growth forms (i.e. graminoids vs. shrubs), or on structural aspects of plant form (woodiness), or life history attributes (annuals vs. perennials), or affinity to the wetland habitat (*sensu* Reed 1988), and origin (indigenous vs. alien). These broad taxa groupings were used in Section 7.2 to search for biodiversity differences between disturbance categories.
2. A combination of these above units was used in a classification of plants by morphological adaptations to habitat requirements (Cook 2004); as well as following the example of Boutin and Keddy (1993) on attributes of life history and morphological characteristics of vegetative propagation, competitive ability and stress tolerance (e.g.

graminoid-helophyte-stress-tolerant vs. graminoid-terrestrial-stress-tolerant taxa). A list of the functional groups developed from plants of the present study is presented in Appendix 10. These functional groups are intended to be recognizable in the field from their growth form and are thought to have particular association with wetland habitats that is at least partially dependent upon their functional type. The plants structural (architectural) aspects that were chosen to group species were based partly on the plant metrics developed in the North American bioassessment programs and partly on traits that were thought to give species a particular strategy for colonizing or occupying space (for detail see Section 2.9.2). The functional group classification attempt made in this study was based on field observation during this study. These groupings were used in place of species in Chapter 6 to determine the difference between disturbance categories. No metrics could be derived from these groups, predominantly as a result of conflicting reactions to disturbance from species included within any group. Although not found relevant in this study, the use of functional groups is potentially very useful and considerable effort should potentially be placed into empirically defining traits and functional and structural aspects that confer advantages on wetland taxa in the South African context (*sensu* Boutin and Keddy 1993). Classification of functional groups characterized by species with uniform responses to disturbance would assist in the determination of useful metrics of wetland environmental condition.

9. PROPOSED FRAMEWORK AND PHYTO-ASSESSMENT DEVELOPMENT PROTOCOL

In this chapter, an outline is presented of the framework for determining units of comparatively homogeneous wetland vegetation in any region of South Africa as well as the phyto-assessment metric development-protocol that can be applied within such homogeneous units of wetland vegetation. This framework and proposed protocol are applicable to any South African biogeographical region in which homogeneous units of wetlands vegetation are determined to occur.

9.1. Framework for the determination of comparable vegetation units

<u>Objective: To identify relatively homogeneous sets of wetland habitat both in terms of biogeography and localized physical habitat:</u>

a. Different biogeographical regions of wetland plants were classified by Mucina *et al.* (2006a) partially on the basis of Cowan's (1995) wetlands regions (see Section 2.9.1) The wetland vegetation units, broadly classified by Mucina *et al.* (2006a) as lowland freshwater, vernal pool, etc. also serve to differentiate wetland habitats.
b. Comparable physical habitats may include wetlands of one or more hydrogeomorphic (HGM) types but have similar hydrological zones and habitat for vegetation as determined by the National Wetland Classification System (SANBI 2009).
c. Within such wetlands or HGM units, comparable hydrological habitat-zones, namely supralittoral, littoral and aquatic, are considered to separate wetland vegetation into distinctly different vegetation units (Section 2.9.2).
d. Different 'structural' units of vegetation should be independently assessed (herbaceous, scrub-shrub and forested: Section 2.9.2.i).
e. Geologically different substrates and associated soil nutrient differences are separated by soil types. Soil types are a parameter used in the separation of different dryland vegetation units (e.g. Cape Flats Sand Fynbos or Cape Flats Dune Strandveld) within the Fynbos Biome (Mucina *et al.* 2006a). Wetlands within such vegetation units should be relatively uniform in terms of substrates (sand, clay, silt: Section 2.9.1) and soil nutrient concentrations (Section 2.8.5). Wetlands as azonal units within the zono-biome scheme may exhibit atypical concentrations of salts from surrounding soils. The separation into different wetland vegetation units (lowland freshwater, vernal pool, saline, etc.) mentioned in point a) above, should however account for such differences.

f. In order to provide sufficient (pseudo)replicates to develop phyto-assessment metrics for each biogeographically homogeneous region of wetlands plants, at least 30 wetlands would need to be sampled for developing metrics and a further 30 sampled to assess whether the metrics are accurate (*sensu* Step 10 Section 2.7.2.1).

9.2. A multi-metric phyto-assessment development-protocol for South Africa

<u>Objective: To develop phyto-assessment metrics for wetlands:</u>

Once wetlands have been identified as comparable using the framework outlined in Section 9.1., the following steps need to be adhered to in order to develop phyto-assessment metrics for wetlands. Protocols governing wetland sampling and subsequent data analysis must be followed in order to ensure the accurate measurement of the vegetation assemblage and accompanying environmental variables as well as a detailed assessment of the difference between disturbance categories.

g. From the wetlands that have been determined to represent comparable habitats, select those wetlands that best represent that type of habitat for the biogeographical region and have the largest number of anthropogenic disturbances impacting upon them.

h. Categorize the wetlands as being in a Reference, Moderate or Worst environmental condition (see Section 3.5.4).

i. Where the wetland-dryland boundary is not apparent within a wetland, a rough delineation procedure should be implemented using the DWAF (2003) method. This method includes soil augering and an examination of the vegetation and signs of past standing water. The delineation process ensures:
   - that all samples and observations recorded from each site are made within the wetland, rather than the dryland areas;
   - the full extent of the wetland area is assessed, including all of the ephemerally- or seasonally-saturated habitat of the supralittoral zone;
   - the approximate extents of the supralittoral, littoral and aquatic hydrological zones are determined; and
   - an accurate description can be made of the hydrological zone (aquatic, littoral or supralittoral) characterizing the habitat at each position where a vegetation sample is made.

j. Within the wetland ‘set’ to be studied, collect data from each wetland in order to categorize environmental conditions (see Section 3.5.5-3.5.7) and vegetation communities (Section 3.5.8). Care should be taken to ensure that wetland samples are taken from comparable depths in the littoral habitat where this hydrological zone is present.

k. In order to ensure the maximum identification potential for herbaceous species, as well as most shrub species, sampling should be conducted during the time period that coincides with the flowering and seed set of most species (or the most dominant species) in the vegetation unit being examined.

l. Analyze the data collected in step "h" following the analysis protocol suggested in Section 2.10.5 and used in Section 7.2 of the present study, in order to determine the patterns of vegetation assemblage that are most strongly associated with the different categories of wetland environmental condition. Those plant species cover/abundance and vegetation diversity attributes that change in empirical value for the different categories of disturbance, represent potential metrics for phyto-assessment index development.

m. Metrics must show signals that are statistically reliable and relevant to the effects of individual gradients of human disturbance or single stressors (e.g. sensitive taxa that show a significant relationship to an increase in human influence such as nutrient enrichment or water extraction) (Fore 2003, DWAF 2004). Metrics that show consistent responses along multiple gradients, such as nutrient enrichment and cumulative disturbance resulting from land use, have a greater level of consistency for index purposes (Fore 2003). Linear correlation can assist in determining the reliability and relevance of metrics.

n. Metrics that show consistent and reliable signals can be considered diagnostic indicators that are representative of the environmental conditions in the study set of habitats.

o. Determine the reference standard for each metric (e.g. % cover or diversity value expected in reference vs. impaired environmental conditions) *sensu* Table 7.36.

p. Successful metrics need to be combined into an Index, formed typically from at least 7 metrics from one biotic assemblage (US EPA. 1998c). The combination of metrics into an Index of Environmental Condition is achieved by assigning a numerical value to each of the metrics. The combination of metrics into an Index Score per Wetland provides a means of ranking wetlands as Reference, Moderate or Worst disturbed.

