Wetland Health and Importance Research Programme

2

The Assessment of Temporary Wetlands During Dry Conditions

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Series Editor: H Malan

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WETLAND HEALTH AND IMPORTANCE RESEARCH PROGRAMME

2 THE ASSESSMENT OF TEMPORARY WETLANDS DURING DRY CONDITIONS

Report to the
Water Research Commission

by

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Front Cover: Wet in winter: The Dreamworld film studios wetland, Cape Town, South Africa.
Inset: Dry in summer: The same wetland.
Photograph: J Day and E Day

PREFACE

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

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

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

An overview of the series

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

Assessing wetland environmental condition using biota

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

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

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

Broad-scale assessment of impacts and ecosystem services

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

Socio-economic and sustainability studies

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

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

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

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

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

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

Miscellaneous

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

EXECUTIVE SUMMARY

INTRODUCTION

Background

In South Africa, methodologies for the formal identification and delineation of wetlands revolve primarily around the use of soil morphological indicators, with the soils of permanently saturated wetlands in particular being easily recognizable by their grey colour and mottled appearance. A number of wetland types and conditions have however been identified for which soil morphological indicators do not readily apply. These include seasonally saturated to inundated wetlands in sandy coastal aquifers dominated by aeolian (wind blown) sands. In many of these systems, both anoxic conditions and the mineral stripping via podzolization that leads to mottling do not occur. The soils of temporary wetlands in very arid areas are also often too shallow, too saline or too temporarily inundated to exhibit typical wetland features in their soils. Such wetlands are called “cryptic”, and cannot reliably be identified as wetlands during the dry season on the basis of standard wetland identification and delineation tools.

Nevertheless, a number of both abiotic and biotic features in such wetlands do indicate periodic wetness, and these form the subject of this report.

Aims of this project

The main aim of this project, which forms one component of the Water Research Commission’s Wetland Health and Importance (WHI) Programme, is to develop a suite of indicators that will complement those already provided in the existing wetland delineation methodology by allowing identification and characterization of cryptic, non-perennial wetlands during the dry season.

Target users

The target users for the outcomes of this project include:

- conservation managers/wetland practitioners;
- wetland researchers;
- national and local authorities; and
- developers and planners seeking early identification of environmental constraints to proposed developments.

THE USE OF WETLAND BIOTA TO ASSESS TEMPORARY WETLANDS IN THE DRY SEASON

Plants

Many species of plants are characteristic of wetlands. The occurrence of some species indicates the presence of saturated and/or inundated soils, while other plants are particularly characteristic of non-perennial wetlands. This study lists a number of plant species known to occur in temporary wetlands in southern Africa.

Invertebrates of temporary wetlands

Various aquatic invertebrate groups survive in temporary wetlands because they have an ability to withstand the dry phase of these wetlands. Crustaceans are well represented in temporary wetlands by the Branchiopoda and the Phyllopoda, with the latter consisting of three Orders – Anostraca, Notostraca and Conchostraca or clam shrimps, of which the Conchostraca occur *only* in temporary waters. Other crustaceans characteristically found in such systems are the Cladocera, the Ostracoda and the Copepoda. The report lists invertebrate species known to inhabit temporary waters in southern Africa.

DEVELOPMENT OF AN APPROACH FOR USING INVERTEBRATES AS INDICATORS OF TEMPORARY WETLANDS

Various invertebrates are able to survive as desiccated propagules in the dry sediments of temporary wetlands. Methods have been developed to hatch these animals by inundating sediments from such wetlands in the laboratory. Since wetland invertebrates will be present only in wetland soils, this phenomenon offers a method for assessing the presence or absence of wetland conditions at a site from which propagule-containing sediments have been taken.

Development of laboratory methods for hatching invertebrates

Methods for incubating and hatching crustacean resting eggs

- Isolation of resting eggs: Hatching success was greatest where eggs had not been separated out from the surrounding substrate.
- Light: Hatching of branchiopod eggs is inhibited by complete darkness. Either constant light or a 12 hours light/12 hours dark regime is recommended.

- Temperature: Maximum numbers of branchiopods hatched at 15°C and the fewest at 25°C.
- Salinity: Similar numbers of individuals hatched at each of the salinities tested between 0 and 1000 mg/L.

Assessment of wetlands

The laboratory methodology developed in the first phase of the project was used to assess a number of seasonal, mainly cryptic wetlands in the Western Cape. Soil samples, collected in the dry season, were incubated for up to 35 days. Soil was also assessed for moisture content. Water quality and invertebrate samples were also collected from the same wetlands during the wet season, and the data from these wetlands compared with the dry season samples.

The key findings of the study can be summarised as follows.

1. The incubation techniques used provide a potential tool for assessment of the seasonally inundated wetlands in their dry state. The presence of any wetland invertebrate fauna in the incubated material indicates the presence of a wetland – although further investigation is recommended into the length of time over which wetland fauna may survive in a resting state, once wetland hydroperiod changes.
2. The presence of phyllopods provides useful evidence of wetlands that experience naturally short hydroperiods and periods of total desiccation.
3. Artificially induced hatching is considered an appropriate method for gauging crustacean assemblages. Seven taxa were represented from hatching trials and all are resistant to desiccation.
4. Good representation of natural invertebrate communities from hatching trials (based on presence/absence of taxa at ordinal level) was observed, suggesting that dry season assessments can provide a low-level surrogate for wet season assessments of biodiversity.
5. Various environmental variables (e.g. soil moisture for branchiopods, the organic content of the soil for ostracods and total ammonium and phosphates for cladocerans) appear to be reasonable predictors of assemblage composition. Turbidity and phosphates are the environmental variables most closely correlated with the species composition of the assemblage. Turbidity also plays an important role in the hatching success of eggs and

the time taken till hatching and could be used as a basic indicator to gauge invertebrate diversity and/or wetland environmental condition ("health").

6. More sites are needed to strengthen statistical results regarding environmental variables and community structure, and to provide a more comprehensive range of anthropogenic effects.

PLANT INDICATORS

DWAF (2005) outlines a method for the use of hydrophytic vegetation as indicators in wetland delineation, based largely on the identification of facultative and obligate wetland plants. Other methodologies interpret >50% cover by facultative and/or obligate wetland plants in either woody or herbaceous vegetation layers as a clear indicator of at least temporarily hydric conditions; the presence of some facultative or obligate wetland plants, but at low rates of cover (<50%), is taken to suggest but not to confirm hydric conditions. However, plants in infrequently and ephemerally inundated temporary wetlands may include annual macrophytes and algae during rare periods of inundation but under more normal, drier, circumstances may consist essentially of terrestrial, often ruderal species. Three possible approaches have been suggested as a way around this problem during dry season assessments. These comprise:

- the use of abiotic indicators such as water level, soil characteristics and the presence of dead plant material;
- the artificial germination of wetland seeds and bulbs in laboratory conditions – this study suggests that this approach is unlikely to be of value in providing a rapid means of assessing wetland character and/or condition; and
- the identification of perennial wetland plant species that would provide clear evidence of wetland inundation or saturation during the wet season, as well as the identification of wetland plant "markers" that would provide evidence that wetland conditions might occur during wetter periods, and which might be interpreted with higher levels of confidence if other wetland indicators are present.

Abiotic indicators in the identification and/or characterization of temporary and other cryptic wetlands

A range of other factors can provide valuable insights into the presence and even the type of cryptic wetlands assessed outside of the wet season. These are listed below.

Topographic indicators – the likelihood of a cryptic wetland being inundated versus saturated during wet season conditions can be determined on the basis of setting, with inundation most likely in depressions on hilltop crests, on hill-slope flats, on plains and in valley bottoms. Clearly, topographic indicators can provide a useful dry-season indication of wetland type, but they are unable to confirm the presence or absence of a cryptic wetland unless water is actually present.

- Soil wetness indicators – usually the least useful for identifying cryptic wetlands, since the soils are by definition not exposed to the specific conditions under which such indicators are formed.
- Other abiotic indicators including:

  the presence of a shallow clay or other impervious layer within 50 cm of the surface;

  the presence of deep polygonal cracks on the surfaces of relatively thick clayey substrata;

  the presence of thin, curled polygons of inorganic fines, collecting on the surface of the substratum;

  a thin “muck” layer on the upper surface of a site, often overlaying sandy soils;

  the presence of sediment deposits on plant stems, leaves, rocks and other objects;

  biotic crusts, comprising the dried remains of free-floating filamentous algae, blue-greens (cyanobacteria) and benthic microflora;

  algal markers;

  water marks on rocks, poles, trees or other fixed objects; and

  the presence of the shells, exoskeletons or bodies of aquatic invertebrates in surface sediment – although these markers should be used with caution as they may remain *in situ* for some time, indicating the presence of a previous wetland that no longer exists.

SUMMARY OF INDICATORS OF WETLAND PRESENCE AND TYPE IN THEIR DRY CONDITION

The indicators of wetland presence and type assessed in this study are listed in Table E1. The study's overall conclusions are as follows.

1. No one indicator provides adequate information about wetland presence, type, hydroperiod, biodiversity, function and principle ecological and hydrological drivers to be useful on its own – particularly with regard to actual or suspected cryptic and/or temporary

wetlands. In fact, assessment of a suite of indicators is required, to build up even a conceptual understanding of wetland ecosystem structure and function.

2. The absence of an indicator does not necessarily equate to the absence of a wetland.
3. Indicators that a wetland is present are usually associated with a higher level of confidence than interpretation of indicators of specific wetland character/habitat type (e.g. seasonally inundated or seasonally saturated) and/or biodiversity.
4. Seasonally/ephemerally inundated wetlands may be identifiable to a higher level of confidence than seasonally saturated systems, as a result of specific indicators for these conditions (e.g. algae and the presence of aquatic invertebrate communities).
5. Detailed delineation of cryptic wetlands is unlikely to be achievable with any useful degree of confidence based on a dry season assessment only.
6. Water chemistry is not easy to assess on the basis of dry season assessments, unless substantial macrophytes and algal material persist into the dry season.
7. Links between crustacean taxa and various water quality, hydrological and physical aspects require further investigation under controlled conditions.
8. Hydroperiod appears to be reflected most accurately by aquatic invertebrate communities.
9. Subtleties in hydroperiod appear to be of great importance in determining wetland crustacean community structure and hence are of biodiversity significance.

Finally, although considerable information can be gleaned about wetland function, structure and character through assessment of the suite of indicators outlined here, the assessment remains at best a surrogate for repeated sampling of a system in its wetted condition. Nevertheless, even where wet-season assessments have been possible, dry-season assessments add an important dimension to the understanding of wetland function, by indicating threshold hydrological, chemical and physical conditions that in many cases constitute actual threshold conditions for the survival of particular species in that habitat.

Table E1: Summary of major physical, chemical and biological indicators available for assessment during the dry season and providing information on particular aspects of wetland condition

Indicator	Condition indicated	Complementary indicators	Confidence
Biotic indicators			
Invertebrates			
• Invertebrates hatched out from dry season sediments under laboratory conditions			
Overall invertebrate assemblage	Crustacean assemblage a surrogate for wet season component – can show expanded faunal component, including sequential colonization effects; insect and other invertebrate components unlikely to be represented in dry season sediment samples. If site known to include wetlands but crustacean component absent from hatched samples – then either hydroperiod is too long or wetland not seasonally inundated, but rather saturated Presence of aquatic invertebrates indicates wetland now or in past subject to seasonal inundation This may be the only indicator of small, cryptic wetlands on rocky substrata with no plants and virtually no soil.	Dry season soil moisture Presence of shells/ exoskeletons of aquatic invertebrates	High
Crustacean component			
Anostraca	Abundant when dry season soils very dry (<6%) Potentially intolerant of high free ammonia concentrations (>0.1 mg/L), high EC (>900 mS/m), summer moisture	Soil moisture Abiotic indicators	Low – data correlative only
Conchostraca	Abundant when dry season soils very dry (<6%) Absent when dry season soils >30% moisture		
Cladocera	Abundant when dry season soils very dry (<30%) Tolerant of wide range of nutrient availability, turbidity and EC		
Ostracoda	Abundant when dry season soils very dry (<30%) Tolerant of wide range of nutrient availability, turbidity and EC		
Copepoda	Abundant when dry season soils very dry (<30%) Tolerant of wide range of nutrient availability, turbidity and EC		
• Presence of old cases, exoskeletons,	Indicative that periodic inundation of the site has taken place in the	Abiotic and	Low

Indicator	Condition indicated	Complementary indicators	Confidence
shells of aquatic invertebrates in sediments	past – not reliable indicator of present hydroperiod unless other factors present	invertebrate indicators	
Macrophytes			
• Presence of perennial or annual hydrophytes – growing or clearly identifiable dried plant remnants during the dry season	Wetland conditions definitely present – plant species habitat requirements (e.g. inundation etc.) will determine wetland type (e.g. seasonally inundated) and (low confidence) range of habitats	Invertebrate and abiotic indicators	High
• Presence of facultative wetland species	Wetlands may be present – in drier climates, presence of facultative wetland species has a higher likelihood of being linked to wetland conditions	Invertebrate and abiotic indicators	Low to medium
• Absence of both dryland and wetland plants from site	Presence of a wetland cannot be ruled out on this basis alone; in absence of invertebrate, soil or other markers, presence of seasonally inundated wetland unlikely, but small wetlands in rocky substrata may have none of these	Signs of recent fire? Abiotic indicators Invertebrate indicators	Low
• Presence of halophytes	Indicate saline soils in and around wetlands – but may also indicate non-wetland saline soils, especially in mesic areas	Other abiotic and/or biotic indicators essential	Low
Algae			
• Algae developing in incubated samples	May simply represent opportunistic propagation of air-borne spores – identification to genus/species level may improve confidence	Abiotic and biotic indicators	Low
• Presence of dried algal remnants	Indicative of wet season water levels – indicates seasonal/periodic inundation	Soil moisture Invertebrates	High
Abiotic indicators			
• Topography	Indicates potential for accumulation of water in wet season – must be interpreted with other indicators	Abiotic and biotic	Low
• Soil wetness	Presence of gleying, mottling: if present as per DWAF (2005) then indicates wetland type (permanent/seasonal etc.) Absence of above, coupled with sandy soil, and/or arid climate	Biotic and abiotic	High

Indicator	Condition indicated	Complementary indicators	Confidence
	and/or perched wetland conditions: cryptic wetland cannot be ruled out Dry season soil moisture data: ➢ >30% and presence of other indicators of wetland conditions: wetland may not support crustacean fauna; lower species diversity ➢ <30% and presence of other indicators of wetland conditions: wetland may support crustacean fauna and potentially linked to higher species diversity/endemism		Low
	Dry season water table <0.5 m from surface OR impermeable layer <0.5 m from surface: indicates wetland presence but not hydroperiod (inundated or not) Dry season water table >0.5 m from surface OR impermeable layer >0.5 m from surface: no strong conclusions can be drawn	Biotic and abiotic – including soil moisture	Medium Low
• Muck layer	Thin layer (<2 cm deep): Presence: wetland conditions in recent past/present Absence: inconclusive Thick layer (<2 cm deep): wetland conditions in past		 Medium Medium
• Sediment deposits on plants and/or rocks	Presence: indicates minimum levels of inundation – wetland assumed to be seasonally inundated Absence: inconclusive		Medium Low
• Biotic crusts	Presence: indicates minimum levels of inundation – wetland assumed to be seasonally inundated Absence: inconclusive		High Low
• Water marks	Presence: indicates minimum levels of inundation – wetland assumed to be seasonally inundated Absence: inconclusive		Medium Low

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ABBREVIATIONS

CCA – canonical correspondence analysis

DEAT – Department of Environmental Affairs and Tourism

DWAF – Department of Water Affairs and Forestry

df – degrees of freedom

EC – electrical conductivity

MAP – mean annual precipitation

MAR – mean annual runoff

MDS – multidimensional scaling

N – nitrogen

NTU – nephelometric turbidity units

P – phosphorus

SANBI – South African National Biodiversity Institute

SD – standard deviation

SE – standard error

TSS – total suspended sediments

USACE – United States Army Corps of Engineers

WHI – Wetland Health and Importance (Research Programme)

WRC – Water Research Commission

1. INTRODUCTION

1.1 Background

The South African National Water Act (Act 38 of 1998) defines a wetland as "land which is transitional between terrestrial and aquatic systems, 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". Based on this definition, the Department of Water Affairs and Forestry (DWAF, 2005) notes that wetlands must have one or more of the following attributes:

- hydromorphic soils that display characteristics resulting from prolonged saturation;
- the presence, at least occasionally, of water-loving plants (hydrophytes); and
- a high water table that results in saturation at or near the surface, leading to anaerobic conditions in the top 50 cm of the soil.

Using these characteristics, a national wetland delineation manual was developed with a view to identifying the outer edges of wetlands – usually the so-called "temporary zone" (DWAF, 2005). The method relies on four specific indicator types, namely:

- a terrain unit indicator (which identifies positions in the landscape in which wetlands are likely to occur);
- a soil form indicator (which identifies soil forms associated with prolonged and frequent saturation);
- a soil wetness indicator (which is based on morphological signatures developed in the soil profile as a result of prolonged and frequent saturation, resulting in interstitial anoxia and its associated chemical and physical effects on soil); and
- a vegetation indicator, which identifies hydrophilic vegetation associated with frequently saturated soils.

Of these indicators, the soil wetness indicator, based on soil morphology, is used extensively in the identification and delineation of wetlands in South Africa. The soils of permanently saturated wetlands in particular are usually easily recognizable by their grey colour and mottled appearance, even when they are not inundated (DWAF, 2005, Job, 2009).

A number of wetland types and conditions have been identified, however, in which the use of the above suite of wetland indicators becomes problematic. DWAF (2005)

provides two examples of “difficult” conditions, namely recent alluvial deposits that are too recent or too sandy for morphological signs of wetness to be readily detectable, and seasonally saturated to inundated wetlands in sandy coastal aquifers dominated by aeolian (wind blown) sands. In these areas, normal markers of prolonged shallow surface saturation, such as soil colour, may be of little use in wetland identification because of other factors such as mineral stripping via podzolization (DWAF, 2005), or simply because anoxia does not develop.

Job (2009) confirmed that hydric indicators were absent from several temporary wetland areas assessed during the dry season in sandy coastal areas in the Western Cape. Very sandy soils usually drain rapidly, so water is retained for relatively short periods and anoxia does not develop. Similar patterns also arise in temporary wetlands elsewhere, including the Free State, and particularly in very dry areas such as the Northern Cape, the Kgalagadi and the Namib Desert. In such areas, soils are often waterlogged for too short a time, and/or do not become sufficiently anoxic to evince signs of waterlogging. Alternatively, the wetlands are “perched” on shallow soils over impermeable layers, and are too shallow to become anoxic, or are too saline for hydric morphological features to develop (USACE, 2006). Many of these systems, moreover, support plants and animals that are highly seasonal, and are not visible outside of periods of inundation, making the use of other indicators such as vegetation similarly problematic during the dry season. Temporary wetlands such as these are sometimes referred to as “cryptic”, in that they cannot reliably be identified as wetlands during the dry season on the basis of standard wetland identification and delineation tools. Whilst their soils may not necessarily display evidence of waterlogging, other features, both abiotic and biotic, do display a variety of very characteristic features. These form the subject of this report.

1.2 Characteristics and importance of temporary wetlands

Temporary wetlands are remarkably varied. They are usually shallow (<1 m at their deepest) and roughly oval in shape, and range in diameter from less than a metre to tens of kilometres. They may receive their water from rain, river flow, ground water, or any combination of these. The substrata can be rocky or can consist of soft sediments. These wetlands may support a wide variety of wetland plants and aquatic animals, and their waters range between very pure and highly saline. In general, large temporary wetlands are known as pans. Pans tend to be fed by rainfall, and sometimes by river flow, and their beds are filled with soft sediments. Such systems in the Free State, for instance, are seasonally inundated and support dense growth of grasses in the dry

season. Very large pans (>1km in diameter), such as the ones in the Northern Cape, are often fairly saline (hence the term 'salt pan') and tend to support few, if any, plants. In the dry season, their surfaces are often smooth and shiny from a fine glazing of salt crystals on the surface. Smaller (<10 m in diameter) temporary systems are commonly called pools (or, in the south-western Cape, vleis). Some of them have sediment-filled bottoms, and support vegetation, while others are depressions in bare rock with virtually no sediment and thus no plant life either. If the sediments contain fine clays and silts, they tend to crack as they dry out and harden.

Partly as a result of the difficulty in identifying (and thus managing and conserving) such systems, coupled with the ease with which they can be filled in or otherwise destroyed as a result of various anthropogenic activities, temporary wetlands are highly threatened ecosystems. They are also often highly significant for biodiversity conservation, many of them providing habitat for highly specialized and sometimes endemic wetland fauna. Given these factors, some means of identifying cryptic temporary wetlands outside of the sometimes brief inundation periods – when they support clearly identifiable wetland fauna or flora – would clearly be a useful progression in wetland management and conservation.

1.3 Aims of this project

The main aim of this project, which forms one component of the Water Research Commission's Wetland Health and Importance (WHI) Programme, has been to develop a suite of indicators that will complement those already provided in the existing wetland delineation methodology by allowing identification and characterization of cryptic, non-perennial wetlands during the dry season. Some of these indicators may theoretically also be applicable to the identification of other wetland types that have been subject to particular forms of disturbance, including fire, extensive grazing or anthropogenic removal of vegetation, each of which can also make the use of normal wetland indicators difficult or unreliable.

The development of indicators of ecosystem condition or "health" for these cryptic wetlands was also an objective of this project, although it was recognized from the outset that compilation of any kind of quantitative scoring system would be an unlikely outcome, and that the identification of broad indicators of wetland character, from which condition can be deduced with reference to an assumed natural state, is the most realistically achievable outcome of this aspect of the project.

1.4 Target users

The target users of a suite of indicators for cryptic/dry season wetland identification and characterization might include:

- conservation managers/wetland specialists engaged in the identification, description and classification of wetlands;
- wetland researchers with an interest in seasonal wetland colonization and succession patterns;
- national and local authorities (e.g. the Department of Water Affairs: DWA) engaged in decision-making regarding land-use in areas potentially including wetlands; and
- developers and planners: the application of this tool might prevent the costly delays involved in carrying out specialist wetland Environmental Impact Assessments only during the wet season.

1.5 Approach to this project

The cryptic wetlands that form the focus of this project do not fall neatly into any of the delineation protocols put forward by DWAF (2005). Job (2009) notes the need to consider a wide range of wetland indicators before assigning (or not assigning) wetland status to so-called "difficult" wetland sites. Delineation and wetland identification guidelines developed for application in arid areas elsewhere in the world (e.g. the arid Midwest of the United States of America: USACE, 2006) also recommend the use of a suite of indicators that might improve the accuracy of estimates of wetland status and condition.

In light of these issues, this project has focused on the collation and, in some cases, testing of a wide range of potential indicators of different types of cryptic wetland, although most of the original research presented revolves around the development of biotic indicators – specifically, the use of plant and invertebrate propagules and specimens that remain present but are usually undetected in wetland soils during the dry season – for the identification of cryptic wetlands.

Development of a method that makes use of biotic propagules in wetland assessments involved an initial pilot phase, in which techniques for the collection, incubation and identification of faunal and floral propagules were developed, based on techniques largely developed elsewhere for use on similar groups of organisms. This phase formed the subject of a BSc Honours dissertation by Anne Ketley (Ketley, 2007). The pilot phase was followed by the collection of dry season soils, which were incubated in the laboratory

using the protocols developed in the pilot study, and compared with invertebrate data collected from the same wetlands during the wet season. Patterns of occurrence between dry and wet season faunal assemblages were explored, as well as links between dry season faunal communities and other indicators of wetland ecological condition, based on wet season water chemistry data. The main objective of the biotic sampling phase was to allow links to be made between dry season data and the kinds of wetlands they represented in the wet phase.

The use of perennial macrophytes to indicate the presence of cryptic wetlands in their dry condition was also investigated, albeit at a broad level, based on existing plant data and the collation of macrophyte data collected as part of the "Macrophyte Index" developed by Corry (2010) in another component of the overall WHI programme.

Finally, a list of abiotic indicators that are evident during the wet season was also compiled, and their roles in providing information regarding both wetland type and character were explored, again based largely on existing data and on broad guidelines for wetland delineation. These indicators include aspects of soil-surface morphology such as cracking, water marks on substrate and vegetation, the presence of shells of dead wetland animals, and the shape and position of the wetland in the landscape.

1.6 The extent of this project

The focus of this project has been on wetland identification and characterization, rather than on delineation. As such, although techniques that are used in various wetland delineation methodologies have been presented here, the criteria outlined in this document should be seen as complementing, rather than substituting for, those provided in the wetland delineation methods described in DWAF (2005). Nonetheless, this project has contributed significantly to our understanding of the biology and biodiversity of temporary wetlands. Furthermore, although we would have liked to examine taxa from a wide range of systems, logistical and time constraints forced us to limit regional studies to the provision of lists of taxa, and experimental work was confined to crustacean taxa from the south-western Cape.

2. THE USE OF WETLAND BIOTA TO ASSESS TEMPORARY WETLANDS IN THE DRY SEASON

2.1 Overview of the biotas of temporary wetlands

Temporary waters, as a whole, may support an abundance of organisms ranging from bacteria to vertebrates, but the most obvious inhabitants when the system contains water are usually invertebrates, particularly crustaceans and insects (e.g. Williams, 1998). While the microbial component of the wetland community is functionally important, virtually nothing is known about its taxonomy or diversity and it is not discussed further in this report.

2.1.1 Plants

Many species of plants are characteristic of wetlands and their occurrence indicates the presence of saturated, if not inundated, soils. Other plants, such as various species of *Ranunculus* and *Aponogeton*, are particularly characteristic of non-perennial wetlands. Appendix 1 lists a number of plant species known to occur in temporary wetlands in southern Africa. While the list is by no means exhaustive, it does provide an indication of the range of relatively common plants, the presence of which (in plant or seed form) would indicate wetland conditions and, in the absence of other hydric indicators, could indicate the presence of a cryptic wetland. If the plants themselves are not present at the time, their seeds could, at least in theory, be germinated in the laboratory and the seedlings identified.

Margaret Brock and colleagues of the Co-operative Research Centre for Freshwater Ecology in Armidale, Australia, are currently world leaders in work on the seedbanks of wetland plants. They have developed a series of useful techniques (e.g. Brock *et al.,* 1994, see also Baskin and Baskin, 1998) for separating and identifying seeds of wetland plants. Using these techniques, they have studied patterns of zonation and drying (Brock and Casanova, 1997), and community structure of wetland plants and invertebrates (Brock *et al.,* 2003).

These methods have also been used to investigate salinisation of Australian wetlands – an issue that has long been of economic and environmental concern in that country (e.g. White, 1997). Using the techniques referred to above, Nielsen *et al.* (2003) examined the effects of increasing salinity on the germination of aquatic plants and the hatching of

micro-zooplankton from two wetland sediments. They found reductions in species richness and abundance of both plants and animals at salinities between 1000 and 5000 mg/L.

2.1.2 Invertebrates of temporary wetlands

Various aquatic invertebrate groups survive in temporary wetlands because they have an ability to withstand the dry phase of these wetlands in the form of a propagule such as a resting egg (e.g. cladocerans: Vandekerkhove *et al.,* 2004a and rotifers: Pourriot and Snell, 1983), a young encysted larva (large branchiopods such as fairy, clam and tadpole shrimps: e.g. Brendonck, 1996), or even as a desiccated larva (e.g. some chironomids: Harrison, 2003 and copepods: Rayner, 2001) or adult (tardigrads: Rayner, 2002).

Crustaceans are well represented in temporary wetlands by the Branchiopoda, a group of primitive crustaceans that includes the Phyllopoda and the Cladocera, or water fleas. The Phyllopoda consists of three Orders: the Anostraca or fairy and brine shrimps, the Notostraca or shield shrimps, and the Conchostraca or clam shrimps, almost all of which are found only in temporary waters. Other crustaceans characteristically found in such systems are the Cladocera, the Ostracoda (seed shrimps) and the Copepoda (e.g. Day, 2001). Within the crustaceans it is only these groups which have propagules that are able to survive desiccation, although an occasional decapod (e.g. crab) or peracarid (isopod or amphipod) will colonize inundated temporary wetlands from nearby permanent systems. In a similar way, a wide variety of insects (adult and juvenile bugs and beetles, immature dragon- and damselflies, mayflies, mosquitoes and other flies) can be found when water is present in a temporary wetland, but these animals will have flown in from permanent wetlands in the vicinity. Just a single species of midge (Diptera: Chironomidae), a remarkable west African species called *Polypedilum vanderplanki*, can survive desiccation as a larva (e.g. Harrison, 2003).

Several species of mollusc (e.g. the introduced *Physa acuta* and some species of *Lymnaea*) are able to resist desiccation as adults (Appleton, 2002), while several unnamed turbellarian (at least some of which are probably of the genus *Mesostoma*: JA Day, preliminary identification), nematode, rotifer and gastrotrich species can also be found in temporary wetlands, surviving dry periods as desiccation-resistant eggs. Leeper and Taylor (1998), examining the invertebrates of a temporary wetland in South Carolina, USA, found that nematodes, rotifers and microcrustaceans were most numerous, while oligochaetes and chironomids dominated the biomass.

2.2 Temporary wetlands in southern Africa

Temporary wetlands occur wherever suitable substrata contain water for periods varying from a few days to several years. Such conditions usually result from rain falling unevenly over time, so that for part of the time rainfall exceeds evaporation, whilst for the rest of the time evaporation exceeds rainfall and the wetlands dry up. Such conditions are common over much of the drier part of southern Africa, from the southern coastline of South Africa to the midlands and higher altitudes of KwaZulu-Natal and Zimbabwe in the east, and northwards to Namibia, southern Angola and Botswana in the west. Some of the most extensive temporary wetlands in the region are the Makgadikgadi Pan in Botswana, Etosha Pan in Namibia, and the huge "vloere" ("floors") such as Verneuk Pan and Groot Vloer, in the Northern Cape. All of these support organisms able to survive dry periods, but very little information is available on most of them. Published literature, some rather old, is available on Makgadikgadi Pan (Tooth and McCarthy, 2007); the temporary pools near Gaborone in Botswana (Brendonck and Riddoch, 1997; 2000); Etosha Pan (see http://www.met.gov.na/maps/Etoshareferences.doc for a list of the literature); the wetlands of the Skeleton Coast of Namibia (Day, 1990); rock pools in Zimbabwe (Weir, 1966); and pools in north-eastern KwaZulu-Natal (Hamer and Appleton, 1991) and the Free State (Meintjies, 1996). Jones (2002), Bird (2010) and Corry (2010) include temporary wetlands in their studies of the wetlands of the south-western Cape of South Africa. Apart from the work listed above, very little indeed is known about the biology or ecology of the biotas of southern African temporary wetlands.

Appendix 2 lists the invertebrate species known to inhabit temporary waters in southern Africa. Note that some of the species listed are not exclusive to temporary wetlands, but colonize them from adjacent permanent water bodies during periods when the temporary wetlands are inundated. Additionally, because temporary wetlands are small and scattered across the landscape, they represent a very scarce type of habitat. For this reason, many of the invertebrate species found therein are also rare (Williams, 1998), although we have little information on the conservation status of most of them.

Species of animals that are able to complete their life cycles in temporary aquatic habitats have various kinds of adaptations. They all exhibit a period of diapause or aestivation during the dry phase, but they are also able to synchronize hatching and reproduction with the wet phase (Elgmork, 1980). The differential timing of diapause and hatching is linked to environmental triggers such as salinity, oxygen tension, illumination and the temperature of the water, while the rates of both embryonic development and maturation

are primary determinants of the patterns of succession in the field (e.g. Hairston and Cáceres, 1996).

Adaptations that enhance survival and reproductive success in the wet phase include effective modes of dispersal, rapid growth, short life span and small size – typical features of 'r'-selected taxa – and opportunistic or generalist feeding modes (Williams, 1998). On the other hand, the phyllopods in particular appear to suffer from poor competitive abilities, colonizing early, growing quickly, and using the favourable conditions whilst available, but often succumbing to predation when large insects colonize the ponds.

2.3 Biogeographical considerations

Despite the fact that several hundred invertebrate species have been recorded from temporary waters within southern Africa (see Appendix 2), it is difficult to come to any sensible conclusions about the biogeographical distribution of the fauna as a whole. It is possible, though, to surmise fairly accurately about some distribution trends within the crustaceans, many of which are obligate members of the temporary wetland fauna. For instance, *Triops granarius*, the single species of notostracan known from the region, seems to occur everywhere but in the extreme south-western Cape. The fact that this area is well sampled suggests that such a distribution pattern is real. Indeed, the number of species of phyllopod (fairy, brine, shield and clam shrimps) in the area is very small indeed, with only *Streptocephalus dendyi* and *S. purcelli* (Anostraca), and *Leptestheria rubidgei* (Conchostraca) having been recorded here. Outside of this well-studied area, it is not possible to say if the apparently narrow distribution ranges of the phyllopods reflect a real phenomenon, or whether they are an artefact of patchy sampling. The situation is different with the ostracods and the cladocerans. Approximately 40 species of each have been recorded from the region. The cladocerans are mostly very widespread, many seeming to occur throughout the area in both temporary and perennial wetlands, including those in the extreme south-west. A third pattern is seen in the ostracods: some species are widespread and some are fairly local endemics in Namibia or the south-western Cape. Both of these areas have been fairly well sampled.

2.4 Resting stages

Permanent inhabitants of temporary wetlands are able to survive in these systems because during the dry period they form propagules (resting stages) that hatch and re-colonize the habitat when favourable conditions return (Brendonck *et al.,* 1998). The resting stages of phyllopods are second-instar larvae encased in a desiccation resistant, protective cyst, which looks like and is commonly called an egg. Turbellarians have dormant eggs, resistant cysts containing young larvae, or cocoons whilst ostracods and cladocerans have quiescent or resting eggs, and copepods have diapausing eggs (Williams, 1998).

The resting stages form an “egg bank” which, like a “seedbank”, can act as a buffer against environmental variability, including unsuitable physical and biological conditions (Brendonck *et al.,* 1998; Brendonck and Riddoch, 2000; Brendonck and de Meester, 2003). Thus egg banks determine the “potential biodiversity and the ecological and evolutionary dynamics” of the assemblages of temporary wetlands (Brendonck and Williams, 2000). Only a portion of the phyllopod resting stages hatches at each inundation (Brendonck, 1996; Davies and Day, 1998; Brendonck and Riddoch, 2000), a phenomenon that has been likened to ‘bet-hedging’, and seems to be an adaptation to the uncertain length of inundation of the pond (i.e. varying hydroperiod). If the entire batch of resting eggs in the egg bank hatches at first inundation, and the pond does not last long enough for them to grow to maturity and themselves lay eggs, then the entire population will die out. It seems that on each inundation only a proportion of the eggs hatch, so it is likely that on at least some occasions the pond will last longer than the life cycle of its inhabitants, who will be able to produce the eggs necessary for the next generation. If there is insufficient water in the wetland for them to grow to adulthood and complete their life cycles for several cycles in a row, the species will die out in that particular pond.

2.5 Hatching

Resting propagules hatch in response to environmental conditions favourable for growth and reproduction (Brendonck, 1996; Brendonck *et al.,* 1998). The conditions required for the resting eggs to hatch are species-specific (Brendonck and de Meester, 2003, Vandekerkhove *et al.,* 2005a) and include different day lengths and intensities of light, the presence or absence of oxygen and carbon dioxide, and salinity.

The following section outlines techniques that have been developed to allow the artificial hatching of resting stages under laboratory conditions.

2.6 *In vitro* hatching of resting stages

Various techniques have been used over many years to hatch resting eggs and to germinate dormant seeds from dry wetland soils. Although one study (Skinner *et al.,* 2001) attempted to use propagules as indicators of wetland environmental condition in Australia, most studies have been confined to biodiversity aspects. For instance, many early crustacean biologists obtained material for study by asking their overseas colleagues to collect dry mud from known wetlands. From this mud they would culture whole populations of various species, which they would then describe. Many of the early records of South African copepods, branchiopods and ostracods are based on specimens hatched from dry mud transported from the Cape to northern Europe. The researcher GO Sars (1916; 1924; 1927) described tens of species in this way. Ironically, some of the wetlands representing his type localities have been obliterated in the intervening years and some of the species described then may have become extinct without ever having been seen in, or collected again from, their native habitats.

Similar techniques have been used quite widely over the years. For instance, Boulton and Lloyd (1992) examined the invertebrates of the Murray River floodplain in Australia by inundating sediments in the laboratory. More recently, Luc Brendonck and his group at the University of Leuven in Belgium have pioneered the use of similar but more sophisticated techniques for a variety of purposes, most particularly for assessing biodiversity (e.g. Vandekerkhove *et al.,* 2005b; 2005c). Their work is particularly useful because it describes culturing methods for obtaining optimal hatching success. These include suitable culture media (Kluttgen *et al.,* 1994); food (Coutteau *et al.,* 1992); photoperiod and temperature (e.g. Vandekerkhove, 2005a); and storage conditions (Centeno *et al.,* 1993). Vandekerkhove *et al.* (2004b) report on methods for isolating propagules from surrounding dry mud, because some do not hatch when inundated if covered in sediment. Furthermore, Vandekerkhove *et al.* (2004a) provide a photographic key to the ephippia (egg cases) of European cladocerans.