In order to check that the metrics accurately reflect the level of wetland condition in the manner in which they were designed to, they need to be tested on another set of data from the same type of wetlands from which the metrics were developed (Step 10 in Section 2.7.2.1.). Such a test must be performed on wetlands from the same biogeographical region and with the same basic geophysical environmental properties (habitat, HGM type and geological substrate). Using data from a different pool of wetlands would introduce natural variability that would confound the metric testing. The procedure for phyto-assessment using metrics developed in the above manner only requires measurement of vegetation parameters and does not require the measurement of disturbance influences or environmental parameters.

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APPENDIX 1

HUMAN DISTURBANCE SCORE FIELD SHEET

1. Intensity of Activities
5 = Poor: currently active and major disturbance
4 = less intense than “poor”, but current or active alteration
3 = active medium intensity disturbance
2 = low intensity alteration causing minor disturbance
1 = low intensity alteration or past alteration that is not currently affecting wetland
0 = natual landscape and/or no evidence of disturbance

2. Landuse characterization

Rate spatial extent: 0 = none, 1 = (< 25%), 2 = (25-50%), 3 = (50-90%), 4 = (>90%); then where impact exists: score as per above activity Intensity table.

Present Landuse / Activity		In wetland							Within 100 m of wetland edge							Within 100 to 500 m of wetland edge						
			Spatial Extent x Intensity = impact							Spatial Extent x Intensity = impact							Spatial Extent x Intensity = impact					
		Ex-tent	WQ	WQ Im-pact	Hydro-logy	Hy-dro im-pact	Phys struc	Phys struc im-pact	Ex-tent	WQ	WQ Im-pact	Hy-drol	Hydro im-pact	Phys struc	Phys struc im-pact	Ex-tent	WQ	WQ Im-pact	Hy-drol	Hydr o im-pact	Phys struc	Phys struc im-pact
Commercial afforestation																						
Agriculture - crops																						
Agriculture - livestock																						
Pugging - impact of livestock hooves																						
Agriculture - irrigation																						
Fish stocking																						
Irrigation release schemes		1	2	2	2	2	0	0	2	1	2	0	0	2	4	2	0	0		0		0
Annual pastures											0											
Perennial pastures											0											
Abandoned lands											0											
Rural development											0											
Suburban gardens									1	1	1	0	0	1	1	1	0	0		0		0
Recreational (sports field, golf estate, etc.) specify		1	2	2	2	2	0	0	2	2	4	3	6	2	4	2	3	6		0		0
Informal settlement											0											
Urban development									1	2	2	0	0	1	1	3	0	0		0		0
Industrial											0											
Infilling		1	1	1	2	2	1	1	2	1	2	0	0	1	2	3	0	0		0		0
Mining / excavation											0											
Deep flooding (too deep for emergent veg.)											0											
Shallow flooding											0											

Stranded aquatic vegetation											0											
Old high water marks											0											
Stormwater outlets											0											
Sewage disposal (note dist. of outflow fr. wetland)											0											
WWTW outlets											0											
Solid waste disposal (incl - dumping and litter)											0											
Weirs											0											
Berms											0											
Dams											0											
Water abstraction											0											
Drainage channels											0			2	0	3	2	6		0		0
Roads / Railway									1	2	2	0	0	1	1	1	0	0		0		0
Culverts											0											
Dredging											0											
Pedestrian paths		1	0	0	0	0	2	2	1	2	2	1	1									
off road vehicle use											0											
Erosion, e.g. gullies / headcuts											0											
Deposition / sediment											0											
Other											0											
SUM OF IMPACTS	**61**		**+**	**5**	**+**	**6**	**+**	**3**		**+**	**15**	**+**	**7**	**+**	**13**		**+**	**12**	**+**	**0**	**+**	**0**

3. Buffer Width vegetation categories								
Width of dryland vegetation buffer		(0) unlimited and natural vegetation cover	(1) natural buffer on average > 50 m	(2) buffer averages 25-50 m from perimeter	(3) Transformed vegetation 25-50 meters	(4) Transformed vegetation 10-25 m width	(5) Transformed vegetation < 10 meters	(6) No vegetated buffer
North side		**0**						
East side			**1**					
West side					3			
South side								6
Buffer Score = Average of four sides	**2.5**	0	1		3			6

Wetland Ken03: HDS =	**63.5**

APPENDIX 2

GENERAL FIELD DATA SHEET

WETLANDS HEALTH & IMPORTANCE RESEARCH PROGRAMME
GENERAL DATA SHEET

Team leader	
Sampling team	
Weather conditions	
Photographs taken	List photo #'s on flashcard:
Other visits made?	+ See table 6 at end:

1. GENERAL WETLAND INFORMATION

Wetland name				
Site code	1st 3 letters	Wetland #	day/month /year	quaternary subcatchment code

Degrees-minutes-seconds /	Decimal degrees /	Degrees & decimal minutes	Altitude:
S ° ' . "	S . °	° . '	Cape datum
E 0 ° ' . "	E 0 . °	0 ° . '	WGS-84 datum

LAT-LONG DATA SOURCE (see note 1)[5]	GPS	GIS	Map	Other:

[5] Please provide information about the map you have used if you have obtained your lat-longs from a map: What is the map source (e.g. Surveyor General's office 1: 50 000 map, AA road map, etc).

Location description							
Length*Breadth dimensions (m) of wetland							
Approx size of whole wetland (see note 2)[6]	<0.5 ha	0.5-1	1-5	5-10	10-20 *	20-50	> 50 ha
Approx size of area assessed	<0.5	0.5-1	1-5	5-10	10-20	20-50	> 50 ha

*** Cowardin (1979) >10 ha & > 2 m deep = lacustrine. Wave cut geophysical formation at water surface edge serves to additionally confirm this definition.**

DESKTOP INFORMATION required before survey conducted

Map Reference (1: 50 000)		Altitude	
Orthophoto ref (1: 10 000)		Aerial photo ref	
Closest town / mapped feature			
Site name (e.g. farm name, reserve name)		Name of Landowner/Manager	
Landowner's contact details			
Ecoregion (DWAF)			
Substrate – geological maps			

[6] Approximate sizes are difficult to gauge, but as a guideline, 1 ha is equivalent to about 1 1/3 rugby fields, i.e. one rugby field is about 0.7 ha.

Veg type: Mucina & Rutherford	

2. WETLAND CHARACTERISATION

GEOMORPHOLOGY					
Position in landscape: **1** mountain top, **2** mountain slope, **3** base of slope (of 2 or 4?), **4** foothill **5**coastal plain, **6** coastal dunes, **7** inland plateau, **8** inland plain					
Gradient at site:	Steep Mountain slope	Foothill slope	Slight slope	Flat	
Predominant landform in wetland:	slope	basin	channel	flat	
Substrate size class: **indicate only 1° 2° & 3°**	Bedrock	Boulders >256 mm Ø of individual rocks	Cobbles 150-256 mm	Gravel 2-150 mm	Sand 0.02- 2 mm
	Mud/Silt </= 0.06 mm	Clay < 0.002 mm	Peat	Organic Detritus	Other

HYDROLOGY						
Water permanence:	Perennial		Seasonal		Ephemeral	
[7]**Inundated or saturated?**	I	S	I	S	I	S
Tick areas sampled today						
Approx. max depth of inundation at present time	0-0.5 m (knee)	0.5-1 m (waist)		1-2 m (chest)	> 2 m (over head)	

[7] **Inundated = surface water is present at some stages, Saturated = surface *soils* are waterlogged**

Approx. max depth of annual inundation	0-0.5 m (knee)	0.5-1 m (waist)	1-2 m (chest)	> 2 m (over head)			
Approx level of inundation at present time	Dry	Nearly dry	< half	Half-full	3/4	Full	
Water source: (If there is a combination determine importance 1°, 2°, 3°)	Water table	Ground water spring	Surface water (precipitation)	Surface water (Riverine)	Sea	Artificial: Septic tanks & garden taps	Other
Water outlet: (determine importance 1°, 2°, 3°)	River or stream	No visible outlet/ Evaporation & Seepage into groundwater	Sea	**Artificial**			
Connectivity / Associated systems:	River	Within 100 year floodplain	Downslope of human land use but upslope of an open water body or channel	Estuary / Lagoon	**Part of wetland – upland complex**		
Wetland context:	**Single, discrete wetland**	**Part of a mosaic of wetlands**					

Wetland Characterization cont'd:

HYDROGEOMORPHOLOGY = WETLAND CLASSIFICATION						
Hydro-geomorphic type (Indicate on sketch map)	Valley bottom **with / without channel**	Flood-plain **meander cut-off / flat / pan**	Depression linked to a channel / drainage network **with / without channelled outflow**	Isolated Depression[8] **Fed by groundwater / Surface runoff**	Seep with channelled outflow **Basin / Hillslope**	Seep without channelled outflow **Basin / Hillslope**
# of HGM units						

VEGETATION: – for HGM & area sampled								
Dominant cover type: (if more than one then indicate 1°, 2° or %)	Open water	Aquatic bed[9] Native / **Alien**	Emergent / Herbaceous	***SCRUB-SHRUB***	Woody Alien **scrub / shrub**	**Forested**	**Mud-flats**	**Salt crust**
Herbaceous Dominated Systems – dominant vegetation type	Sedges	Restios	Grasses	Reeds - *Phragmites*	Rushes -*Juncus*	Herbs	Non-vascular	Aquatic sub merged / floating

Presence of Non-Vascular Plants	Lichens	Mosses	Fungi	Ferns	Algae	None
Indigenous monospecific stands of opportunistic species coverage: i.e. *Typha capensis* or *Phragmites australis / grasses*	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)

[8] An inland system that is hydrologically isolated from a drainage network that receives water from rainfall events and groundwater

[9] Aquatic bed: submerged or floating leaved plants rooted in a distinct zone on the openwater side of the emergent vegetation. Excludes floating aquatic species e.g. duckweed not rooted in the substrate

Horizontal Plan View – Heterogeneity or ° interspersion of distinct plant communities & thus habitats within the wetland	High hetero-geneity (0)	Moderately High (1)	Moderate (2)	Moderately Low (3)	Low (4)	None (5) No veg / mono-specific veg

LIST ALIEN SPECIES IN DIFFERENT ZONES: IF you don't know the plants, collect specimens

Inundated:..

....

....

Seasonally saturated:

....

....

Temporarily saturated:

....

....

Within 100 m – i.e. in buffer around wetland:

……………………………………………………………………………………………….

Within 500 m:

………

………

EVIDENCE OF ALIEN CLEARANCE:

Other data sources? (e.g. WfWet data)

Brush piles :

LIST INDIGENOUS DOMINANT SPECIES IN DIFFERENT ZONES: IF you don't know the plants, collect specimens

Inundated:..

....

Seasonally saturated:

....

....

Temporarily saturated:

....

....

Within 100 m – i.e. in buffer around wetland:

……………………………………………………………………………………………….

Within 500 m:

………

……

SPECIAL WETLANDS (score all and sum)	
	Rare habitat type, e.g. Acid sand plain Fynbos / Renosterveld **(1)**
	Known occurrence of vulnerable or endangered species **(1)**
	Significant migratory bird or water fowl habitat or usage **(1)**
	Artificial wetland **(4)**
	Acid Mine Drainage **(5)**
	Other

3. LANDUSE CHARACTERISTICS

CATCHMENT CONDITION AND LANDUSE (* INDICATE WHERE POSSIBLE ON SKETCH MAP) MARK RELATIVE BOX WITH A CROSS				
Intensity of surrounding land use in immediate area (to 500 m)	**Very Low: (0)** Untransformed or old growth natural vegetation or conservation area	**Low: (1)** Transformed natural or old abandoned agricultural lands, residential gardens recreational parkland	**Moderate: (3)** Residential, fenced pasture, conservation tillage or low intensity/subsistence agriculture, new fallow field	**High: (5)** Urban, industrial, intensive agricultural, mining, construction, afforestation, alien veg infestation
Intensity of surrounding land use in greater catchment area	**Very Low: (0)** Untransformed or old growth natural vegetation or conservation area	**Low: (1)** Transformed natural or old abandoned agricultural lands, residential gardens recreational parkland	**Moderate: (3)** Residential, fenced pasture, conservation tillage or low intensity/subsistence agriculture, new fallow field	**High: (5)** Urban, industrial, intensive agricultural, mining, construction, afforestation, alien veg infestation

SCORE TABLE: – **see Appendix 1**

4. WATER QUALITY INDICATORS

Water description:		**Clear**		**Cloudy**		**Muddy**		**Polluted**	**Other**
Water colour:	**No colour**	**Brown**	**Black / tea coloured**		**Green**		**Yellow**	**Polluted**	**Other**

Does the water smell (and of what)? H_2S, algae, sewage, none, other		Presence of salt-tolerant veg (e.g. Sarcocornia)	
Presence of indicator spp? **e.g. Chara, sphagnum**		Presence of salt encrustation?	
Water flow velocity	**Stagnant water**	**low stream power**	**high stream power**

5. WATER CHEMISTRY

WATER CHEMISTRY DATA COLLECTED ON SITE				
Describe habitat where sampled (1)			Description of site where sampled (2)	
Variable	**UNIT**	**VALUE**	**UNIT**	VALUE
pH	pH		pH	
Temperature	°C		°C	
Conductivity	mS		mS	
Turbidity	NTU		NTU	
Redox	mV		mV	

Dominant plant habitat unit assessment

Seasonality Temp/seasonal/permanent	**Hydrology** Dry/ Moist / Saturated/Inundated	**Dominant Substrate type** Sand/ Silt-Clay/ Peat/ Cobble/ Rock/ Salt crust	**Dominant landform in habitat unit** Basin/ Flat/ Channel/ Slope

6. ASSOCIATED WHI ACTIVITIES

Activity	**Yes/ No**	**Person responsible**	**Comments**
Water quality sampling (see above)			
Delineation (soils)			
Macrophyte identification			
Sediment samples taken			
Diatom sample taken			
Invertebrate sample taken			
Dry season samples previously taken			
Fine Scale Planning site			
WET-Health Assessment			
WET-EcoServices Assessment			
EIS Assessment			
Resource-economics Assessment			
Wetland Index of Habitat Integrity			

7. OTHER POINTS TO NOTE

8. SKETCH MAP OF WETLAND AREA **Try to indicate the salient features of the wetland including the different hydrogeomorphic units, roads, areas of cultivation or alien plant infestation, dams, weirs, etc.** NB: Indicate north with an arrow on your sketch. AND Indicate sampling sites on map.