Using these techniques, members of Brendonck's team has investigated the population and community structure of anostracans in temporary wetlands in Botswana. Among other things, the number of eggs found in the egg banks is astonishing: Brendonck and Riddoch (1997; 2000), for instance, found egg densities up to 220 000/m^2 for

Branchipodopsis wolfi in Botswanan rock pools. More recently Hulsmans *et al.* (2006), investigating two species of anostracan (*Phallocryptus spinosa* and *Branchinella ornata*) in Makgadikgadi Pan, also in Botswana, estimated egg densities of each species of up to 50 000/m^2 and to a depth of 130 mm into the sediment. These authors suggest that the depth to which the eggs were found implies that they have been using the pan for as long as it has taken for this depth of sediment to accumulate over them (possibly thousands of years). While we do not dispute the length of time during which the species have inhabited the pan, an alternative explanation for the depth to which the eggs are buried is simply that the sediments are regularly perturbed by the feet of birds visiting the pan when it is inundated with water. This sort of information ultimately assists in our understanding of the biology of the group (e.g. Brendonck, 1996; Brendonck and de Meester, 2003).

Hatching of resting stages *in vitro* may be achieved by incubation of sediments or of isolated propagules under standardized conditions (Vandekerkhove *et al.,* 2004b). Vandekerkhove *et al.* (2004b) found that hatching success was higher when the resting eggs were isolated from the sediment in which they had been collected, and these authors described methods for doing so. Propagules are tiny and can therefore be easily covered by sediment, so isolation is required in the case of propagules that do not hatch when covered with sediment – for example if they need light and high oxygen levels for hatching (Brendonck, 1996). Isolation may also be needed to "concentrate" eggs when there are few eggs present and sediment volumes are large.

The ease with which one can induce propagules to hatch depends on whether dormancy is generated from within (in which case it is referred to as diapause) or as a result of external conditions (in which case it is referred to as quiescence; Brendonck, 1996). In the case of quiescence, hatching will be stimulated by favourable external conditions, so the use of "quiescent" cysts rather than resting eggs is an advantage as no diapause-deactivating techniques are needed for hatching: the cysts simply hatch on hydration (Brendonck *et al.,* 1993). Inducing diapausing eggs to hatch requires a much greater understanding of their biology and the cues required to break diapause. This difference may explain some of the results described below, where certain diapausing taxa (copepods in particular) were very seldom found in our experimental tanks even though they occurred in large numbers live in the same wetlands from which the sediments were collected.

If eggs have been isolated from the surrounding sediment, a culture medium is required to supply the nutrients that have been removed with the removal of the sediment. Kluttgen *et al.* (1994) describe the use of ADAM, an 'artificial fresh water', for the culture of zooplankton. It may also be necessary to provide a food supplement if insufficient algae grow in the hatching tanks under laboratory conditions. Coutteau *et al.* (1992) found that yeast can be used as an algal substitute, but only 75% of the algae can be replaced with yeast for maximal hatching to occur.

Suitable conditions are necessary for storing the sediments that contain resting eggs. For the resting eggs of *Streptocephalus proboscideus* (an anostracan from Botswana), Centeno *et al.* (1993) found that hatching was not significantly affected by different humidity conditions or storage for long periods of time, but they did emphasize that eggs should be stored dry and at room temperature to ensure viability.

Hatching of propagules is light and temperature dependent (Brendonck and Riddoch, 2000; Vandekerkhove *et al.,* 2005a). Vandekerkhove *et al.* (2005a) found that a temperature of 15ºC allowed the greatest proportion of cladoceran resting eggs from Denmark, Belgium and Spain, to hatch. Brendonck *et al.* (1998) hatched anostracans from Botswana under different temperature, conductivity and photoperiod conditions. They found that hatching was stimulated by light, and maximum hatching also occurred at 15°C.

Salt enters wetland systems from the atmosphere, from erosion of sediments and in saline groundwater (Nielsen *et al.,* 2003). In temporary wetlands, salinity can increase with the evaporation of water during the dry period but will decrease when rain dilutes the salts. Nielsen *et al.* (2003) found that salinities between 1000 and 5000 mg/L decreased species richness and abundance of organisms in both a temporary and a semi-permanent wetland in Australia. They concluded that increasing salinity in wetlands would result in a loss of biodiversity. Salinity can affect the hatching of individual species from resting eggs. For example the brine shrimp, *Artemia* (Crustacea: Class Branchiopoda: Order Anostraca), can hatch only in saline waters, whereas some other taxa (which can survive brackish water as adults) can only hatch in very fresh water (JA Day, pers. obs.).

It is worth noting that the techniques used in the present project can be used for assessing the biodiversity, the extent and the biotic integrity of temporary wetlands.

Vandekerkhove *et al.* (2005c), for instance, comment that investigations of dormant propagule banks in Europe have already uncovered ‘hidden’ species that might normally emerge only intermittently in seasons with particularly favourable conditions. Euliss *et al.* (2002) have used the presence of invertebrate remains to delineate the extent of seasonal and temporary wetlands in the Prairie Pothole region of the USA, and Angeler and Garcia (2005, and references therein) discuss the advantages and disadvantages of using plant and animal propagules for assessing the “ecological integrity” of wetlands.

The following section of this report outlines an approach to hatching out resting stages of temporary wetland inhabitants in the laboratory as part of the current WHI programme. Its application in wetland characterization, biodiversity and so-called “wetland health” assessments is also discussed.

3. DEVELOPMENT OF AN APPROACH FOR USING INVERTEBRATES AS INDICATORS OF TEMPORARY WETLANDS

From Section 2 it is clear that various invertebrates are able to survive as desiccated propagules in the dry sediments of temporary wetlands, and that it is possible to hatch these animals by inundating sediments from such wetlands in the laboratory. Since wetland invertebrates will be present only in wetland soils, this phenomenon offers a method for assessing the presence or absence of wetland conditions at a site from which propagule-containing sediments have been taken. An examination of the particular species that hatch may also provide information on the environmental condition of the site concerned. This section describes first the development of standard laboratory methods for hatching invertebrate propagules from experimental sediments, and then details the results and implications for wetland assessment of inundation experiments, based on the use of these standard methods.

The term 'propagule' refers to any life stage that will propagate itself and thus form part of the next generation. The propagules of the invertebrates under discussion are distinguished by being able to resist desiccation. The 'eggs' of the phyllopods, in turn, are in fact metanauplius larvae encased in desiccation-resistant cysts. For the sake of convenience, in the rest of this document we use the word 'egg' or 'resting egg' as a vernacular term for the more correct, but more clumsy, terms 'propagule' and 'cyst'.

3.1 Development of laboratory methods for hatching invertebrates

The laboratory methods described here were developed by a member of the project team (AK) during a research project for an Honours degree in Freshwater Biology from the University of Cape Town, and are reported in full in Ketley (2007).

3.1.1 Study Site

Sediment samples needed for the development of laboratory methods were collected from a known temporary cryptic wetland within an area known as "Dreamworld" on the Cape Flats in the Western Cape of South Africa (S 34°02'05.8"/E 18°43'16.9"). This site, code-named DW, is described in more detail in Section 3.2.

The Western Cape lies within the winter rainfall region of South Africa. Geologically, the wetland is situated on deep, old marine sands at the interface between Table Mountain Sandstones and Malmesbury Shales. At the time that soil samples were collected, the grey sediments of the sampled area were dry and cracked, with the dry remains of large ostracods (*Megalocypris princeps*) present on the surface. The wetland forms part of a broad mosaic of pans on the Kuils River floodplain. It is fed seasonally by rainfall, which collects in the shallow sands overlying expanses of impervious calcrete. In places, this perched water table reaches the surface, forming broad expanses of shallow wetland pools. The wetland is also fed irregularly by flood waters from the Kuils River. The site

seems to have been little affected by human activities even though it is situated in a peri-urban area.

3.1.2 Methods for incubating and hatching crustacean resting eggs

3.1.2.1 Isolation of resting eggs

If the density of eggs in the sediment is low, then large quantities of sediment need to be inundated in order to obtain sufficient hatchlings for the experiments to be statistically valid. Under these circumstances it is necessary to separate the eggs from the sediment before they are inundated. This is a tedious process, but may sometimes be necessary. The technique for separating resting eggs was therefore tested and a comparison made between the numbers of hatchlings produced from isolated resting eggs, and the number produced from resting eggs left in sediment, in order to identify which method would be more suitable for further experiments.

Preparation of ADAM medium (Kluttgen *et al.,* 1994)

ADAM (also referred to as 'artificial fresh water': Vandekerkhove *et al.,* 2004b) is a medium used for hatching branchiopod eggs. It is prepared with 1.665 g sea salt, 11.6 ml $CaCl_2$ stock solution and 11.1 ml $NaHCO_3$ stock solution added to 5L of distilled water. The stock solutions were 117.6 g/L $CaCl_2*2H_2O$ and 25.25 g/L $NaHCO_3$ prepared with distilled water and stored at 4ºC. Freshly prepared ADAM medium was aerated for at least one hour before being used.

Isolation process

Resting eggs were isolated from the sediment using the Onbe-Marcus method (Vandekerkhove *et al.,* 2004b). Four replicates, each comprising 8 g of dry sediment, were sonicated for 30 seconds in a UNC 5 sonicator then filtered dry through a 62µm plastic mesh. The material caught in the mesh was then centrifuged in a 1 g/ml sucrose solution at 3000rpm for 3 minutes in a Beckman CS-6 Centrifuge. The supernatant was washed through a 62µm plastic mesh using ADAM medium, allowing the collection of resting eggs in the mesh.

Comparison of hatching success of isolated and non-isolated resting eggs

Eggs that were isolated as outlined above were incubated in small plastic tubs of dimensions 160 mm x 105 mm and 65 mm deep, which were inundated with ADAM medium to a depth of 20 mm. Non-isolated eggs were retained in a core of sediment

(approximately 80 mm diameter x 100 mm deep) taken during December 2006, dried at room temperature and well mixed. Five 8 g samples of this sediment were placed in separate plastic tubs of the same dimensions as those used for isolated egg samples. All samples were placed in a temperature-controlled room in constant light at a temperature of 15°C.

Statistics

The distribution of the numbers of hatchlings per sample was not normal and so the non-parametric Mann-Whitney U test (Zar, 1999, run on STATISTICA version 7) was used to test for significant differences between the maximum numbers of hatchlings from the isolated eggs and from those that had not been isolated from the sediment.

3.1.2.2 Ascertaining suitable conditions of light, temperature and salinity

Twenty core samples, 100 mm deep, were taken from an area within a radius of approximately 20 m from what was estimated as being the deepest point of the Dreamworld wetland when inundated, and pooled. The samples were stored under dry conditions at room temperature for three weeks to allow the sediment to dry out completely.

For each experiment, ten replicates of 25 g of dry sediment were placed in small plastic tubs of dimensions 160 mm x 105 mm and 65 mm deep, and inundated with deionized water (conductivity <1µS/cm) to a depth of 20 mm. Three experimental conditions were tested: light, temperature and salinity. When any one of the three experimental conditions was tested, the other two were kept constant.

The total number of hatchlings in each tub was counted every day over a 25 day period. After the first 15 days at a temperature of 15°C, they were moved to a room kept at 25°C to allow them to grow to a size at which they could be identified to species level. Statistical tests were all performed only on the data for the first 15 days in each case. While the hatchlings were growing, new hatchlings were also counted daily. At the end of the experiment (i.e. after 25 days), all the animals were collected, preserved in alcohol and identified.

Light, temperature and salinity

- *Light:* Two light regimes were chosen: a 'diurnal' cycle of 12 hours of light followed by 12 hours of darkness, and constant light. Dark cycles were created by covering containers with aluminium foil to omit light. Temperature was kept constant at 15ºC and salinity was set at the salinity of deionized water.
- *Temperature:* Experiments were run at 10, 15 and 25ºC with constant light, and with salinity kept constant with deionized water.
- *Salinity:* Salinities of 0, 100, 500, 1000 and 5000 mg/L were achieved by dissolving sea salt in deionized water. The conductivities of these solutions were 0, 0.2, 1.2, 2.4 and 10.5µS/cm respectively. Sea salt was used since its major ions are present in the same proportions as those of most wetlands of the Western Cape (Day and King, 1995). Light was constant and the temperature was kept at 15ºC.

Statistical analyses

For the light experiment, where there were only two levels (24 and 12 hours of light), the non-parametric Mann-Whitney U test was used to test for differences between treatments. For temperature and salinity experiments, where there were more than two levels, the Kruskal-Wallis and *post-hoc* tests were used (Zar, 1999), with the *post-hoc* test identifying the levels that contributed to the significant differences.

3.1.3 Results

A total of five species of crustaceans hatched from the samples: three species of ostracods, one conchostracan and one cladoceran. A few specimens of a species of a turbellarian platyhelminth, possibly of the genus *Mesostoma*, were also found. A few seedlings germinated but did not reach a size adequate for identification. Appendix 3 provides descriptions of some of the life history characteristics of the major groups of crustaceans that hatched.

3.1.3.1 Comparison between isolated and non-isolated resting eggs

On average, fewer eggs hatched from samples that had been separated from the sediment than from those that had not (Figure 3.1), and hatchlings from eggs in sediment survived for longer than those from isolated eggs. A significantly greater maximum number of eggs hatched from resting eggs in sediment than from isolated eggs ($U = 2$, $p = 0.05$, $n1 = 5$ and $n2 = 4$).

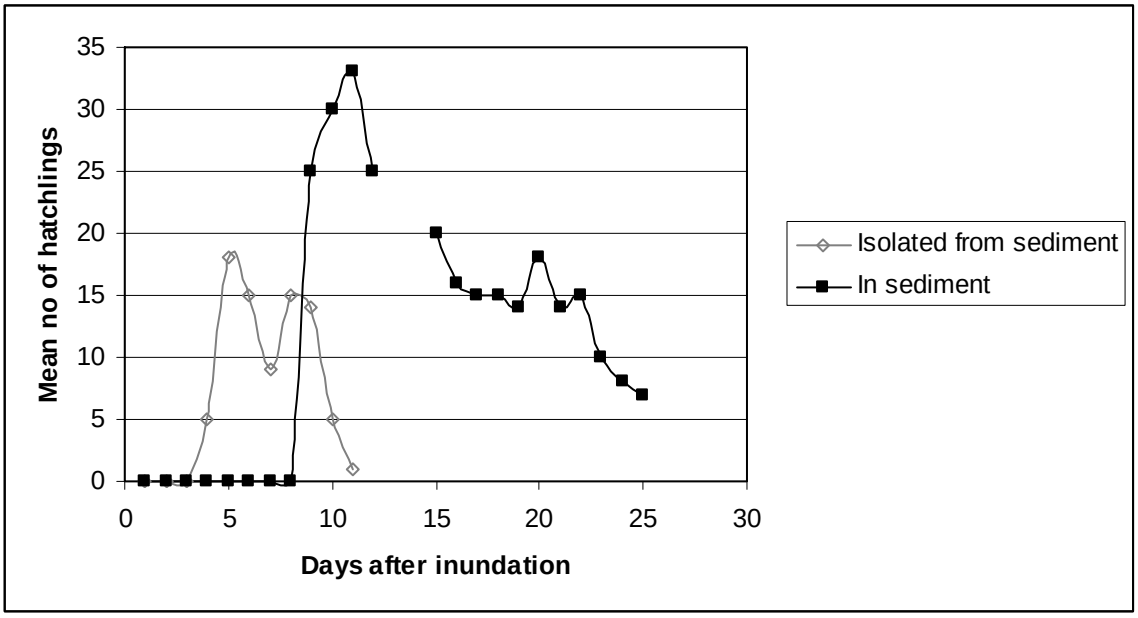

Figure 3.1: The mean number of hatchlings counted each day over a 25 day period from resting eggs in sediment (n = 5) and isolated from sediment n = 4). Incubation temperature = 15ºC for days 1-15 and 25ºC for days 16-25.

3.1.3.2 The effect of light on hatching success

Numbers of hatchlings followed the same pattern for both the 24 and the 12 hour light conditions (Figure 3.2). The larvae began hatching on day 3 after inundation and maximal numbers were reached between days 6 and 8, after which the numbers started to decrease. There was a significant difference in the day on which the maximum number of hatchlings was counted (U = 22.5, p = 0.038, n1 = 10 and n2 = 10), the mean calculated as 6.5 days in constant light and 7.4 days under a 12 hour light regime. The mean maximum number of hatchlings over the 15 day period was slightly greater for those kept in constant light (mean maximum number of hatchlings = 52.4) than for those kept under the 12 hour light regime (44.4) but the difference in maximum number of hatchlings was not significant (U = 30.5, p = 0.14, n1 = 10 and n2 = 10).

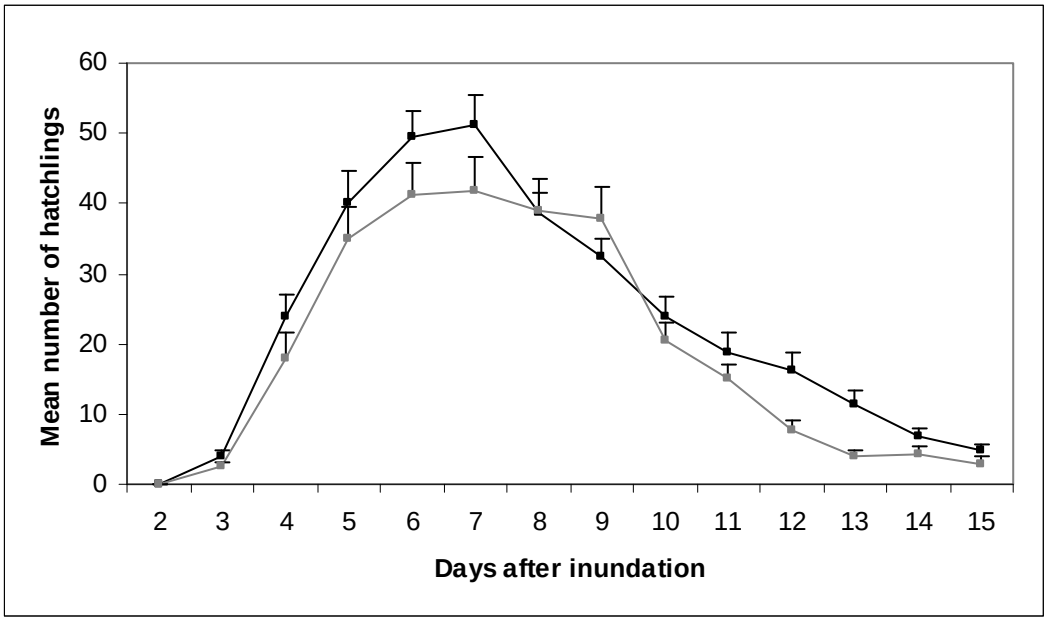

Figure 3.2: The mean number of hatchlings counted each day over the 15 day period, for the constant light conditions (black) and 12 hour light conditions (grey; n = 10 for each data set).