APPENDIX 3

WETLAND PLANT SPECIES INVENTORY FIELD SHEET

Wetland plant inventory field sheet used while sampling to create the list of Western Cape wetland taxa added to Glen's National List of Taxa with Wetland Affiliations (Appendix 5 and accompanying CD)

WETLAND SPECIES LIST – Wetland Code:			
Species name	**Collection #**	**Cover Value**	**Notes**
		wetland average	
Juncus kraussii		2a	Supralittoral – littoral interface
Grass	FC001	x	

"r": 1-2 specimens (ex) / "+" : 3-10 ex / "1": 11-100 ex / 2m: > 100 ex, < 5% / 2a: 5-12.5% / 2b: 12.5-25% / 3: 25-50% / 4: 50-75% / 5: 75-100%

APPENDIX 4

WETLAND VEGETATION SAMPLE (FIELD) SHEET

Vegetation sampling form: Wetland Research Program

Site Code: Wetland Name: Area:		**Environmental Data** **HGM Type:** **Slope:**
Sample No(s). Long: Lat: Altitude: Author(s):	Plot size: Total Cover % Open soil % Open water % Litter depth (cm)	**Aspect:** **Landform:** Basin / Flat / Channel / Slope **Hydrological regime:** Perm / Seas / Temp **Current hydro'l condt'n:** inund'd/satur'd/moist/dry **Depth to water table (cm):**
Habitat description (1): **Dominant vegetation structure (2):** **Estimated Plot Roughness (3):**		**Inundation depth (cm):** **Water velocity:** stagnant / slow / fast **Water fluctuation:** stable / low / high
Vegetation structure (4):		**Drought stress:** none / temporary / seasonally

Layer type (see note 4):	Cover %	Av. height (m)	
herb:			**Soil pH:** **Soil redox (mV):**
shrub:			**Soil depth:** <20 cm / 0.2-1.5 m / >1.5 m
moss:			**Grazing/Mowing:** none, grazed/mowed, overgrazed
Presence of: Mottles / Organic streaks / Peat / Leached layer			**Burial:** none / sand / clay

Bedrock type: Shale**/** Granite / Limestone / Sandstone / Recent Sand / Leached sand / Silcrete / Laterite
Substrate type: Sand / Silt / Clay / Cobble / Boulder / Rock / Peat / Saltcrust
Soil texture: loam / sandy loam / silty loam / clay / silty clay loam / clay / sandy clay / silty clay / peat / other (specify)
Sedimentation: Erosion / balance / chemical / clastic / organic
Rockiness: None / pebbly to bouldery / bedrock

Cover-abundance scales Braun-Blanquet (1928) or presented in brackets (Barkman et al. 1964)

"r":	(or 1)	1-2 specimens (ex)	2m:	(or 4)	> 100 ex, < 5%	3:	(or 7)	25-50%
"+":	(or 2)	3-10 ex	2a:	(or 5)	5-12.5%	4:	(or 8)	50-75%
"1":	(or 3)	11-100 ex	2b:	(or 6)	12.5-25%	5:	(or 9)	75-100%

Species (Coll. #)	Layer (4)	Cover	Field Notes:
			Notes: Habitat / structure / roughness /layers
			(1) e.g.: Hypersaline flat, channel, microdepression, aquatic
			(2) Restioid, Cyperoid, Graminoid, Bryophyte, Woody, Bulbous, Fern
			(3) 1=open soil, 2=sparse graminoid, 3=reedbed/cyperoid,
			4=dense grassland, 5=Shrub graminoid combination,
			6=palmiet scrub combination;
			(4) moss layer, herb layer, shrub layer.

APPENDIX 5

LIST OF WESTERN CAPE WETLAND TAXA ADDED TO GLEN'S NATIONAL LIST OF TAXA WITH WETLAND AFFILIATIONS

Background to the list found on the CD – please see inside back cover of this report

Review of existing lists of plants with wetland association

A review of existing lists of wetland plants in the different regions of South Africa was undertaken in order to determine affinity for particular habitat and to search for species indicative of reference and disturbed conditions. The sources used in this review were as follows:

- A national list of plants with wetland affiliation, produced by Rene Glen from the PRECIS records of SANBI (unpublished *in toto*);
- Aquatic Plants of southern Africa. (Glen et al. 1999);
- Cape Plants. A Conspectus of the Cape flora of South Africa (Goldblatt & Manning 2000);
- Problem Plants of South Africa (Bromilow 2001);
- Alien Weeds and Invasive Plants (Henderson 2001);
- Aquatic and Wetland Plants of Southern Africa (Cook 2004); and
- Inland Azonal Vegetation, (Mucina et al. 2006) in: The Vegetation of South Africa, Lesotho and Swaziland. (Mucina & Rutherford (eds) 2006).

Glen's national list of wetland plant taxa with additional plants added from these other listings and from the present study are reported in the attached CD:

- A national list of plants with wetland affiliation, produced by Rene Glen of SANBI (cf. Glen et al.1999, but unpublished *in toto*) from herbarium records, that contains basic distributional information and hydrological affinity was reviewed and nomenclature updated where change was apparent. The plants of the Western Cape in this list were updated and increased using the Prospectus on Plants of the Cape Floristic Region (Goldblatt & Manning 2000). It is noteworthy that there have been a considerable number of name changes, particularly in the Cyperaceae, which are pertinent to this National List of Wetland Associated Taxa (*Mariscus* was sunk in *Cyperus* and *Schoenoplectus* and *Isolepis* split off from *Scirpus* (Dr Muthama Muasya, UCT, pers. comm.).
    a. The designation of obligate & facultative taxa is based on percentage occurrence

(correlation) in wetland habitat relative to upland habitat. The accuracy of this designation therefore relies on the number of observations for a given taxa. This list of Glens should therefore be adapted into a database that could be updated with herbarium records of distribution and habitat affinity to maintain the accuracy of these designations.

b. Whilst based on herbarium specimens and published reports the limited accuracy of the distribution of many species was realized during the sampling phase of this project. During this project, new data has been collected and for many of the 549 recorded species this meant an expansion of the published distribution range. New records for distribution of the recently described species *Aponogeton fugax*.

- David Hoare's unpublished list of obligate wetland taxa from the different regions of South Africa was also reviewed (Wetland Consulting Services (Pty) Ltd). The taxa in this list have obvious affiliation to wetland environments being predominantly aquatic taxa. No habitat or other ecological information is present in this listing;
- A list of invasive alien and indigenous ruderal (opportunistic invaders) taxa was created for the Western Cape and was added as an appendix to WET-health (Macfarlane et al.2008) (Appendix 6 in present volume).
- Limited accuracy of the reported distribution of many wetland species was realized during the sampling phase of this project. This emphasized the limited research that has been completed on these taxa and further suggests the potential value of a national database of wetland plant taxa.

NB considerable further additions to the list of Western Cape wetland taxa would be possible by taking into account the work of Mucina, et al. (2006a) and the SasFlora database of Barrie Low (SaSFlora 1998-2009).

Photographic record and field guides

Whilst considerable photographic record was kept of many of the plants sampled during this project, the development of keys and photographic guides to the vegetation of wetlands was not undertaken as existing botanical literature was, for the most part, sufficient for identification purposes (Cook 2004).

APPENDIX 6

APPENDIX 6.1. INVASIVE ALIEN AND INDIGENOUS PLANT SPECIES COMMONLY FOUND IN WETLANDS OF THE WINTER RAINFALL REGION OF SOUTH AFRICA

By Corry, F.

2009

These tables were compiled by extracting relevant species from the references listed at the end of this appendix. Considerable information was obtained and cross referenced with Rene Glen's database of Wetland Plants of South Africa. This work forms part of the Wetland Health and Importance Project for the development of macrophyte based indices of wetland ecological condition and as such these lists are still under revision and any comments or additions would be most welcome. In this regard please contact Fynn Corry of the Freshwater Research Unit via email Fynn.Corry@uct.ac.za

Table 6.1: Invasive alien plant species commonly found in wetlands in the winter rainfall areas of South Africa

Scientific name	Common name	Hydric status (see Table 6.2)
Terrestrial Species		
Arundo donax	Spanish reed	f
Acacia baileyana	Bailey's wattle	fd
Acacia dealbata	Silver wattle	fd
Acacia elata	Peppertree wattle	fd
Acacia longifolia	Long leafed wattle	f
Acacia mearnsii	Black wattle	fd
Acacia melanoxylon	Black wood	fd
Ageratina adenophora	Crofton weed	fd
Ageratum conyzoides	Invading ageratum	f
Araujà sericifera	Moth catcher	fd
Arundinella nepalensis	River grass	fw
Canna indica	Indian shot	f
Canna X generalis	Garden canna	f
Cirsium vulgare	Scotch thistle	fd
Cortaderia jubata	Purple pampas	fd
Cortaderia selloana	Pampas grass	f
Cuscuta campestris	Common dodder	fd
Hedychium coronarium	White ginger lilies	fd
Hypericum perforatum	St. John's wort	fd

Lantana camara	Lantana	fd
Lolium rigidum	Rye grass	f
Lythrum salicaria	Purple loosestrife	f
Melia azedarach	Syringa	fd
Metrosideros excelsa	New Zealand Christmas Tree	fd
Paraserianthes lopantha	Stink bean	fw
Paspalum dilatatum	Common paspalum / Dallis grass	fw
Paspalum distichum	Water couch grass	fw
Paspalum urvillei	Giant paspalum / Vasey grass	fw
Paspalum vaginatum	Brak paspalum	fw
Pennisetum purpureum	Napier fodder	fd
Populus canescens	Grey poplar	fw
Ricinus communis	Castor-oil plant	fd
Rosa rubiginosa	Eglantine, sweetbriar	fw
Rubus fruiticosus	European bramble	fd
Salix babylonica	Weeping willow	fw
Sesbania punicea	Red sesbania	fw
Solanum mauritianum	Bug weed	fd
Syzigium paniculatum	Australian water pear	fd
Verbena bonariensis	Purpletop verbena or vervain	fd
Aquatic Species		
Alternanthera philoxeroides	Alligator weed	o
Azolla filiculoides	Red water fern	o
Azolla pinnata	Red water fern	o
Eichornia crassipes	Water hyacinth	o
Pistia stratiotes	Water lettuce	o
Nasturtium officinale	Watercress	o
Salvinia molesta	Salvinia	o
Myriophyllum aquaticum	Parrot's feather	o

While these listed invasive alien plants are generally favoured by disturbance, they also readily invade areas that have not been disturbed. These plants are perennial and once they are well established they generally persist at the expense of the indigenous vegetation.

APPENDIX 6.2. CLASSIFICATION OF PLANTS ACCORDING TO OCCURRENCE IN WETLANDS

(Based on the US Fish and Wildlife Service Indicator Categories: Reed 1988)

Table 6.2: Classification of plants according to occurrence in wetlands, based on U.S. Fish and Wildlife Service Indicator Categories (Reed, 1988)

Obligate wetland species	o	Almost always grow in wetlands (>99% of occurrences)
Facultative wetland species	fw	Usually grow in wetlands (67-99% of occurrences) but are occasionally found in non-wetland areas
Facultative species	f	Are equally likely to grow in wetland and non-wetland areas (34-66% of occurrences)
Facultative dryland species	fd	Usually grow in non-wetland areas but sometimes grow in wetlands (1-34% of occurrences)
Dryland species	d	Almost always grow in drylands (>99% of occurrences)

APPENDIX 6.3. RUDERAL SPECIES COMMON TO WETLANDS

Ruderal species are typically species that are adapted to rapidly colonized areas with disturbed soils (e.g. in cultivated lands). As such, these species typically increase in abundance in response to disturbance, but are gradually replaced by later successional species as a site recovers. As with alien plants, specific species vary in their hydric status (Table 6.2) and occurrence between different geographic areas.

Table 6.3 Some weedy (ruderal) species commonly found in wetlands in the winter rainfall areas of South Africa

Species	Common Name	Hydric status (see Table 6.2)	Alien	Annual/ perennial
Ageratina adenophora	Crofton weed	fd	A	P
Aizoon canariense		fd		A-P
Anagallis arvensis	Scarlet pimpernel	fw	A	A
Arauja sericifera	Moth catcher	fd	A	?
Bidens formosa	Cosmos	fd	A	A
Bidens pilosa	Common blackjack	fd	A	A
Centella asiatica	Waternael	fw		P
Centella eriantha		fw		P
Cerastium capense	Horingblom	f		A
Chenopodium album		fw	A	A
Chenopodium ambrosioides		f	A	A

Table 6.3 Some weedy (ruderal) species commonly found in wetlands in the winter rainfall areas of South Africa

Species	Common Name	Hydric status (see Table 6.2)	Alien	Annual/ perennial
Circium vulgare	Scotch thistle	fd	A	A
Commelina africana	Wandering Jew	fw		P
Commelina bengalensis	Benghal Dayflower	fw	A	A
Conium chaerophylloides		fw		biennial
Conyza pinnata		f		P
Conyza pinnatifida		f		P
Conyza scabrida		fd		P
Cyperus dives	Mat sedge	o		P
Cyperus esculentus	Yellow flowered watergrass	fd	A	P
Cyperus rotundus	Purple watergrass	fd	A	P
Echinochloa crusgalli	Barnyard millet	o	A	A
Eragrostis cilianensis	Stinkgrass	fw		A
Eragrostis curvula	Weeping Love Grass	fd		P
Eragrostis plana	Fan lovegrass	fd/fw		P
Eragrostis planiculmis	Broom lovegrass	o		P
Gnaphalium gnaphalodes		fw		P
Heliotropium curassavicum		o	A	A-P
Heliotropium supinum		o	A	A
Hibiscus trionum	Flower of an hour	fd	A	A
Holcus lanatus	Common velvetgrass	fw		P
Hyparrhenia dregeana	Silky thatching grass	f		P
Ischaemum fasciculatum	Red vlei grass	o		P
Imperata cylindrica	Cottonwool grass	o		P
Ipomea purpurea	Morning glory	fd	A	A
Juncus effusus	Soft rush	o		P
Juncus tenuis	Wire rush	o	A	P
Leersia hexandra *	Southern cutgrass	o		P
Leysera tenella	Vaalteebossie	fw		A-P
Lolium rigidum	Rye grass	f	A	A
Mariscus congestus		fw		P
Oxalis corniculata	Creeping woodsorrel	fd	A	P
Panicum maximum	Guinea grass	d		P
Panicum schinzii	Sweet buffalo grass	fw		A
Paraserianthes lopantha	Stink bean	fw	A	P
Paspalum distichum *	Couch paspalum	w	A	P
Phalaris aquatica	Towoomba canary	fw	A	P

Table 6.3 Some weedy (ruderal) species commonly found in wetlands in the winter rainfall areas of South Africa

Species	Common Name	Hydric status (see Table 6.2)	Alien	Annual/ perennial
	grass			
Phalaris minor	Small canary grass	f	A	A
Persicaria aviculare	Knotgrass	o	A	A
Persicaria lapathifolia *	Pale persicaria	o		A
Phyllopodium bracteatum Benth.		f		A
Phyllopodium cuneifolium		f		A
Plantago spp	Plantain	fd	A	P
Polypogon monspeliensis	Brakbaardgrass	f	A	A
Pseudognaphalium luteo-album	Jersey cudweed	fd	A	A
Pucinellia angusta	Vinkbrakgrass	f		P
Pucinellia distans		f	A	P
Pucinellia fasciculata		f	A	P
Pycreus polystachyos	Field sedge	o		P
Ranunculus multifidus	Wild buttercup	o		P
Ranunculus muricatus	Spiny-fruited buttercup	f	A	A
Ricinus communis	Castor-oil plant	fd	A	P
Rumex acetosella	Common sheep sorrel	fw	A	P
Setaria sphacelata	Common bristle grass	f		P
Setaria verticillata	Bur bristle grass	fd	A	A
Spergularia media	Perennial sea spurrey	fw	A	P
Sorgum halapense	Johnson grass	fd	A	P
Tagetes minuta	Khaki weed	fd	A	A
Verbena bonariensis	Purpletop verbena or vervain	fd	A	P
Vulpia bromoides	Squirrel tail fescue	fw	A	A
Vulpia myuros	Rats tail fescue	fw	A	A
Xanthium strumarium	Large cocklebBurr	fd	A	A

It is important to highlight, that those species marked with an "*" are also well adapted to colonizing shallow open water areas, even where soil disturbance is entirely lacking.