3.1.3.3 Temperature

The numbers of individuals hatching did not follow the same pattern for the three temperature conditions (10, 15 and 25°C; Figure 3.3). At 10°C, hatching began only on day 8 after inundation, the number of hatchlings then increased until day 14 or 15, and then decreased again. At both 15°C and 25°C, hatching began on day 3 after inundation and the number of hatchlings increased until a maximum was reached between days 6 and 8, after which the numbers started to decrease.

There was a significant difference in the day on which the maximum number of hatchlings was counted for the three different temperature conditions ($H = 21.94$, $df = 2$, $p < 0.0001$). The *post-hoc* test showed that the difference was significant for the 10°C temperature condition compared to the 15°C ($p = 0.00003$) and 25°C ($p = 0.0045$) samples, but not the days of maximum hatching for temperatures of 15°C and 25°C ($p = 0.61$).

The mean maximum numbers of individuals that hatched at 10ºC, 15ºC and 25ºC were 37.1, 52.4 and 20.7 respectively, and the difference between the maximum number of hatchlings in different treatments was significant ($H = 16.06$, $df = 2$, $p = 0.0003$). The *post-hoc* test showed that the significant difference occurred between the maximum

numbers counted for the 15°C and 25°C temperature conditions only (p = 0.0002). Overall, the greatest number of individuals hatched at 15°C and the fewest at 25°C.

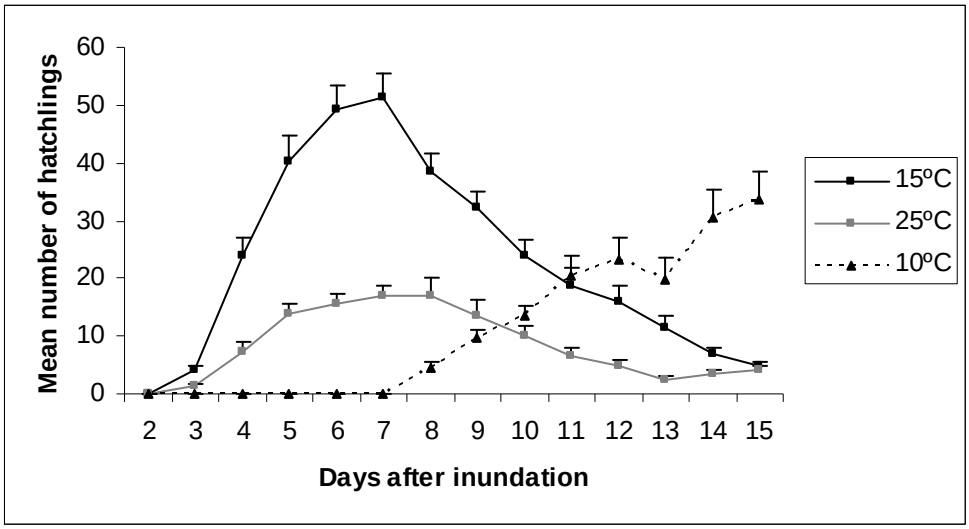

Figure 3.3: The mean number of hatchlings counted each day over the 15 day period at 10°C, 15°C and 25°C; n = 10 for each data set.

3.1.3.4 Salinity

Numbers of individuals hatching over time (Figure 3.4) followed the same pattern for the lower salinity levels (0, 100, 500 and 1000 mg/L) throughout the 15-day period, while no individuals hatched at 5000 mg/L. Hatching began on day 3 or 4 and increased until a maximum was reached between days 7 and 9, after which the numbers of new hatchlings started to decrease. Data for the 5000 mg/L salinity level were excluded from further statistical analyses.

There was a significant difference in the day on which the maximal number of hatchlings was counted for the four salinity levels (H = 16.73, df = 3, p = 0.0008). The difference was significant between the 0 mg/L and 100 mg/L salinity levels (p = 0.0008), as well as for 0 mg/L and 1000 mg/L salinity levels (p = 0.039). The fastest hatching rate occurred at the lowest salinity level, where the maximum number of hatchlings was counted first at 6.6 days after inundation. The greatest number hatching occurred later for the 100 mg/L and 1000 mg/L salinity levels at an average of 8.6 and 8 days after inundation respectively.

Little difference was apparent between mean maximum numbers of individuals that hatched at salinity levels between 0 and 1000 mg/L (Table 3.1) and there was no

significant difference between the maximum number of individuals that hatched at the four salinity levels (H = 7.55, df = 3, p = 0.0562). Thus salinity seems to have affected hatching abundance only above 1000 mg/L.

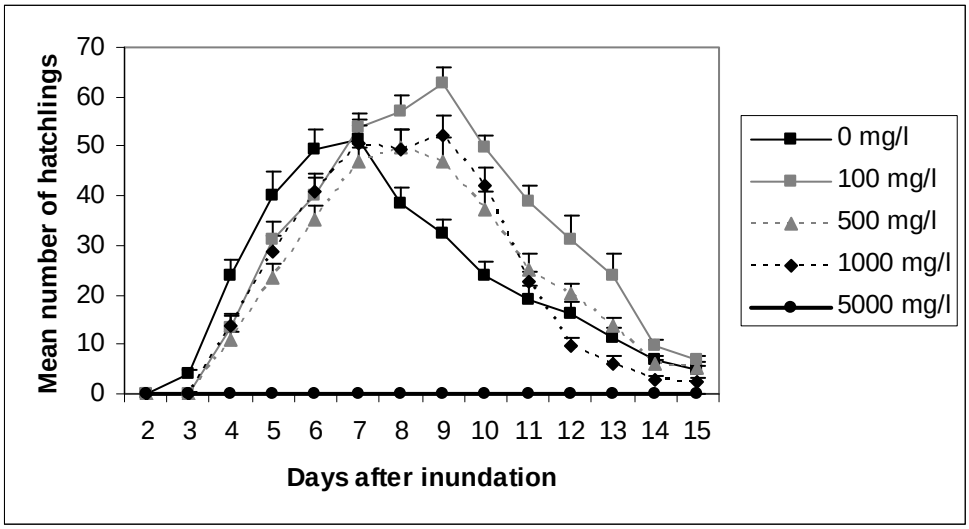

Figure 3.4: The mean number of hatchlings counted each day over the 15-day period, for the 0, 100, 500, 1000 and 5000 mg/L salinity conditions (n = 10 for each data set).

Table 3.1: The mean maximum number of hatchlings, with standard error, for the 0, 100, 500, 1000 and 5000 mg/L salinity conditions

Salinity level (mg/L)	Mean maximum number of hatchlings	Standard error
0	52.4	4.01
100	64.8	2.95
500	55.0	3.13
1000	60.8	2.90
5000	0	0

3.1.3.5 Identification of hatchlings

Different crustacean species hatched at different times and grew at different rates (Figure 3.5) and most individuals died before they grew to a size at which they could be identified. Numbers of conchostracans, which made up most of the individuals that first hatched, decreased rapidly until there were very few left. Ostracods were first recognized from day 17 after inundation and the numbers increased until the end of the experiment. Cladocerans were first recognized on day 18 after inundation, where after numbers increased until the end of the experiment. In all, only nine conchostracans (*Leptestheria*

rubidgei) grew to adulthood, compared to 35 cladocerans (*Macrothrix propinqua*) and 158 ostracods (117 *Cypricercus*, 28 *Zonocypris cordata* and 13 *Megalocypris princes*).

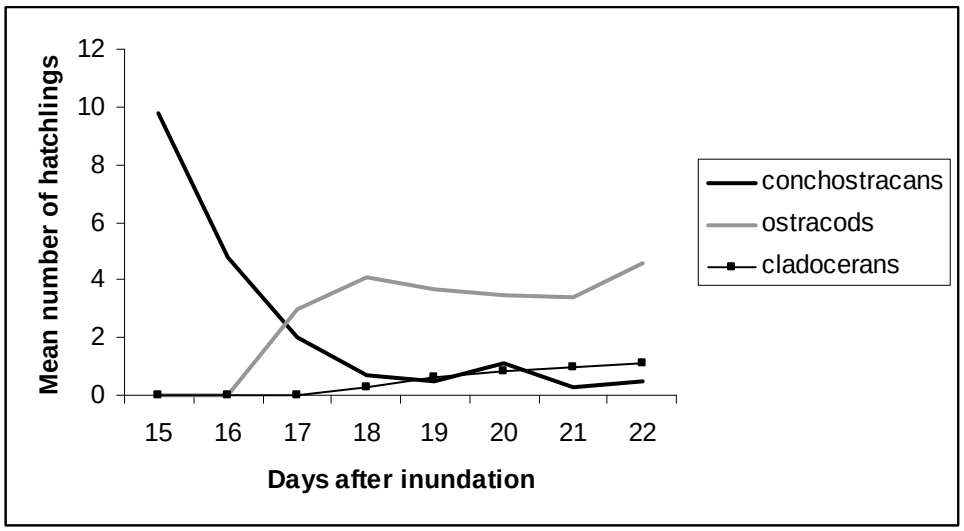

Figure 3.5: Daily counts of the mean number of hatchlings of the different taxa grown at 25ºC under conditions of constant light, for all experiments combined.

3.1.4 Discussion

3.1.4.1 Isolation of resting eggs

More hatchlings hatched from the resting eggs in sediment, and hatchlings survived for longer, than those hatching from isolated eggs. This result is different from that of Vandekerkhove *et al.* (2004b), who found isolation of resting eggs to be advantageous for overall hatching success. The numbers of eggs in our samples were far fewer than the numbers encountered by Vandekerkhove's group. It is likely that eggs are lost during the process of separating them from the sediment and where initial numbers are low, this would have a significant effect. In any event, hatching success was greater in the non-isolated samples. We therefore recommend that future experiments should be carried out without attempting to isolate the propagules from the sediment, a process that is inefficient with regard to time and cost, given the small volumes of sediment used. In situations where insufficient numbers of propagules are obtained from the sediment, it may be necessary to isolate resting eggs from larger volumes of sediment than recommended in the standard protocol described here.

The hatchlings from the isolated resting eggs also died off fairly quickly. The concentration of Se in the ADAM medium may be important. For instance, Kluttgen *et al.*

(1994) used 0.5 ml of SeO_2 stock solution for the preparation of 5L of medium, while Ebert *et al.* (1998) suggested that a twentieth of this concentration should be used, and Vandekerkhove *et al.* (2004b) suggested that the ADAM medium itself should be diluted 1 in 5. Because of the lack of clarity with regard to these issues, SeO_2 was not used in our experiments. Regardless of the contents of the medium, it is unable to provide food for the developing hatchlings. This means that if the hatchlings are to grow to a stage at which they can satisfactorily be identified, food of some kind must be provided. This can be an artificial food such as an algal culture or yeast, but it is more satisfactory to use some of the sediment in which the eggs themselves were collected because sediment of this kind will contain the propagules of natural algal and fungal foods for the hatchlings.

3.1.4.2 Light

It has long been known (e.g. Brendonck *et al.,* 1998) that hatching of branchiopod eggs is inhibited by complete darkness. The reason may be that in a newly inundated wetland complete darkness will occur only within the sediments. Buried propagules will thus not hatch but be retained in the sediment and may hatch only during some future inundation when conditions may be more favourable.

The maximum number of hatchlings was similar in sediments kept in constant light and those kept in 12 hours light and 12 hours dark, so either regime can be used for hatching experiments. The day on which maximum hatching occurred was, however, earlier in constant light conditions. From our data we cannot say if this is because of some effect of light on the propagules themselves, or if it is merely the effect of light on the growth of algae that developed in the containers and were used as food by the developing crustaceans. For practical reasons we recommend that incubations be carried out in constant light because algae grow faster under such conditions and thus provide more food for the larvae, which will in turn grow faster.

3.1.4.3 Temperature

Both Brendonck *et al.* (1998) with Botswanan branchiopods, and Vandekerkhove *et al.* (2005a) with Belgian branchiopods, found that maximum hatching occurred at 15°C. The same was found in the present study, where most branchiopod eggs hatched at 15°C and fewest at 25°C. The Western Cape falls within a winter rainfall region, where the average maximum daily temperature during winter is between 17°C and 19°C and the average water temperature ranges between 12°C and 16°C. The resting eggs in temporary wetlands in the Western Cape are adapted to hatching during winter and one might thus

expect hatching abundances to be greatest at moderately low temperatures. Experiments should thus be carried out at 15°C if branchiopods are to be hatched.

3.1.4.4 Salinity

Nielsen *et al.* (2003) found that salinities between 1000 and 5000 mg/L decreased the species richness and abundance of organisms found in a temporary and a semi-permanent wetland in Australia. They concluded that increasing salinity in wetlands would result in a loss of biodiversity, but they did not mention what the natural salinity of the wetlands was. Similar results were found in the present study, where salinities somewhere between 1000 and 5000 mg/L reduced the proportion of eggs hatching to zero. Similar numbers of individuals hatched at each of the salinities tested between 0 and 1000 mg/L, though. For hatching experiments, therefore, it seems that the actual salinity of the medium is not important as long as it does not greatly exceed 1000 mg/L for freshwater wetlands. (Clearly the same constraint does not apply when dealing with salt pans.)

As a matter of interest, after the experiment the sediments used in the 5000 mg/L replicates were dried and re-inundated with deionized water, which diluted the salt concentration, hatchlings started to appear. The salinity effect is thus not permanent.

3.1.4.5 Identification of hatchlings

After day 15, the hatchlings were grown at 25°C and new hatchlings appeared because different species hatch at different times after first inundation. The ostracods and cladocerans only began to hatch after the samples were moved to the 25° temperature level, by which time the conchostracan larval counts were decreasing. The ostracod and cladoceran numbers increased until the end of the experiment, when they were collected and preserved for later identification.

From the hundreds of conchostracan larvae originally hatched at the start of the experiment, only nine grew to adulthood. In other experiments using larger tanks, conchostracans have survived very satisfactorily (JA Day, pers. obs.) so it may well be that insufficient food was generated for these relatively large crustaceans in the very small containers used in the present experiments.

3.1.4.6 Food source

Different foods (yeast and algae) were tested for interest's sake. There was no apparent difference in the survival of the hatchlings when different individuals were given algae or yeast, or no additional food, but it was interesting to note that the cladocerans hatched only when yeast or algae were present. Future experiments should thus be conducted using different foods if laboratory culture of temporary pool crustaceans is to be pursued.

3.1.5 Recommended methods for use in developing a dry season wetland index

As a result of the experiments described above, we performed all the experiments described in the next section using constant light, a salinity of 1000 mg/L or less, and a temperature of 15°C, which is necessary for conchostracans to hatch. Note, however, that a temperature of 25°C has been shown to produce more ostracods and cladocerans.

3.2 Artificial incubation as a tool for wetland characterization and assessment during the dry season

This section of the report investigates the feasibility of hatching invertebrate propagules, using the method outlined in Section 3.1, as a tool for confirming the presence of temporary wetlands in the dry phase, and for assessing their condition. As far as possible, we attempt to link various abiotic variables with the invertebrate assemblages that hatched during the incubation trials.

3.2.1 Selection of sites

A total of 24 seasonally inundated, permanently to seasonally saturated wetland sites (Figure 3.6 and Table 3.2) were selected for this study. Broadly speaking the sites represent two main geographic areas in the Western Cape: the west coast, and the Cape Flats (a low-lying area stretching between the mountains of the Cape Peninsula and the Cape Fold Mountains to the east). Sites were selected to coincide with those used in the vegetation (Corry, 2010) and invertebrate (Bird, 2010) components of the Wetland Health and Importance Programme, and thus were all sites for which wet season invertebrate and plant data were available. As far as possible the wetlands were chosen to represent different lengths of hydroperiod, degree of human disturbance, and underlying substrate. Site choice was also partly dictated by logistical factors such as site accessibility and budget, and the number of sites assessed was constrained by the amount of laboratory space available for incubating propagules.

3.2.2 Study sites

All of the study sites receive rain mainly in winter as a result of low pressure frontal systems or "cold fronts" carried onshore by north-westerly winds. Towards the north and

west, wetlands such as those near Piketberg tend to be fed by rainfall and to experience a drier climate and lower average rainfall than those to the south and east. In contrast, those such as the Lotus River and Mfuleni wetlands on the Cape Flats receive on average more rain than those on the West Coast and are fed mostly by ground water as the water table rises to the surface. A few Cape Flats sites comprise shallow sandy soils, perched on impermeable layers of clay or calcrete. When compared to the more arid systems on the west coast and further north, these south-western wetlands tend to retain moisture in their soils for longer and thus to experience longer hydroperiods. The perched wetlands already described and certain groundwater fed systems along the West Coast are exceptions. In addition, a few of the more permanent systems on the Cape Flats are artefacts of artificially raised water table levels resulting from urban impacts such as increased storm water runoff and sewage effluent.

In general, nutrient-poor sands derived from the Cape Granite Suite and quartzites of the Table Mountain Sandstone formation, interspersed with elements of peat and loam, constitute the underlying substrate of most of the wetlands of the Cape Flats (Norman and Whitfield, 2006), although certain sites, such as Baden Powell and Dreamworld (Table 3.2), are located on clay soils derived from Cape granites. With distance northwards and westwards from the Cape Flats, soils tend to contain higher proportions of fine sands and somewhat more nutrient-rich clays derived from the Malmesbury Group (Norman and Whitfield, 2006).

Figure 3.6: Seasonal wetland sites sampled in their dry season in March 2008. See Table 3.2 for an explanation of site code numbers.

3.2.3 Sampling protocol

3.2.3.1 Field Procedures

Dry soil samples were collected from the selected sites over a period of eight days in March 2007, towards the end of the dry season. At each site, ten soil cores, each 50 mm deep x 80 mm in diameter, were collected using a custom-built steel auger with a serrated head. Soil cores were collected from within a radius of approximately 20 m from the estimated deepest point of the wetland under inundated conditions. The ten replicate samples collected from each site were subsequently pooled and mixed, providing a single heterogeneous sample from each site. In addition to these samples, a single 100 mm deep x 80 mm diameter soil core was collected from the lowest lying and therefore potentially wettest area of each wetland for soil moisture analysis. Samples for measurement of soil moisture were stored in airtight "ziploc" bags at room temperature for approximately two days before being analysed. The remaining pooled samples, which were to be used for the incubation trials, were stored in the laboratory in open bags under dry conditions at room temperature for three to four weeks to ensure that the sediments would be dry at the start of the incubation experiments.

3.2.3.2 Laboratory procedures

Soil properties

Sealed soil samples were remixed to reconstitute evaporated moisture after which a single handful (approximately 85 grams) of sediment from each sample was placed separately in a tinfoil tray about 70 mm in diameter and weighed. Three replicate samples from each of the 25 sites were prepared in this manner. Initial wet weights were recorded, after which samples were placed in a drying oven at 60°C until constant weight was achieved. They were weighed again, and then burnt in a muffle furnace at 400°C for four hours, after which a final ash-free dry weight was obtained. The organic fraction has been expressed as a percentage of the total dry mass of the sample. The results of particle size analyses for soils from a number of the selected dry-season wetland assessment sites are provided in Appendix 4, using data from Corry (2010).

Table 3.2: Site codes and locality data for seasonal wetland sites sampled in March 2007 in the Western Cape, South Africa. Note site codes as used by Bird (2010) and Corry (2010)

Site Name	Code	GPS Coordinates		General Locality	#Estimated Water Source	Land-use Intensity	Substrate
Zeekoevlei 1	LOT 01	S 34°03'29.3	E 018°30'16.4	Cape Flats (Rondevlei)	WT/SW	Low	Sand
Zeekoevlei 2	LOT 02	S 34°03'30.6	E 018°30'12.6	Cape Flats (Rondevlei)	WT/SW	Low	Sand
Lotus River 4	LOT 04	S 34°03'14.3	E 018°30'18.9	Cape Flats (Grassy Park/Philippi)	WT/SW	High	Sand
Lotus River 5	LOT 05	S 34°02'55.2	E 018°30'37.5	Cape Flats (Grassy Park/Philippi)	WT/SW	High	Sand
Lotus River 6	LOT 06	S 34°02'17.2	E 018°32'08.3	Cape Flats (Grassy Park/Philippi)	WT/SW	High	Sand
Lotus River 11	LOT 11	S 34°04'10.4	E 018°29'52.7	Cape Flats (Grassy Park/Philippi)	WT/SW	Very Low	Sand
Kenilworth 11	KEN 11	S 33°59'39.2	E 018°29'00.8	Cape Flats (Kenilworth)	WT/SW?	Very Low	Sand
Kenilworth 13	KEN 13	S 33°59'47.0	E 018°29'05.4	Cape Flats (Kenilworth)	WT/SW?	Very Low	Sand/Clay
Baden Powell 1	BAD 01	S 34°02'16.5	E 018°43'30.0	Cape Flats (Khayelitsha)	WT/SW	Low	Sand/Clay
Dreamworld 1	DRE 01	S 34°01'50.9	E 018°43'29.6	Cape Flats (Khayelitsha)	SW/GW	High	Sand/Clay
Dreamworld 2	DRE 02	S 34°02'04.5	E 018°43'17.6	Cape Flats (Khayelitsha)	GW	High	Sand/Clay
Mfuleni 1	MFU 01	S 34°00'44.7	E 018°40'52.7	Cape Flats (Khayelitsha)	WT/SW	Moderate	Sand
Mfuleni 3	MFU 03	S 34°00'34.7	E 018°40'42.7	Cape Flats (Khayelitsha)	WT/SW	High	Sand
Koeberg 2	KOE 02	S 33°41'07.0	E 018°26'05.6	West coast (Koeberg)	SW/WT	Very Low	Sand/Calcrete
Koeberg 5	KOE 05	S 33°41'12.8	E 018°26'13.0	West coast (Koeberg)	SW/WT	Very Low	Sand/Calcrete
Sout 1	SOU 01	S 33°42'34.6	E 018°27'15.9	West coast (Koeberg)	SW/WT	Moderate	Sand/Clay

Piketberg 6	PIK 06	S 32°41'18.8	E 018°55'57.6	West coast (Piketberg)	SW/WT	High	Sand/Clay
Piketberg 11	PIK 11	S 32°38'27.2	E 018°53'27.3	West coast (Piketberg)	SW/WT	High	Sand/Clay
Darling 3	DAR 03	S 33°17'15.2	E 018°26'42.4	West coast (Darling)	SW/WT	High	Sand/Clay
Yzerfontein 2	YZE 02	S 33°20'25.5	E 018°11'01.4	West coast (Yzerfontein)	WT/SW	Low	Sand/Clay
Diep River 4	DIE 04	S 33°38'52.3	E 018°34'08.4	West coast (Malmesbury)	SW/WT	High	Sand/Clay

SW = surface water, WT = water table, GW = ground water; codes are displayed in order of greatest influence (e.g. “SW/WT” = surface water having greater influence than water table, while WT/SW = water table having greater influence than surface water). Land-use intensity refers to land-use within ~500 m of the wetland.

Invertebrate incubations

Dried soil samples collected in the field were inundated in small plastic tubs 160 x 105 x 65 mm deep. Ten replicates were prepared for each site. 25 g of mixed, dried sediment was placed in each tub and inundated with de-ionised water (conductivity <10µS/m) to a depth of approximately 20 mm. Inundated tubs were incubated at 15°C with permanent lighting and water levels were maintained at approximately 20 mm depth. Although Ketley (2007), as described in Section 3.1, developed the hatching and incubation methodology on the basis of 25 day trials, a more conservative approach was taken in the present study, and a total incubation period of 40 days was allowed for.

In addition to the standard incubation trials, a simple test was performed to evaluate the effect of drying of soil samples on hatched invertebrate assemblages. A double set of soils from a single site (LOT01) were collected. One set was treated as outlined above, and dried for 30 days. The second set was inundated immediately on collection – that is, without a drying period. This site is believed to have been affected by an artificially raised water table, resulting in prolonged damp to saturated conditions during the dry season.

Identification of hatched invertebrates

Cladocerans hatch in the form of miniature adults, which can be recognized immediately as cladocerans. Ostracods, copepods and phyllopods, on the other hand, hatch as minute, 6-limbed nauplius larvae, which are not distinguishable from each other. It is therefore necessary to wait for several days for characters to appear that allow identification even of Order, while identification to species level usually requires the presence of adult males or females, depending on the taxon. For the purposes of this study, visual (naked eye) identifications were carried out daily, with hatchlings being identified only to Class or Order (Cladocera, Copepoda, Ostracoda, Anostraca, Conchostraca), while species level identification of voucher specimens took place after the hatching period (~40 days). The total number of hatchlings in each tub was counted every day over a 30 day period. Following development from the naupliar stages, organisms were identified *in situ* as far as possible and numbers of each taxon recorded. Hatching data were summarized over time, with the mean maximum number of all hatchlings as well as the mean maximum number for each individual taxon (Anostraca, Cladocera, Conchostraca, Copepoda and Ostracoda) recorded for each wetland. The number of days from inundation to emergence of the first hatchlings was also noted. For convenience, after the 30 day test period the surviving animals from each wetland were placed together in a small tank at 25°C to allow more rapid growth to adulthood so that individuals could be identified to species level. (While 15°C is the optimal temperature for

hatching branchiopods, all of the taxa grow much more quickly at higher temperatures.) Ethanol-preserved voucher specimens were kept for future reference.

Water chemistry

Mean values for electrical conductivity and pH in the laboratory were calculated from three replicates taken from each sample in order to assess differences between values *in situ* and in the laboratory, with *in situ* values being based on once-off conductivity, pH, nutrients, dissolved oxygen, temperature and turbidity data recorded from the "open water" habitat at each site during wet season sampling of the invertebrates of these wetlands. Details of the methods used are available in Bird (2010). It is recognized that once-off data do not provide information on seasonal differences in physical and chemical variables that result from dilution, evapo-concentration and/or biological processes such as photosynthesis, respiration or decomposition. Such data are useful, however, for comparing wet season conditions with those in the laboratory experiments reported here.

3.2.3.3 Data Analysis

Hatching data were analyzed using multivariate analyses in the form of a simple community analysis procedure in the statistical package PRIMER v6 (Clarke and Gorley, 2006). Non-parametric multidimensional scaling (MDS) as well as cluster analyses were used to explore differences between sites based on the suite of species hatching during the incubation experiments. For these analyses, the data were square-root transformed and compared using the Bray-Curtis resemblance measure. Certain variables such as soil moisture and organic content were then overlaid to examine trends between biotic and abiotic components among sites.

Pearson correlations and canonical correspondence analyses (CCA) were performed in ECOM v. 1.37 (Pisces Conservation Ltd., 2000) to explore links between all environmental variables (e.g. pH, conductivity, turbidity, soil moisture) and hatching data. CCA is a multivariate technique that ordinates sites or samples in terms of their biological components in relation to the influence of various environmental variables. Variables identified as having appreciable multicollinearity ($r \geq 0.8$) were removed from the CCA and analysed separately in order to eliminate as far as possible the effect of each non-biological variable on each of the others. Sites for which there are either no hatching data or no environmental data were excluded from the CCA. Significance of the CCA Eigen values obtained was calculated using a Monte Carlo randomization test, set at 1000 simulations. This test gives the probability that the observed magnitude of obtained Eigen values was generated by chance.

Following preliminary analyses, the variables that were found best to explain the variance in community structure were analyzed using the BIOENV test in PRIMER 6. BIOENV is a multivariate technique that examines the relationship (reported as Spearman Rank Correlations) between a set of unrelated environmental variables and biological data. Environmental data were first normalized, given the variety of measurement scales across the measured variables and then the Euclidean distance measure was used to create a resemblance matrix of the environmental data. Essentially, this measure quantifies the difference between sample sets in terms of distance in multivariate space. A strong similarity between sets relates to a low Euclidean distance and vice versa. The BIOENV analysis relates this resemblance matrix comprising environmental data to a second, fixed, resemblance matrix comprising biological data (Bray-Curtis similarity measure; Clarke and Warwick, 2001). The individual environmental variables as well as combinations of variables that best explain the variability in the biological data are returned.

In order to gauge similarities in invertebrate assemblages among sites between the wet and dry seasons, hatching data from dry season sediment samples were compared to wet season invertebrate data at ordinal level using a presence/absence transformation. Using the Bray-Curtis similarity measure, a resemblance matrix was then formed which provided the basis for a cluster analysis and MDS plot using PRIMER 6.

3.2.4 Results and discussion

3.2.4.1 Soil Moisture

Table 3.3 lists the percentage soil moisture and the percentage organic content of soils collected in the dry summer from each of the wetlands. In general, soils from sites on the west coast contained less soil moisture than those on the Cape Flats. The west coast is thus assumed (albeit on the basis of limited data) to represent wetlands with shorter hydroperiods than those on the Cape Flats (Box 1). Soils from Dreamworld and Baden Powell on the Cape Flats were exceptions, however, in that they contained very little soil moisture. Wetlands at these sites are known, however, to be perched on an impervious calcrete layer, rather than being groundwater dependent. Both are seasonally fed by groundwater and irregularly by flood waters from the Kuils River.

Sites containing high proportions of clay and silt, and low organic content, generally retained less soil moisture than those with sandy soils (Figure 3.7). The relationship

between turbidity, soil moisture and clay content is discussed in more detail in Section 3.2.4.4.

Table 3.3: Mean percentage soil moisture and percentage organic matter of soils collected in the dry summer from each of the wetlands (n = 3)

Site	Mean summer soil moisture (%)	Mean organic content (g/kg)
BAD 01	0.974	20.117
PIK 11	1.128	31.811
DRE 02	1.411	6.332
YZE 02	1.593	50.915
DAR 03	1.728	56.833
DRE 01	2.060	51.713
KOE 05	2.758	14.432
DIE 04	4.413	67.895
KOE 02	5.064	20.503
SOU 01	12.677	18.445
PIK 06	14.282	47.882
LOT 2B	14.632	13.049
LOT 06	15.337	38.512
KEN 13	17.072	41.463
LOT 04	18.717	23.385
MFU 03	20.820	27.513
MFU 01	25.922	21.253
KEN 11	26.448	59.416
LOT 05	27.891	114.005
LOT 11	30.402	54.237
LOT 01	32.448	112.281
LOT 2A	42.742	114.673

Box 1: Geology and organic content

Sandy soils, such as those found on the Cape Flats and derived from the Table Mountain Sandstone formation, are porous and have good drainage. These factors promote the growth of roots, in turn providing a suitable medium in which a wide range of plant species can grow. As a result these soils often contain high levels of organic matter, in the form of living or decayed plant matter, or peat.

By contrast, clay-rich soils found in some areas of the west coast are derived from granites or Malmesbury Shales. They are characteristically poorly drained as a result of their low porosity. These soils retain water in the upper layers for longer periods than sandy soils. This in turn makes them unsuitable soils for many plants species, as the roots of the plants cannot easily grow in waterlogged conditions. Clay soils therefore tend to have low levels of organic matter. Details from Norman and Whitfield (2006).

An analysis of dry soil samples revealed that soil moisture was positively correlated with soil organic content ($r = 0.59$; $p < 0.05$), supporting the theory that organic matter assists in retaining moisture in the soil during the dry season and in some cases prevents the soil from drying out completely. This in turn might prevent the total desiccation required by certain branchiopod cysts (e.g. anostracans) for successful hatching, thereby providing unsuitable conditions for survival. Conversely, a higher organic content, often coincident with increased primary productivity, might favour the presence of other taxa such as ostracods.

While soil moisture and soil organic content are clearly not the only factors affecting the survival of branchiopods, this example highlights the potential importance of just two easily measurable abiotic variables in controlling the species composition of wetland invertebrate communities.

3.2.4.2 Incubation experiments

Water chemistry

No significant differences were noted between measurements of pH and conductivity *in situ* and in the laboratory (t-test, $n = 10$, $df = 9$; $p = 0.7$ and $p = 0.1$, respectively). While these differences were not significant, they were sometimes still large (up to 1.5 pH units and up to 1000 mS/m higher in the field than recorded in incubation experiments).

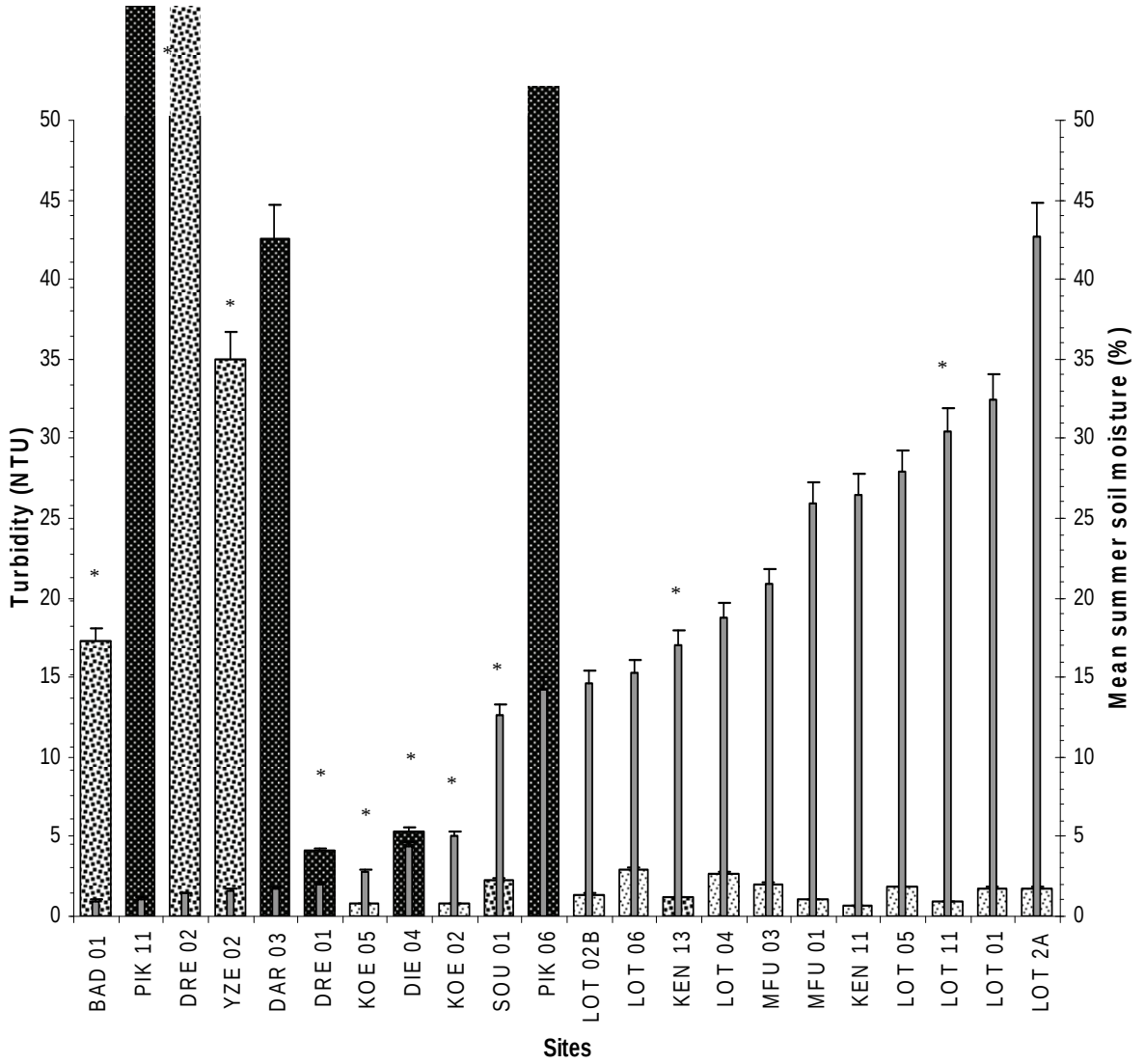

Figure 3.7: Mean soil moisture in the dry season (thin grey bars) and turbidity in the wet season (thick bars) shown in relation to clay content (shading) for seasonal wetland sites sampled during March 2008. Dark shading indicates sites with highest relative proportions of clay, medium shading indicates sites with a soil/clay mix while light shading indicates sites with highest relative proportions of sand. Asterisks (*) denote sites for which mechanical soil analyses are available. Turbidity values were out of range for the following sites: PIK 11 (706 NTU), DRE 02 (130 NTU) and PIK 06 (52 NTU).

Hatching of individual taxa

All but one of the sites (PIK 11) yielded invertebrate hatchlings and the data revealed successional trends, examples of which are shown in Figure 3.8, using data from the inundated soil samples of a wetland site located near Koeberg Power Station on the West Coast (KOE 02). Clearly evident from this figure is the peak in the mean maximum number of hatchlings of each taxon occurring between the 19th and 27th days after inundation. This trend was exhibited in approximately half of the inundated samples. In the others (DRE 01, DRE 02, PIK 06, BAD 01, MFU 03, LOT 01, DIE 04, DAR 03,

LOT 06, MFU 03, LOT 05), maximum numbers of hatchlings continued to increase after 27 days.

Within the 30-40 day period over which the incubation trials were run, it was mostly crustaceans (ostracods, anostracans, cladocerans and conchostracans) that hatched, although several gastropods and a single leech were also recorded (Table 3.4). Owing to the very small numbers of gastropods and leeches obtained throughout the duration of the incubation trials, members of these two taxa were excluded from multivariate analyses. No insects hatched from any of the sites throughout the duration of the incubation trials.