The annual species are generally only abundant in the first year or two following a disturbance. However, the perennial species may take longer to reach their greatest abundance and often continue to persist, particularly where they provide tall and dense cover to the exclusion of other species.

APPENDIX 6.4. COMMON INVASIVE INDIGENOUS SPECIES FOUND IN WETLANDS OF THE WINTER RAINFALL REGION OF SOUTH AFRICA

While introduced and invasive plants generally have the most obvious impact on wetland vegetation, there are also a variety of indigenous species that tend to increase in abundance in response to disturbance events. A list of some common species are listed in Table 4.12, along with some brief notes outlining some of the common factors affecting their abundance.

Table 6.4: Invasive indigenous plants commonly found in South African wetlands (see Table 6.2 for a description of different hydric statuses)

Species	Hydric status	Notes
Typha capensis	w	Favoured by increased nutrients, stabilized and increased wetness and/or increased disturbance
Phragmites australis	w	Favoured increased nutrients, stabilized and increased wetness and/or increased disturbance
Phragmites mauritianus	w	Appears to be favoured by increased disturbance and/or increased sediment deposition
Leucosidea sericea	fd	Favoured by exclusion of fire and/or wetland desiccation.
Pteridium aquilinum	fd	Favoured disturbance/or and exclusion of fire

It is important to stress that the above species, especially *Typha capensis* and *Phragmites australis* occur naturally across many wetlands in South Africa, and many wetland areas are naturally dominated by a single species (e.g. *Phragmites australis*). In the permanently wet portions of a wetland, where only species tolerant of intense water logging are able to grow, it is common to naturally find a single species dominating, and pollen analysis from wetland sediment strata show that some wetlands have been dominated naturally by species such as *Phragmites australis* for thousands of years.

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APPENDIX 7

ABIOTIC CHARACTER OF EACH WETLAND ASSESSED IN THE CAPE COASTAL LOWLANDS

**Two national zonal units are shown for wetlands on the interface between these two units.

Sub-region	Wetland	Hydrogeomorphic unit	Wetland Size (ha)	max depth (mm)	Hydrology Group	Hydrological regime	Drainage category	Intrazonal Vegetation Unit	National Zonal Vegetation Unit***	Hydrological zones	Disturbance category	Hydrological zones
West Coast	Ber01	Depression	<0.5	0-500	Winter Inundated, Summer dry	Seasonal	Endorheic	Vernal Pool	Saldanha Flats Strandveld	supralittoral & littoral	worst	supralittoral & littoral
West Coast	Ber02	Floodplain	>50	1000-1500	Winter Inundated, Summer dry	Seasonal	Endorheic	Cape Estuarine Salt Marsh	Hopefield Sand Fynbos	supralittoral, littoral & aquatic	worst	supralittoral, littoral & aquatic
West Coast	Vel02 in (Berg River)	Depression	<0.5	0-500	Winter inundated, summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Hopefield Sand Fynbos	supralittoral & littoral	worst	supralittoral & littoral
West Coast	Dar01	Depression	0.5 to 1	500-1000	Winter Inundated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Swartland granite Renosterveld	supralittoral & littoral	worst	supralittoral & littoral
West Coast	Dar01B	Depression	0.5 to 1	500-1000	Winter Inundated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Swartland granite Renosterveld	supralittoral & littoral	worst	supralittoral & littoral
West Coast	Dar02	Depression	<0.5	0-500	Winter Inundated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Swartland granite Renosterveld	supralittoral	worst	supralittoral

West Coast	Dar03	Floodplain	0.5 to 1	0-500	Winter Inundated, Summer dry	Seasonal	Endorheic	Lowland Alluvial	Swartland alluvium Renosterveld	supralittoral	moderate	supralittoral
West Coast	Dar05	Depression	1 to 5	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Vernal Pool	Hopefield sand Fynbos	supralittoral	moderate	supralittoral
West Coast	Dar10	Floodplain	0.5 to 1	0-500	Winter saturated, Summer dry	Seasonal	Exorheic	Lowland Alluvial	Swartland alluvium Renosterveld	supralittoral	reference	supralittoral
West Coast	Dar11	Depression	<0.5	0-500	Winter saturated, Summer dry	Seasonal	Endorheic	Vernal Pool	Atlantis sand Fynbos / Swartland alluvium Renosterveld	supralittoral	moderate	supralittoral
West Coast	Ver01A	Floodplain	10 to 20	500-1000	Winter inundated, summer dry	Seasonal	Exorheic	Cape Lowland Freshwater	Leipoldtville sand Fynbos	supralittoral & littoral	moderate	supralittoral & littoral
West Coast	Ver01B	Floodplain	10 to 20	500-1000	Winter inundated, summer dry	Seasonal	Exorheic	Cape Lowland Freshwater	Lamberts Bay Strandveld	supralittoral & littoral	worst	supralittoral & littoral
West Coast	Ver02A	Floodplain	10 to 20	1000-1500	Winter inundated, summer saturated	Perennial	Exorheic	Cape Lowland Freshwater	Leipoldtville sand Fynbos	supralittoral, littoral & aquatic	moderate	supralittoral, littoral & aquatic
West Coast	Ver02B	Channelled valley bottom	10 to 20	0-500	Winter inundated, summer saturated	Perennial	Exorheic	Cape Lowland Freshwater	Leipoldtville sand Fynbos	supralittoral, littoral & aquatic	moderate	supralittoral, littoral & aquatic
West Coast	Ver02C	Channelled valley bottom	10 to 20	0-500	Winter inundated, summer saturated	Seasonal	Exorheic	Cape Lowland Freshwater	Leipoldtville sand Fynbos	littoral & aquatic	moderate	littoral & aquatic

West Coast	Ver03	Channelled valley bottom	5 to 10	0-500	Winter inundated, summer dry	Seasonal	Exorheic	Cape Lowland Freshwater	Leipoldtville sand Fynbos	supralittoral, littoral & aquatic	worst	supralittoral, littoral & aquatic
Cape Flats	Dri01	Depression	0.5 to 1	1000-1500	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral, littoral & aquatic	moderate	supralittoral, littoral & aquatic
Cape Flats	Dri03	Depression	0.5 to 1	1000-1500	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral, littoral & aquatic	moderate	supralittoral, littoral & aquatic
Cape Flats	Dri05	Depression	0.5 to 1	0-500	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral & littoral	reference	supralittoral & littoral
Cape Flats	Dri06	Depression	1 to 5	500-1000	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral & littoral	moderate	supralittoral & littoral
Cape Flats	Dri07	Depression	1 to 5	0-500	Winter saturated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral	moderate	supralittoral
Cape Flats	Dri08	Depression	0.5 to 1	0-500	Winter inundated, Summer saturated	Perennial	Exorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral & littoral	moderate	supralittoral & littoral
Cape Flats	Dri09	Depression	0.5 to 1	0-500	Winter saturated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral	moderate	supralittoral
Cape Flats	Mfu01 in Driftsands	Depression	<0.5	500-1000	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral, littoral & aquatic	worst	supralittoral, littoral & aquatic