Generally the anostracans were the first group of invertebrates to be identifiable to species after inundation (15 days for positive identification), with cladocerans following shortly thereafter (16 days). Ostracods and conchostracans were identifiable after about 24 days and 25 days respectively, and copepods after 26 days. These results highlight the rapid rates of development of these invertebrates that allow them to inhabit ephemeral environments.

Table 3.4: Invertebrate taxa hatching after the incubation of soil samples from the seasonal wetland sites. The number of sites in which representatives from each taxonomic group were recorded is given in parentheses

Class	Order	Sub order	Average time until recognition (days)	Earliest day of recognition	Latest day of recognition
Unidentified nauplii	Unidentified nauplii	Unidentified nauplii (n=22)	8	1	22
Branchiopoda	Anostraca (n=8)		15	2	32
Branchiopoda	Diplostraca	Conchostraca (n=8)	25	10	35
Branchiopoda	Diplostraca	Cladocera (n=21)	16	5	34
Ostracoda (n=21)	Podocopida		24	8	34
Copepoda (n=9)			26	19	37

The organisms listed in Table 3.4 in fact represent the primary component of the temporary wetland fauna of southern Africa, together with the phyllopod Order Notostraca (shield shrimps), which occur only in very seasonal wetlands that dry out completely (Davies and Day, 1998), and have not been recorded from the south-western Cape (Rayner, 2000). As such, these results are particularly useful in providing a basic time frame estimate for the positive identification of a seasonally inundated wetland that dries out in the in the dry season. In essence, based on the figures of earliest recognition of these taxa (Table 3.4), and noting that hatching of copepods takes longer than hatching of other groups, it could potentially take between 19 and 37 days to positively identify a suite of invertebrate fauna found exclusively in temporary wetlands.

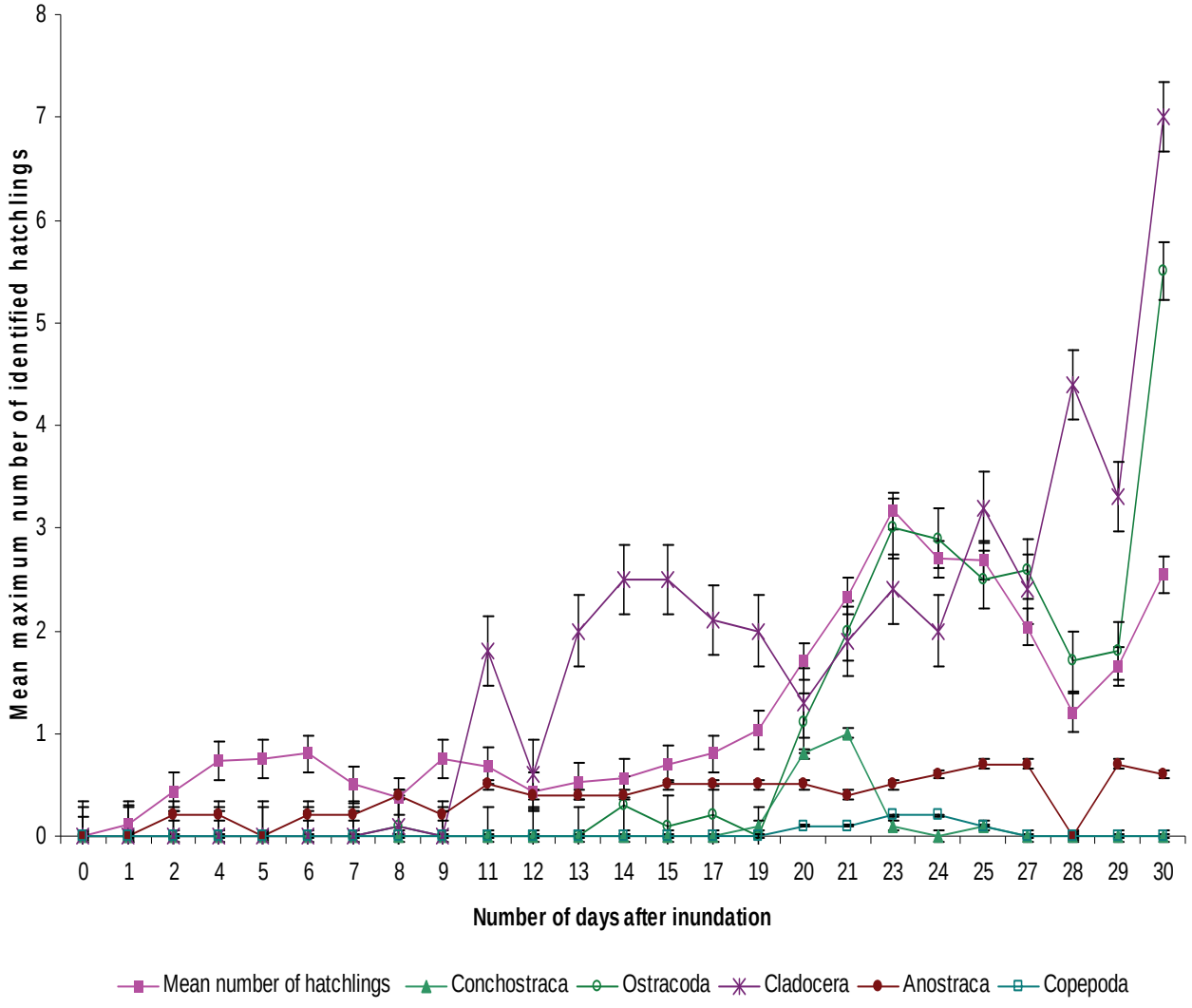

Figure 3.8: Typical successional trends observed in invertebrate hatching trials: mean maximum number of hatchlings, and numbers for each taxon (± std. error) for site KOE 02.

The successional trends observed in the hatching experiments described here showed contrasting results to those obtained in a study by Quintana *et al.* (2006), working on temporary wetlands in Spain. Their results revealed that ostracods were the dominant heleoplankton (littoral fauna that swim free near the edges of water bodies) fauna shortly after inundation. If a high water turnover rate (water flow over and through a wetland), normally experienced only during the filling stage of a wetland, was maintained for approximately three weeks, however, ostracods were replaced by copepods and cladocerans. As water turnover rate was reduced towards the end of the hydroperiod, cladocerans and copepods tended to maintain their dominance. For the most part, our results showed that cladocerans were the dominant organisms following inundation, with ostracods becoming dominant after 25 to 28 days. While overall numbers of copepods in the experiments were low, their numbers generally increased towards the end of the experiments.

In addition to the standard incubation trials, a simple test was performed to evaluate the effect of drying of soil samples on hatched invertebrate communities collected from site LOT 01, one of the more permanently saturated wetland sites, which we presumed to have a long hydroperiod. It was observed that when soil samples had been dried for 30 days, unidentified nauplii appeared 15 days subsequent to inundation, while ostracods and cladocerans were visible after 32 days and copepods after 35 days. By contrast, incubation of undried soil revealed the presence of unidentified nauplii after just 12 days and cladocerans after 17 days, although copepods and ostracods were absent from the sample for the entire duration of the incubation trial. Overall, diversity and abundance of taxa were higher in the dried sample, but a longer time period was required for positive identification of taxa. These results might reflect the emergence of different generations, or specific crustacean groups that require desiccation for hatching. Alternatively, different species might be adapted to different hydroperiods and therefore exhibit differential maturation rates as hydroperiod fluctuates. This issue is worth following up with subsequent experiments, as it has implications for wetland rehabilitation and management. If permanent saturation/moisture in naturally seasonal wetlands “switches off” certain taxa, then protection of such wetlands from changes in hydroperiod becomes a critical aspect of biodiversity conservation.

Invertebrate assemblages

The degree to which soil moisture affects invertebrate community composition is demonstrated in the MDS analyses presented in Fig 3.9. While overall similarity among all sites is negligible (<20%), specific groupings of closely related sites are clearly visible.

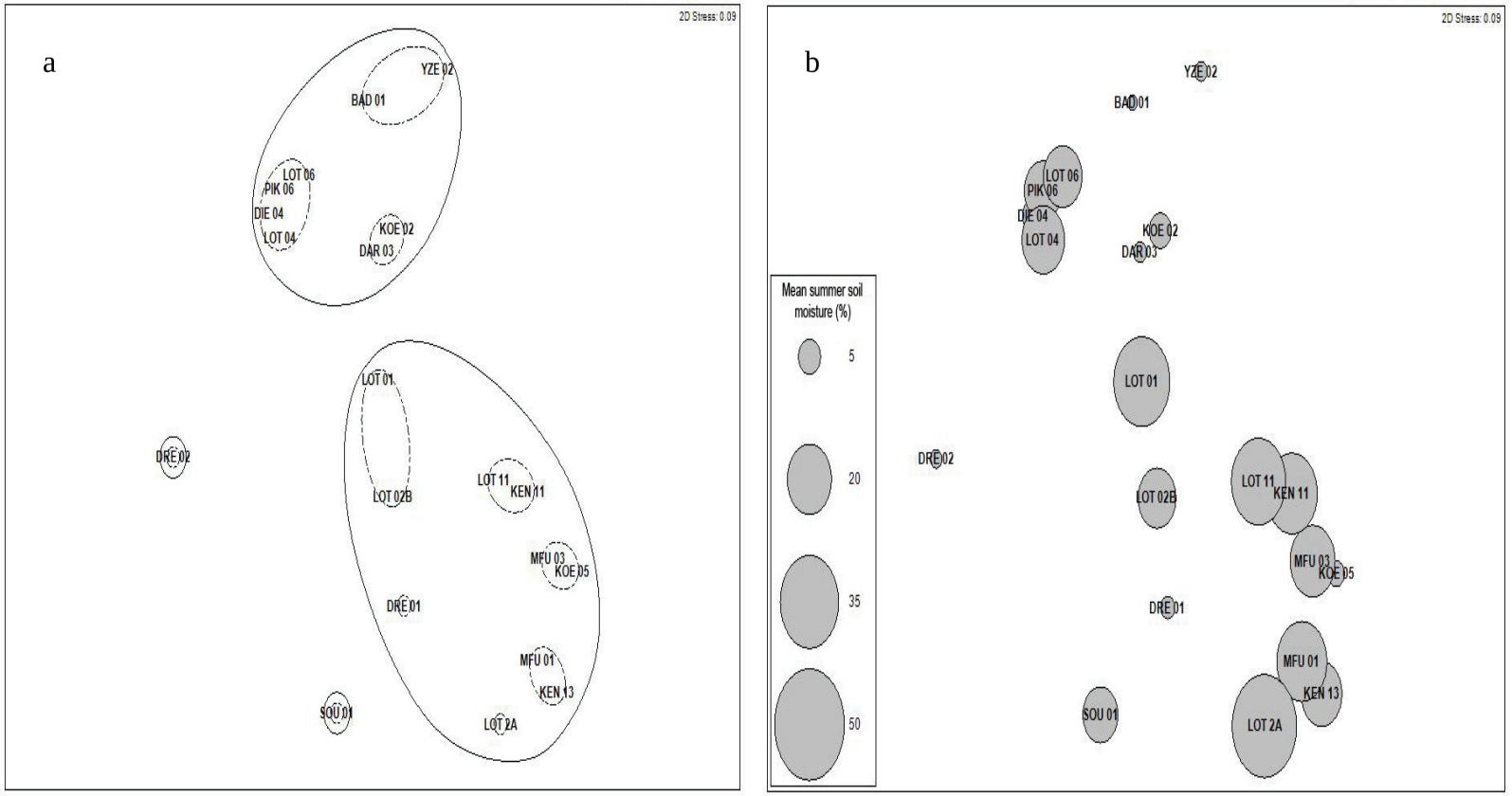

Figure 3.9: Links between crustacean assemblages from laboratory hatchings and dry season soil moisture from hatchling sites.

Cluster analysis based on the community composition and abundance of hatched invertebrates illustrates the levels of similarity among sites which have been grouped at similarity levels of 60 and 80% in Figure 3.9a. Figure 3.9b illustrates the same site groupings, overlaid by summer soil moisture data. This figure suggests that sites with comparable invertebrate communities share similar levels of soil moisture during the height of the dry season.

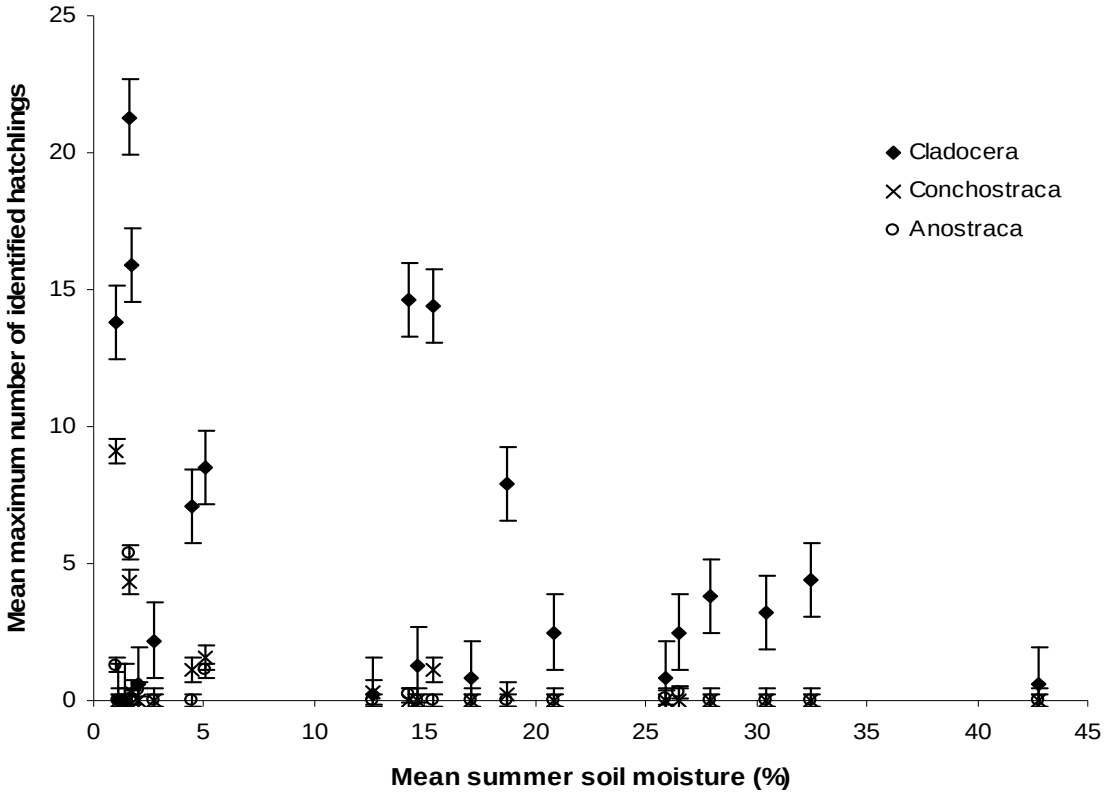

Figure 3.10: Abundance (±1 SD) of branchiopods hatched from incubation trials in relation to mean dry season soil moisture at hatchling sites.

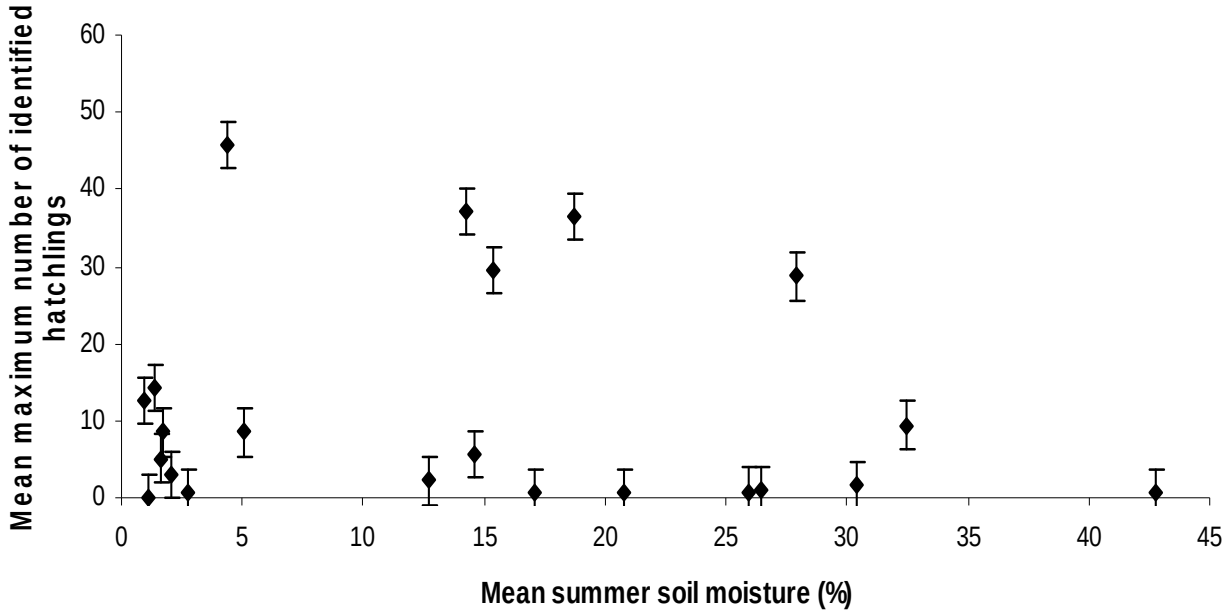

Figure 3.11: Abundance (±1 SD) of ostracods hatched from incubation trials in relation to mean dry season soil moisture at hatchling sites.

This finding becomes particularly evident when one examines the number of branchiopods (anostracans, conchostracans and cladocerans; Figure 3.10) as well as ostracods and copepods (Figures. 3.11 and 3.12 respectively) hatching in relation to the dry season soil moisture at the sites from which their eggs were collected. Most branchiopods were present in, and hatched from, soils exhibiting low moisture levels (generally <20%).

The close correlation between soil moisture and organic content (Table 3.3) explains the similar patterns observed when soil organic content was plotted against numbers of branchiopod hatchlings (Figure 3.10). For organic content (x) versus soil moisture (y) the equation $y = 0.2279x + 4.1402$ was used ($R^2 = 0.35$). Generally, the highest numbers of branchiopods hatched from soils with organic content of less than 60 g/kg. Ostracod and copepod hatchlings (Figures. 3.11 and 3.12) were recorded from sites with a range of soil moisture and organic content values, however, showing no clear trends with soil moisture or organic content. Ostracods are some of the most ubiquitous and widespread aquatic invertebrates in southern Africa, while copepods in most cases constitute the majority of zooplankton in lentic freshwater habitats (Rayner, 2001) and therefore are expected to have relatively wide tolerances to different environmental conditions.