Cape Flats	Mfu03 in Driftsands	Depression	1 to 5	0-500	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral, littoral & aquatic	worst	supralittoral, littoral & aquatic
Cape Flats	Mfu04 in Driftsands	Depression	<0.5	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Vernal Pool	Cape Flats Dune Strandveld	supralittoral & littoral	moderate	supralittoral & littoral
Cape Flats	Mfu05 in Driftsands	Depression	<0.5	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Vernal Pool	Cape Flats Dune Strandveld	supralittoral, littoral & aquatic	worst	supralittoral, littoral & aquatic
Cape Flats	Ken01_1	Depression	<0.5	500-1000	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos	supralittoral & littoral	worst	supralittoral & littoral
Cape Flats	Ken01_2	Depression	<0.5	0-500	Winter inundated, Summer saturated	Seasonal	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos	supralittoral & littoral	worst	supralittoral & littoral
Cape Flats	Ken04	Depression	1 to 5	0-500	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos	supralittoral & littoral	worst	supralittoral & littoral
Cape Flats	Ken05	Depression	0.5 to 1	0-500	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos	supralittoral & littoral	moderate	supralittoral & littoral
Cape Flats	Ken06_1	Depression	<0.5	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos	supralittoral & littoral	moderate	supralittoral & littoral
Cape Flats	Ken06_2	Depression	<0.5	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos	supralittoral & littoral	moderate	supralittoral & littoral

Cape Flats	Ken06_3	Depression	<0.5	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos	supralittoral & littoral	moderate	supralittoral & littoral
Cape Flats	Ken10	Depression	0.5 to 1	500-1000	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos	supralittoral & littoral	reference	supralittoral & littoral
Cape Flats	Ken11	Depression	0.5 to 1	0-500	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos	supralittoral & littoral	reference	supralittoral & littoral
Cape Flats	Ken20	Flat	1 to 5	0-500	Winter saturated, summer dry	Seasonal	Endorheic	Cape Lowlands Freshwater	Cape Flats Sand Fynbos	supralittoral	moderate	supralittoral
Cape Flats	Ken21	Depression	<0.5	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Vernal Pool	Cape Flats Sand Fynbos	supralittoral	moderate	supralittoral
Cape Flats	Lot01	Depression	0.5 to 1	0-500	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos / Dune Strandveld	supralittoral & littoral	moderate	supralittoral & littoral
Cape Flats	Lot02	Depression	1 to 5	500-1000	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos / Dune Strandveld	supralittoral & littoral	reference	supralittoral & littoral
Cape Flats	Lot03	Depression	0.5 to 1	0-500	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos / Dune Strandveld	supralittoral & littoral	worst	supralittoral & littoral
Cape Flats	Lot04	Depression	0.5 to 1	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos	supralittoral, littoral & aquatic	worst	supralittoral, littoral & aquatic

Cape Flats	Lot05	Depression	0.5 to 1	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos	supralittoral & littoral	worst	supralittoral & littoral
Cape Flats	Lot06	Depression	1 to 5	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos	supralittoral & littoral	worst	supralittoral & littoral
Cape Flats	Lot10	Depression	0.5 to 1	500-1000	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral, littoral & aquatic	reference	supralittoral, littoral & aquatic
Cape Flats	Lot11	Depression	0.5 to 1	500-1000	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral, littoral & aquatic	reference	supralittoral, littoral & aquatic
Cape Flats	Lot12	Depression	0.5 to 1	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral	reference	supralittoral
Cape Flats	Lot13	Depression	<0.5	0-500	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Cape Flats Dune Strandveld	supralittoral, littoral & aquatic	reference	supralittoral, littoral & aquatic
Cape Flats	Lot14	Depression	<0.5	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Cape Lowland Freshwater	Cape Flats Sand Fynbos / Dune Strandveld	supralittoral & littoral	worst	supralittoral & littoral
Overberg	Her01	Hillslope seep	1 to 5	0-500	Winter saturated, Summer saturated	Perennial	Exorheic	Cape Lowland Freshwater	Overberg Sandstone Fynbos	supralittoral & littoral	moderate	supralittoral & littoral
Overberg	Her02	Hillslope seep	1 to 5	0-500	Winter saturated, Summer saturated	Perennial	Exorheic	Cape Lowland Freshwater	Overberg Sandstone Fynbos	supralittoral & littoral	worst	supralittoral & littoral

Overberg	Her03	Hillslope seep	1 to 5	0-500	Winter saturated, Summer saturated	Perennial	Exorheic	Cape Lowland Freshwater	Overberg Sandstone Fynbos	supralittoral & littoral	moderate	supralittoral & littoral
Overberg	Mel01	Depression	1 to 5	0-500	Winter inundated, Summer dry	Seasonal	Endorheic	Vernal Pool	Elim Ferricrete Fynbos	supralittoral, littoral & aquatic	moderate	supralittoral, littoral & aquatic
Overberg	Rat02	Flat	>50	0-500	Winter saturated, Summer dry	Seasonal	Endorheic	Cape Inland Salt Pan	Overberg Sandstone Fynbos	supralittoral & littoral	moderate	supralittoral & littoral
Overberg	Rat03	Depression	20 to 50	1000-1500	Winter inundated, Summer inundated	Perennial	Endorheic	Cape Lowland Freshwater	Overberg Sandstone Fynbos	supralittoral, littoral & aquatic	reference	supralittoral, littoral & aquatic
Overberg	Rat04	Depression	20 to 50	500-1000	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Agulhas Limestone Fynbos	supralittoral, littoral & aquatic	reference	supralittoral, littoral & aquatic
Overberg	Uyn01	Depression	<0.5	1000-1500	Winter inundated, Summer saturated	Perennial	Endorheic	Cape Lowland Freshwater	Elim Ferricrete Fynbos	supralittoral, littoral & aquatic	reference	supralittoral, littoral & aquatic
Overberg	Was01	Depression	>50	1000-1500	Winter inundated, Summer saturated	Perennial	Exorheic	Cape Lowland Freshwater	Central Ruens Shale Renosterveld	supralittoral, littoral & aquatic	reference	supralittoral, littoral & aquatic
Overberg	Was02	Floodplain	>50	0-500	Winter saturated, Summer dry	Seasonal	Exhorheic	Cape Inland Salt Pan	Central Ruens Shale Renosterveld	supralittoral	worst	supralittoral

APPENDIX 8

WETLAND SPECIES FUNCTIONAL TYPES

Please see attached CD. It can be found inside the back cover of this report

APPENDIX 9

APPENDIX 9.1. DETECTION LIMITS OF SOIL NUTRIENT ANALYSES

Detection limits of soil nutrient analyses.

Analyte	Lower Limit of Detection	Lowest Quantifiable Concentration	Uncertainty of Measurement (%)	Calibration Range
P- Olsen	0.10 ($mg.kg^{-1}$)	0.32 ($mg.kg^{-1}$)	14.7	0-5 ($mg.kg^{-1}$)
P- Bray No. 2	0.14 ($mg.kg^{-1}$)	0.46 ($mg.kg^{-1}$)	3.4	0-10 ($mg.kg^{-1}$)
C% – Walkley-Black	0.01%$^{m}/_{m}$	0.05%$^{m}/_{m}$	14.6%$^{m}/_{m}$	0-12%
Resistance	-	-	15%	10-10,000 (Ω)
N (Leco)	0.04%$^{m}/_{m}$	0.053%$^{m}/_{m}$	6.9%$^{m}/_{m}$	0-100%$^{m}/_{m}$
C (Leco)	0.01%$^{m}/_{m}$	0.04%$^{m}/_{m}$	11.3%$^{m}/_{m}$	0-100%$^{m}/_{m}$
Ca (ICP-OES)	N/A	N/A	3.9%	0-1.50%$^{m}/_{m}$***
K (ICP-OES)	N/A	N/A	3.7%	0-0.05%$^{m}/_{m}$
Mg (ICP-OES)	N/A	N/A	3.4%	0-0.80%$^{m}/_{m}$
Na (ICP-OES)	N/A	N/A	21.6%	0-0.08%$^{m}/_{m}$

(%m/m = the mass of analyte per mass of sample expressed as percentage)

*** A calibration range of 0-1.50%$^{m}/_{m}$ for Ca means a range of 0 to 15000 $mg.kg^{-1}$ and is thoroughly sufficient for current analysis purposes.

APPENDIX 9.2. SUMMARY OF WATER QUALITY SAMPLE NUMBERS

Water quality variables and number of wetlands for which measurements were taken. Total wetland count n = 60.