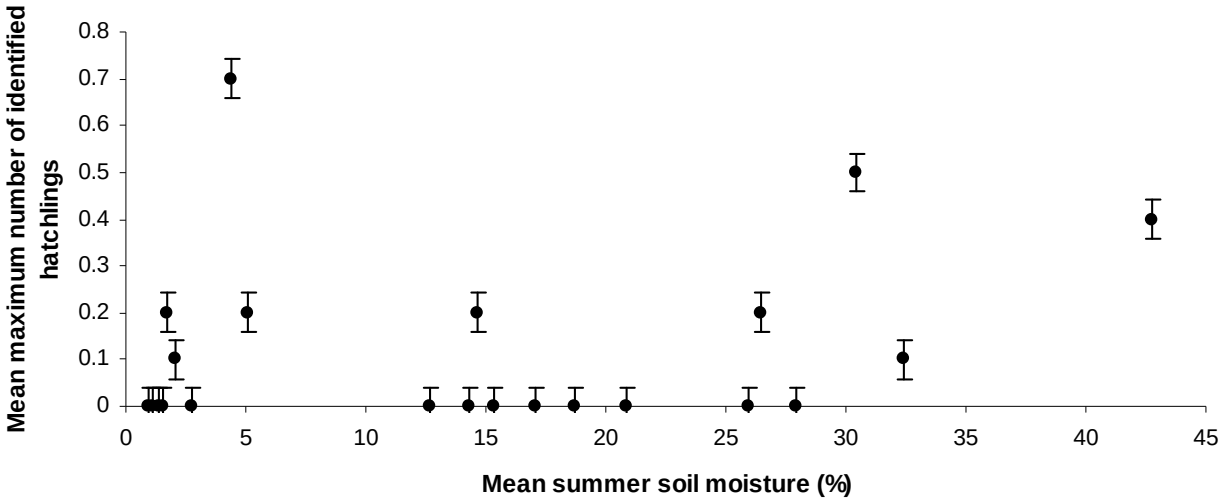

Figure 3.12: Abundance (±1 SD) of copepods hatched from incubation trials in relation to mean dry season soil moisture at hatchling sites.

From these results, it seems likely that environmental conditions (e.g. soil moisture) favouring a particular branchiopod taxon such as the Anostraca would favour also the Conchostraca and Cladocera because of similarities in life histories and developmental rates within this group (Day, 2001). This supposition is supported by the finding that the abundances of branchiopod groups (Anostraca, Cladocera and Conchostraca) were strongly positively correlated ($r = 0.56$, $p < 0.05$).

In essence, our data show that soil moisture and soil organic content affect the numbers of individuals and the taxonomic composition of the crustacean assemblages of the study wetlands.

3.2.4.3 Correlations with environmental variables

Several physical and chemical variables (electrical conductivity, pH, turbidity, dissolved oxygen, temperature and nutrients) that were measured during the wet season are analysed below in relation to the numbers and kinds of invertebrates hatching during the incubation of dry season soil samples. The following trends were observed.

Electrical Conductivity

Electrical conductivity (EC) values ranged from 18 to 1580 mS/m at the different sites in the wet season. Cladocerans and ostracods showed the widest tolerance, being present at sites over a wide range of EC values. Generally, copepods occurred in waters at the lower end of the EC range (from 18.3 mS/m) and were absent from sites with EC values

greater than 700 mS/m. Branchiopods occurred at sites with both high and low EC values. Conchostracans were absent from sites with EC values below 50 mS/m but were present, along with cladocerans and ostracods, at SOU 01, the site with the highest EC value (~1580 mS/m). In general, branchiopods occurred in slightly higher numbers at seasonally drier sites characterised by higher EC values than in moister soils with very low EC values.

While EC is considered to be one of the primary factors controlling the composition of wetland invertebrate assemblages, very little information is available regarding the salinity tolerances of wetland organisms (Dallas and Day, 1994; Quintana *et al.,* 2006), although ostracods are known to occupy habitats ranging from fresh to highly saline conditions (Martens, 2001). We do not have sufficient data on salinity tolerances of the crustaceans in question to draw further conclusions on the basis of the present study.

Dissolved Oxygen

The amount of oxygen that can dissolve in water varies with temperature. Wet season sampling data show that oxygen levels at different sites ranged from 2.3 to 15 mg/L. Cladocerans, ostracods and copepods were most abundant between about 2 and 8 mg/L, and conchostracans and anostracans between about 4 and 8 mg/L. While the higher concentrations of oxygen are unlikely to be limiting, the minimum concentrations often are. These data indicate the relatively low levels of dissolved oxygen in which temporary wetland invertebrates can occur, in comparison with riverine ones. The levels observed in this study thus illustrate the broad tolerance levels exhibited by temporary wetland crustaceans.

Nutrients

Orthophosphate (PO_4-P) levels in inundated wetlands ranged from <0.001 to 1.277 mg/L and crustaceans mostly occurred at concentrations below 0.450 mg/L. Levels of orthophosphate are usually low (<0.01 mg/L) in unpolluted waters (e.g. Dallas and Day, 1994), although phosphate levels may be much higher than this in small isolated wetlands (Malan and Day, 2005). Interestingly, cladocerans, ostracods and conchostracans were recorded at site LOT 06 when orthophosphate levels were as high as 1.277 mg/L.

Nitrate and nitrite together (NO_3^-+ NO_2^-)-N ranged between values of <0.01 and 8.2 mg/L; the greatest number of taxa was observed in sites where nitrate values were below 0.015 mg/L. Anostracans were absent from sites at which the combined value exceeded 0.005

mg/L and copepods from sites where combined values exceeded 0.015 mg/L. Ostracods, cladocerans and conchostracans were all present at the site where the combined concentration of (NO_3^-+ NO_2^-) -N was 8.24 mg/L.

Phosphorus (P) and nitrogen (N) determine the trophic conditions in aquatic ecosystems and DWAF (1996; 2002) has defined the trophic status of rivers based on the ranges of P and N as indicated in Table 3.5. (It should be noted that these were developed specifically for rivers and may not necessarily apply to isolated wetlands.) Figures 3.13 and 3.14 indicate the mean maximum number of identified hatchlings from each taxonomic group occurring in each of DWAF's[1] trophic states, based on P levels.

Table 3.5: Ranges of P- and N-based nutrients associated with different trophic conditions in aquatic ecosystems. Nitrogen ranges are taken from DWAF (1996) and P ranges from DWAF (2002)

Trophic State	Average summer inorganic N concentrations (mg/L)	Average summer inorganic phosphorous concentrations (mg/L)
Oligotrophic	<0.5	<0.015
Mesotrophic	0.5-2.5	>0.015-0.047
Eutrophic	2.5-10	>0.047-0.130
Hypertrophic	>10	>0.130

Total ammonium (NH_4-N) ranged between 0.002 and 1.087 mg/L. Most taxa occurred at sites where ammonium values were less than 0.02 mg/L. Cladocerans and ostracods showed greatest tolerance levels, being recorded in reasonable numbers from sites with levels as high as 1.09 mg/L N. Conchostracans, while also being recorded at this concentration, were fewer in number. Anostracans and copepods occurred in wetlands with concentrations of ammonium up to 0.85 and 1 mg/L respectively.

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

In general, total numbers of crustacean individuals were greater at higher concentrations of nutrients, as might be expected for sites where relatively high nutrient concentrations resulted in greater algal productivity. The highest levels of nitrate and nitrite, ammonium and orthophosphates were recorded at site LOT 06, from which the highest mean maximum number of hatchlings was recorded. Ostracods, cladocerans and conchostracans were represented along with a large number of unidentified nauplii.

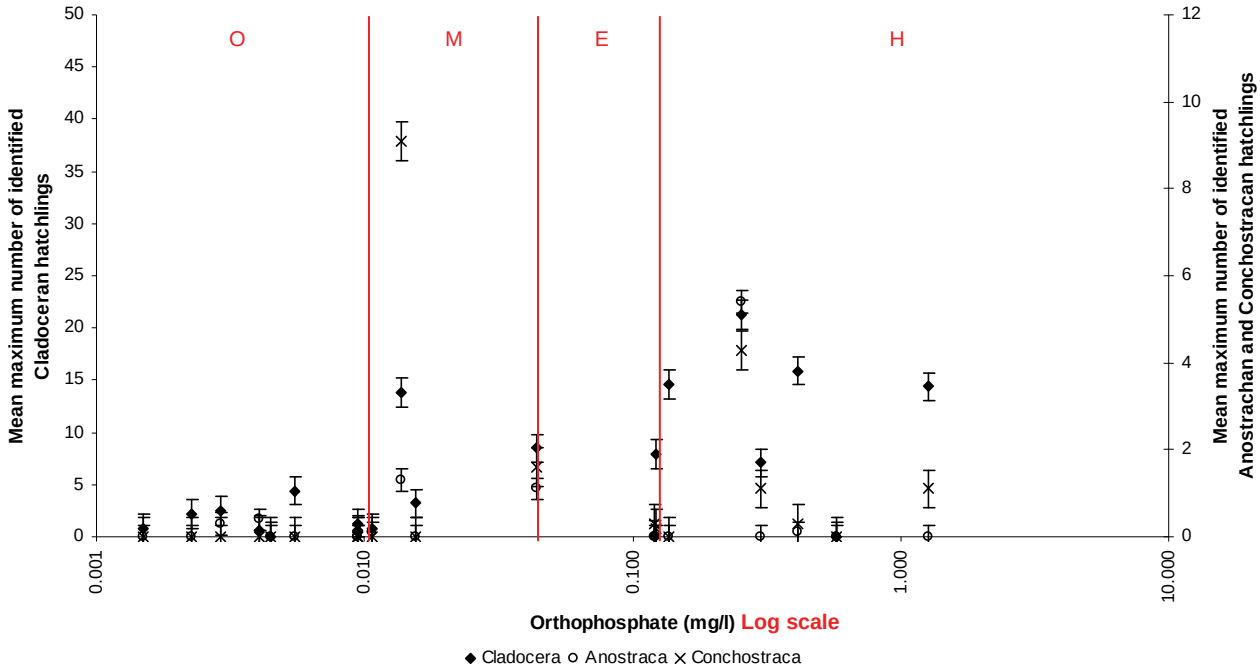

Figure 3.13: Abundance of branchiopods hatched from incubation trials in relation to trophic status based on wet season orthophosphate values. Trophic status is based on levels outlined in DWAF (2002). Solid lines denote ranges of trophic status indicated by the letters O (oligotrophic), M (mesotrophic), E (eutrophic) and H (hypertrophic).

In terms of trophic status, the greatest numbers of taxa were, however, recorded at concentrations of P ranging from oligotrophic to eutrophic. In contrast, with regard to N compounds, the greatest numbers of taxa were recorded from oligotrophic systems. All taxa exhibited greatest mean maximum numbers of individuals in hypertrophic conditions, but greatest numbers of occurrences in oligotrophic to mesotrophic conditions.

pH

In situ pH values ranged between 4.6 and 9.1. The two sites from the Kenilworth area (KEN 13 and KEN 11) showed the lowest pH values, at 4.6 and 6.6 respectively, and are considered typical of acidic wetlands in the south-western Cape, which derive from leaching of weak organic acids from decaying fynbos vegetation (e.g. Dallas and Day, 1994).

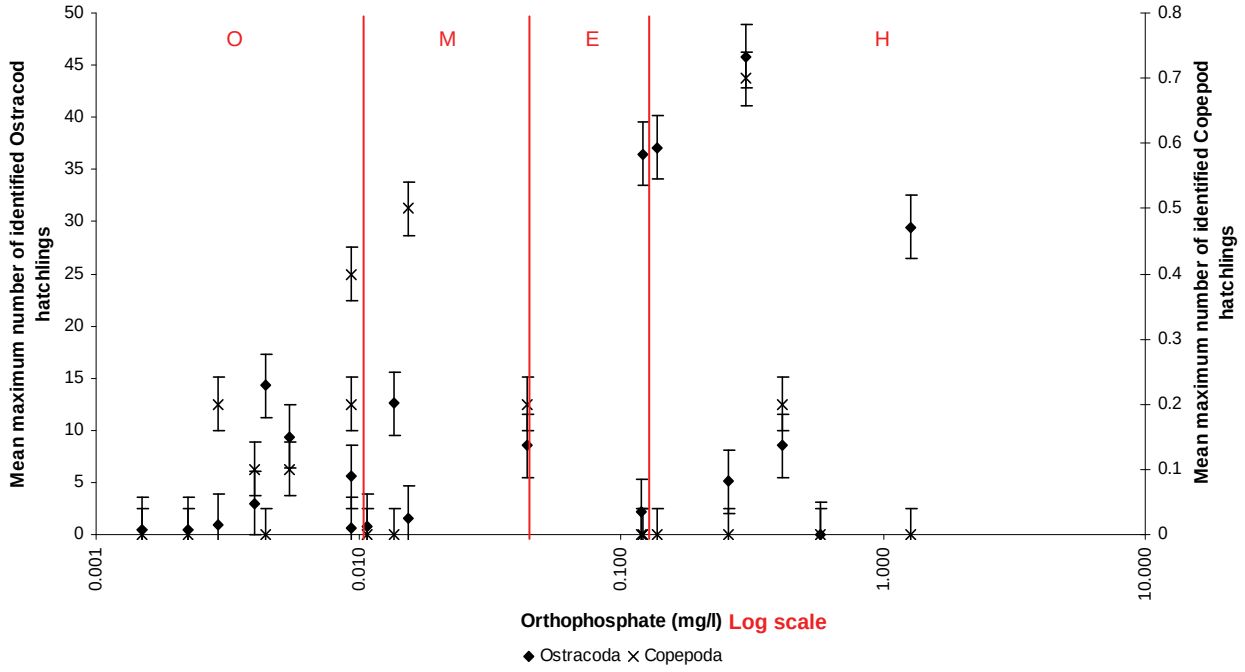

Figure 3.14: Abundance of ostracods and copepods hatched from incubation trials in relation to trophic status based on wet season orthophosphate levels. Trophic status is based on levels outlined in DWAF (2002). Solid lines denote ranges of trophic status indicated by the letters O (oligotrophic), M (mesotrophic), E (eutrophic) and H (hypertrophic).

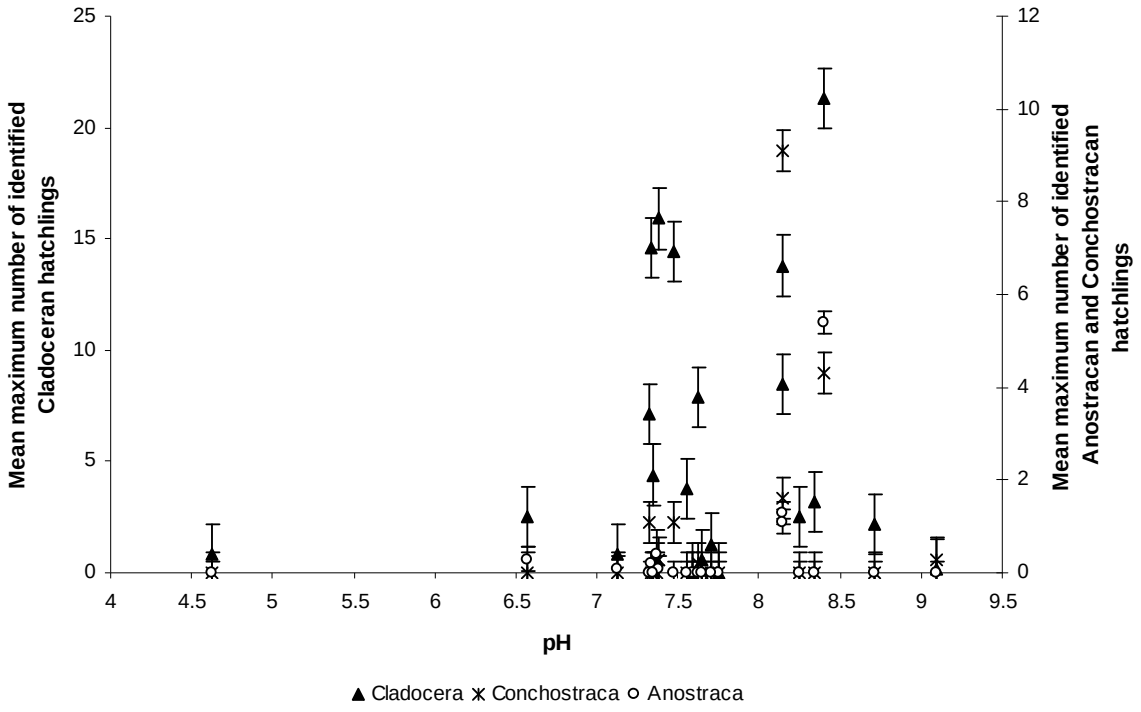

Figure 3.15: Numbers of branchiopods hatched from incubation trials in relation to wet season pH.

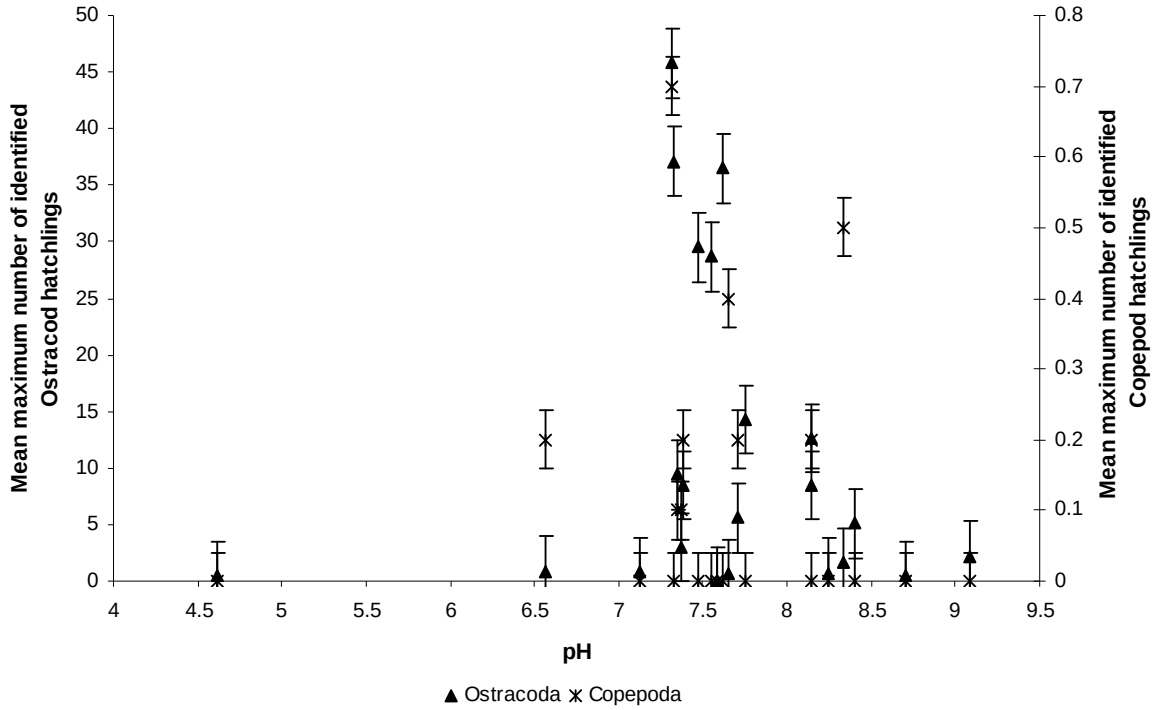

Figure 3.16: Abundance of ostracods and copepods hatched from incubation trials in relation to wet season pH.