Water Variable	# of Wetlands
pH	50
Temperature (^{0}C)	50
Conductivity ($mS.m^{-1}$)	50
Turbidity (NTU)	43
Redox (mV)	23
Dissolved Oxygen $mg.L^{-1}$	32
Dissolved Oxygen %	14
NH_4^+ ($\mu g.L^{-1}$)	34
NO_3^{-2} + NO_2^- ($\mu g.L^{-1}$)	33
PO_4^{-3} (µg.L-1)	33

APPENDIX 10

DESCRIPTION OF COMMON DISTURBANCE CLASSES IN SOUTH AFRICAN WETLANDS AS FROM WET- HEALTH

Used to help fill in the Landuse Characterisation Table in section 3.

Disturbance class	**Description**
Land uses commonly associated with complete transformation of wetland habitat	
Infrastructure	This includes houses, roads and other permanent structures that have totally replaced wetland vegetation.
Deep flooding by dams	This includes situations where flooding is too deep for emergent vegetation to grow.
Land uses commonly associated with substantial to complete transformation of vegetation characteristics.	
Crop lands	These lands are still in use and when active are generally characterized by almost total indigenous vegetation removal (predominance of introduced species). Examples include maize lands, sugarcane lands & madumbi fields, etc.
Commercial plantations	Common plantations include pine, wattle, gum, poplar. Other land uses such as vineyards and orchards may have a similar impact on wetland vegetation.
Annual pastures	These areas are characterized by frequent soil disturbance with a general removal of wetland vegetation. Some ruderal wetland species may become established but are removed on a frequent basis.
Perennial pastures	Although such areas generally include a high abundance of alien terrestrial grasses or legumes, the reduced disturbance frequency may permit the establishment of some wetland species.
Dense alien vegetation patches.	Where dense patches of alien plants can be identified within a wetland system, they should be identified as a separate disturbance class and evaluated as a unit.
Shallow flooding by dams	Such areas can often be identified at the head or tail end or edges of dams.
Sports fields	These include cricket pitches, golf courses and the like, where a species such as Kikuyu have been introduced and are maintained through intensive management. These are often located within areas of temporary wetland where terrestrial species generally dominate.
Gardens	Gardens are generally associated with urban environments.
Sediment deposition/ infilling and excavation	Deposition includes sediment from excessive erosion or human disturbance (e.g. a construction site) upstream of the wetland, which is carried by water and deposited in the wetland. Infilling is the placement by humans of fill material in the wetland (e.g., for a sports field). Excavation is the direct human removal (usually with heavy machinery) of sediment from the wetland, which is commonly associated with mining and sand winning
Eroded areas	In wetlands this typically occurs as gully erosion
Land uses commonly associated with moderate transformation of vegetation characteristics.	

Old / abandoned lands	These secondary vegetation areas have typically been altered through historic agricultural practices, but are in the process of recovering. They are generally characterized by a high relative abundance of ruderal species, but this abundance may vary greatly depending on time since cultivation ceased. In cases where this varies greatly within an HGM unit, it may be worthwhile to distinguish between vegetation classes comprising recently abandoned lands and vegetation classes comprising older lands that are at a more advanced successional stage of recovery.
Land uses generally associated with low transformation of wetland vegetation.	
Seepage below dams	Earthen dams used for agricultural purposes often allow water to leak through the wall, creating artificial wetter areas below the dam wall. Such areas are typically characterized by an increase in hydric species.
Untransformed areas	These primary vegetation areas have not been significantly impacted by human activities. This may include wetland areas within game or extensive grazing management systems. Small pockets of untransformed vegetation may also be set aside as streamside buffers on commercial landholdings.
Note: Scattered alien plants may occur in most of the above disturbance classes. Where this occurs, alien plants are considered as part of the larger disturbance class of which they are part (e.g. scattered bramble occurring within an old land), and the intensity of disturbance score is modified to account for the fine grain disturbances within them.	

APPENDIX 11

Table 11. Permutational analysis of dispersion of environmental variables measured at the vegetation sample scale between the spatial medians of wetlands as a grouping factor. Significant dispersion suggests greater variation of soil variables within rather than between wetlands.

All variables that had heterogeneous dispersion within the samples from each wetland are marked * for significance levels 0.05 to 0.001												
Variable	**West Coast**				**Cape Flats**				**Overberg**			
	F	*df 1*	*df 2*	*P*	*F*	*df 1*	*df 2*	*P*	*F*	*df 1*	*df 2*	*P*
Potential water depth	8.4	11	63	0.001*	1.98	24	179	0.007*	5.4	6	59	0.002*
Soil depth	24.3	7	53	0.001*	constant				no test, too few replicate samples			
Aspect	0.78	7	53	0.6	3.3	24	179	0.001*	2.2	6	59	0.06
Slope	0.75	7	53	0.6	2.7	24	179	0.003*	6.3	6	59	0.003*
ph Field measurement	1.95	9	49	0.06	1.8	27	178	0.02*	3.1	2	25	0.04*
Soil Redox	0.23	2	15	0.8	1.7	22	159	0.05*	3.1	2	25	0.06
Soil Variables: laboratory analysed soil parameters sampled in each vegetation sample												
% Clay particles	2.7	2	15	0.05*	1.9	21	149	0.02*	5	2	22	0.02*
% Sand particles	0.93	2	15	0.4	1.9	21	149	0.037*	0.5	2	22	0.6
% Silt	0.23	2	15	0.9	1.9	21	149	0.024*	2.7	2	22	0.06
Bulk Density	0.7	2	15	0.5	1.5	21	149	0.08	no test, too few replicate samples			
pH (KCl)	2.5	2	15	0.1	3.1	21	149	0.001*	0.26	2	22	0.8
Resistance	2.3	5	15	0.2	2.7	25	155	0.003*	0.35	2	22	0.7
Exchangeable cations of H+	4.4	5	24	0.01*	2.7	25	161	0.01*	5.4	2	22	0.008*
Exchangeable cations of Na+	2.4	5	24	0.04*	3.5	25	161	0.002*	1.3	2	22	0.3
Exchangeable cations of K+	2.1	5	24	0.07	3.7	25	161	0.001*	0.46	2	22	0.9
Exchangeable cations of Ca++	1.2	5	24	0.3	3.9	25	161	0.001*	3.4	2	22	0.06
Exchangeable cations of Mg++	1.2	5	24	0.3	5.3	25	161	0.001*	0.48	2	22	0.6
Phosphorus	1.6	5	24	0.2	4.1	25	161	0.004*	0.8	2	22	0.4
Potassium	2.1	5	24	0.07	3.8	25	161	0.001*	0.4	2	22	0.9
% Nitrogen	**1.4**	**5**	**24**	**0.2**	**1**	**25**	**161**	**0.4**	**0.3**	**2**	**22**	**0.8**

% Carbon	**1.4**	**5**	**24**	**0.3**	**1.4**	**25**	**161**	**0.08**	**1.2**	**2**	**22**	**0.4**
T-value	1.1	5	24	0.4	3.1	25	161	0.001*	0.36	2	22	0.7
Cation exchange capacity	1.4	5	24	0.18	1.1	25	161	0.3	no test, too few replicate samples			
Na water soluble	2.8	5	24	0.05*	4.97	25	161	0.001*	no test, too few replicate samples			
K water soluble	0.64	5	24	0.7	5.5	25	161	0.001*	no test, too few replicate samples			
Ca water soluble	6.1	5	24	0.002*	6.08	25	161	0.001*	no test, too few replicate samples			
Mg water soluble	4.3	5	24	0.008*	4.8	25	161	0.003*	no test, too few replicate samples			

d.f.1 = (number of wetlands less one); d.f.2 = (number of samples less number of wetlands)