The greatest average number of branchiopods was observed at a higher pH range than that observed for ostracods and copepods (Figures. 3.15 and 3.16). Branchiopod abundance was greatest within a pH range of 8-9, while most ostracods and copepods occurred within a range of 7-8.5. These pH ranges are of interest with regard to ammonia concentrations. Ammonia exists in two forms – ammonium (NH_4^+) ions and un-ionised or free ammonia (NH_3). Ammonia (but not ammonium) is toxic to many aquatic organisms at even low concentrations (0.1 mg/L; *sensu* DWAF, 1996). Its proportional contribution to total ammonia varies with temperature and pH, with high pH values (>8) and high temperatures dramatically increasing the concentration of toxic NH_3 in a water body. The correspondence between cladocerans and conchostracans, both of which occurred in water with total ammonia concentrations >1 mg/L, and the high pH values of many of these waters (>8) suggests that these groups of crustaceans in particular appear to have relatively high tolerances for free ammonia. At 15°C and pH 8.5, 1 mg/L total ammonia has a concentration of 0.1 mg/L, and thus falls within the acute toxicity range defined by DWAF (1996).

Turbidity

Turbidity at different sites ranged between 0.64 and 706 NTU. Observations made during wet season *in situ* sampling suggested that turbidity was a result of suspended inorganic sediments rather than algae in the water column (M Bird, 2009, pers. comm. to V Ross-Gillespie, University of Cape Town, Cape Town). More branchiopods were observed at high turbidities (between 15 and 50 NTU) than were ostracods and copepods (between 0 and10 NTU). Representatives from all taxonomic groups were recorded from a site with a turbidity level of 42.6 NTU, but only ostracods and cladocerans were recorded at turbidity levels higher than 50 NTU, and only ostracods at a level of 130 NTU. Turbidity was also found to be higher at sites with lower soil moisture and higher clay content. This is to be expected, as finer sediment particles (such as clays) are readily suspended in the water column following initial rains or physical disturbance of the sediment (Figure 3.4). As was also observed by Seaman (2002), the most turbid waters (e.g. 706 NTU and 52.1 NTU) had a relatively low salinity (24.9 and 35.2 mS/cm respectively) because high salinities usually cause precipitation of the finely divided clay particles that make water turbid.

3.2.4.4 Relationships between biological and environmental data

Data for sites LOT 05 (no environmental data available) and PIK 11 (no hatchlings) were excluded from the analyses reported on below.

Significant Pearson correlations were observed between various aspects of the incubation experiments and turbidity, orthophosphate concentrations and ammonium concentrations in the wet phase (Table 3.6).

Table 3.6: Significant Pearson correlations between environmental variables and invertebrate hatching data

Correlation	r value	Significance
Turbidity (NTU) – Days until first hatchling emergence	0.63	$p<0.01$
Orthophosphates (PO_4-P) – Mean max number of Cladocera	0.54	$p<0.05$
Total ammonia (NH_4-N) – Mean max number of Cladocera	0.53	$p<0.05$

The data provided in Table 3.6 indicate that the higher the turbidity, the longer it took for nauplii to hatch. Continuous turbidity is believed to have serious impacts on various members of the freshwater biota (Dallas and Day, 1994). We know that elevated turbidity

values reduce light penetration. We also know (e.g. Brendonck *et al.,* 1993; 1998) that darkness inhibits hatching. If, in this case, light is a cue for hatching, then turbidity would have a significant impact on the hatching response. As an adaptation, this may be rooted in the fact that reduced light penetration leads to reduced primary production, which could impact on feeding success, thereby slowing development and productivity rates. Turbidity may also be linked to smothering of small organisms.

Table 3.6 also indicates positive correlations between numbers of cladocerans hatching, and concentrations of orthophosphate and ammonium in the wet phase. Most cladocerans are filter feeders on phytoplankton, and both N and P promote primary productivity, so the correlation, at least at intermediate nutrient concentrations, is not surprising. This result could also help to explain the trend observed in our hatching experiments (Section 3.2.4.2), in which – unlike reports in the literature from elsewhere – ostracods were not initially the dominant faunal group. An abundance of nutrients might have promoted sufficiently rapid phytoplankton growth to allow the rapid colonization by plankton feeders.

The results of the CCA are displayed in Figure 3.17. The plot displays those unrelated environmental variables (i.e. variables with negligible multicollinearity: $r < 0.8$) that best explain the variance observed in the mean maximum number of each taxon emerging in the hatching experiments. Variables omitted from the CCA analysis because of multicollinearity are pH, ammonia, nitrite and nitrate, and dissolved oxygen.

Figure 3.17 displays taxa and sites in relation to environmental gradients (represented by vectors), which allows for the interpretation of the similarity between sites with regard to taxonomic composition and the environmental requirements of those species. The plot displays the site and taxonomic data constrained by environmental variables along two axes. In most cases these two axes account for the majority of the variance present within the biological data. In this case, the cumulative percentage of variance explained by the two environmental axes accounted for approximately 45.3% of the total variance observed in the invertebrate data, with axes 1 and 2 accounting for 31.41 and 13.96% respectively. Eigen values calculated for these axes give an indication of the amount of variance explained. In this case Eigen values for canonical axis 1 and 2 were 0.169 and 0.075 respectively. Essentially, the length and the direction of the vectors in the ordination plot indicate the importance of the environmental variable and the correlation of the environmental variable with the biological data respectively (taxonomic composition). Thus, long vectors almost horizontal to the x-axis represent those

environmental variables having the greatest individual influence on the biological data or species composition. The position of the sites in relation to the vectors gives an indication of the environmental characteristics of the site, while species preferences for environmental conditions can be interpreted from their position in relation to the vectors.

The variables, “days until first hatching” and EC account for most of the spread observed along the first axis, while the variables Turbidity (NTU) and orthophosphates (PO_4-P), with additional effect from soil moisture and organic content, account for the majority of the variability along the second axis. These environmental variables accounted for the most variance in the invertebrate hatching data.

The Monte Carlo randomization test revealed a high probability ($p > 0.05$) that the magnitudes of the Eigen values observed could have been generated by chance, so the environmental variables analyzed in the CCA cannot significantly explain trends (i.e. cannot account for the variance) in the invertebrate abundance data obtained from the hatching experiments. This does not mean, of course, that the variables are unimportant in structuring the crustacean assemblages of the sites in question.

In order to gain an alternative perspective of the effect of the same environmental variables identified in the CCA (Figure 3.17) on the invertebrate community structure from the hatching trials, the multivariate statistical package PRIMER was used to analyse the invertebrate data in terms of presence or absence of taxa, rather than of abundance. (Site PIK 11 could therefore be included in the analysis.) The same environmental variables when submitted to the BIOENV function of PRIMER revealed the following best results: significant Spearman rank correlations between invertebrate composition and (a) the turbidity variable ($r = 0.488$; $p < 0.05$), and (b) a combination of turbidity and orthophosphates ($r = 0.441$; $p < 0.05$).

These results make sense when one considers that orthophosphates play an important role in determining algal and plant growth and therefore affect organic composition and trophic status, both of which affect the structure of the assemblage. It may also be linked indirectly to soil moisture, as increased organic material may result in an extended hydroperiod. Turbidity may play a role by limiting light for rooted plant growth (in deeper systems usually) or, since turbidity is a reflection of particulate material in the water, by delaying or preventing hatching of eggs and cysts or by directly clogging the gills of invertebrates. It is worth noting, too, that the PO_4^{3-} ion adheres readily to finely divided clay particles, which are often the major cause of turbidity.

Summary data showing the trends in invertebrate assemblages hatched from incubation trials in relation to the various environmental variables discussed in this report are displayed in Appendix 5. In essence, the results suggest that turbidity and orthophosphate are the key physical and chemical properties that have the greatest influence on the composition of crustacean assemblages and their numbers, while pH, salinity, EC and ammonia have important secondary affects.

3.2.4.5 Comparison of crustacean assemblages in the wet and dry phases

Figure 3.18 summarises the results of similarity analyses carried out using presence/absence data only, and comparing wet- and dry season data. Similarity was relatively high between crustacean assemblages hatched in the laboratory and those sampled in the field in the wet season. The major split into two clusters is probably because copepods are greatly underrepresented in the hatching experiments. This in turn may be a reflection of the fact that copepod eggs undergo diapause and are not easily induced to hatch under laboratory conditions. The lowest group average similarity recorded between individual nodes (sites) was 71.0%. When gastropod data were incorporated, the lowest group average dropped to 62.2%. These results indicate that hatching experiments in the laboratory provide a good representation of natural crustacean assemblages.

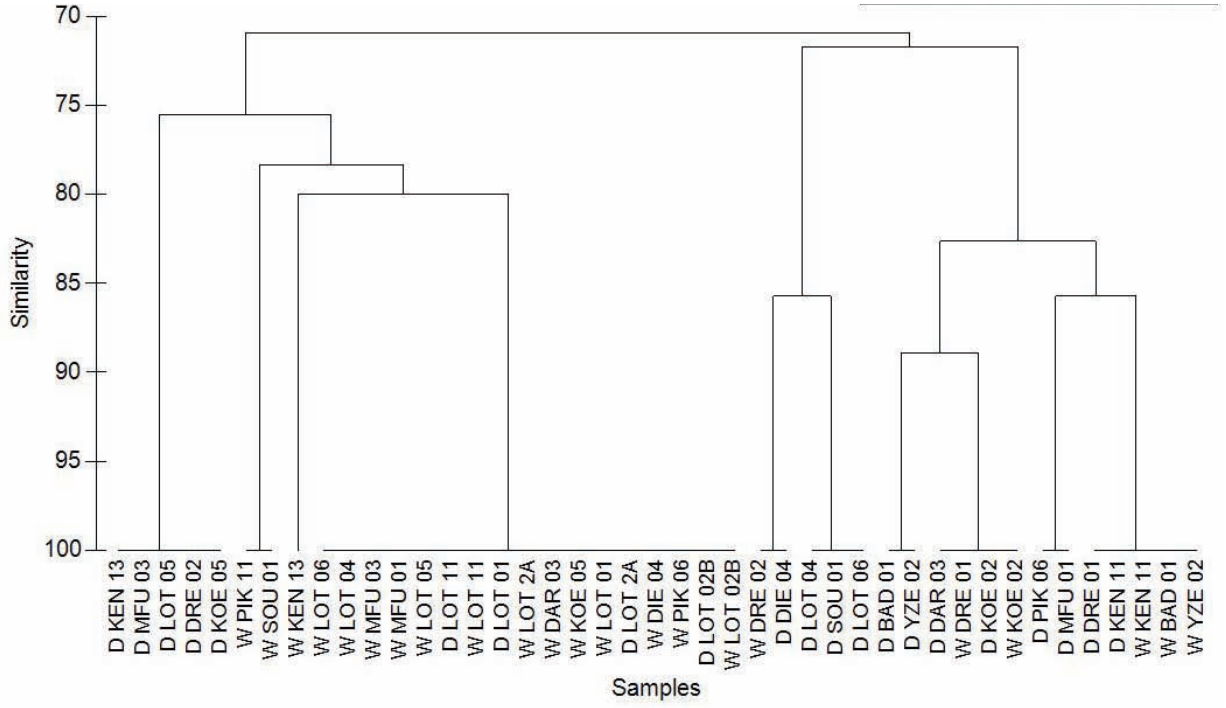

Figure 3.17: Cluster analysis of invertebrate taxa (presence/absence) from incubation trials and from wet season samples. D denotes incubated samples and W denotes wet season samples.

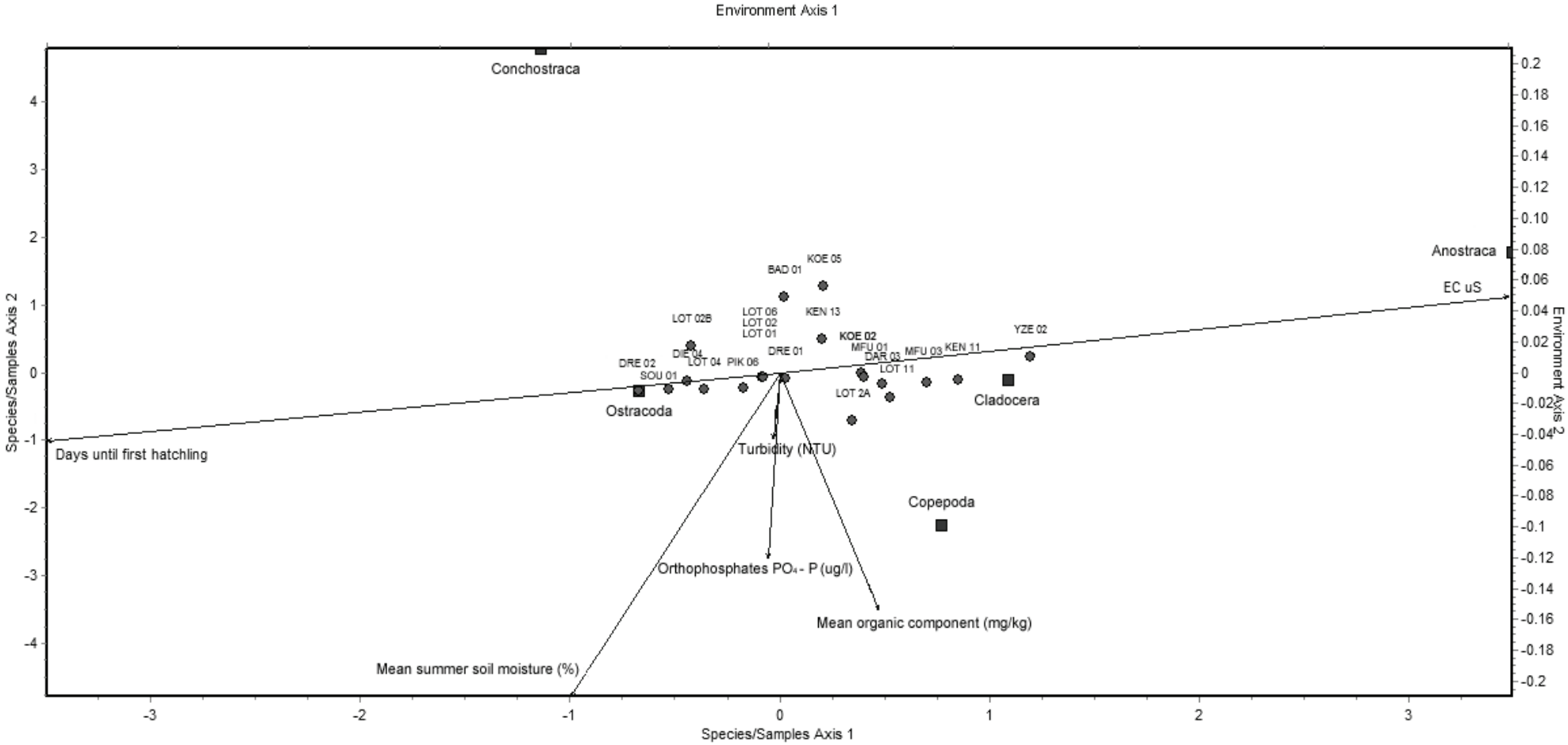

Figure 3.18: Canonical Correspondence Analysis (CCA) ordination of invertebrate abundance/community composition from incubation trials in relation to environmental variables measured in the wet phase.

3.2.5 Conclusions

The key findings of this study can be summarised as follows.

1. The incubation techniques described here provide a potential tool for assessment of the seasonally inundated wetlands in their dry state. The presence of any wetland invertebrate fauna in the incubated material indicates the presence of a wetland – although further investigation is recommended into the length of time over which wetland fauna may survive in a resting state, once wetland hydroperiod changes (e.g. in the event of increased or decreased hydroperiod). The presence of phyllopods ('large branchiopods': anostracans, conchostracans and notostracans) provides useful evidence of wetlands that experience naturally short hydroperiods and periods of total desiccation. The hatching method is easy to carry out with simple sampling procedures and is, overall, relatively quick to complete (approximately 20-30 days hatching – quicker if microscope identification of nauplii is performed). Wet sediment samples may require drying but this process can be speeded up by using a drying oven at 40°C.
2. Artificially induced hatching is considered an appropriate method for gauging crustacean assemblages. Seven taxa were represented from hatching trials and all are resistant to desiccation.
3. It should be noted that for the purposes of this study only a basic visual identification, with the naked eye, was carried out on a daily basis while species level microscope identification of voucher specimens took place after the hatching period (~40 days). It is therefore expected that identification of nauplii to Order can be made at an earlier stage with the use of a microscope, which in turn may provide further insight and clarification of successional hatching trends. The cost, in terms of time spent, *vs.* the benefits of early identification will need to be considered.
4. Good representation of natural invertebrate communities from hatching trials (based on presence/absence of taxa at ordinal level) was observed (~70% similarity between wet and dry communities), suggesting that dry season assessments can provide a low level surrogate for wet season assessments of biodiversity.
5. Various environmental variables (e.g. soil moisture for branchiopods, the organic content of the soil for ostracods and total ammonium and phosphates for cladocerans) appear to be reasonable predictors of assemblage composition. Turbidity and phosphates are the environmental variables most closely correlated with the composition of the assemblage and the abundance of eggs hatching. Additionally turbidity plays an important role in the hatching success of eggs and the time taken till hatching. As turbidity is related to soil composition (i.e. presence of clays), it could be

used as a basic indicator to gauge invertebrate diversity and or wetland environmental condition (“health”).

6. More sites are needed to strengthen statistical results regarding environmental variables and community structure, and to provide a more comprehensive range of anthropogenic effects.
7. Ten replicate soil core samples were adequate for our purposes in accounting for the entire wetland area (including all zones identified in the delineation method of DWAF, 2005). It may, however, be more efficient to take egg-containing sediment samples only from the part of the wetland that would be deepest when inundated, and would be likely to contain the greatest number of eggs.

4. PLANT INDICATORS

4.1 Categorisation of wetland plants

Some 482 species, subspecies and/or varieties of macrophytes that occur in southern Africa (either naturally or as a result of human introduction) have been described by Cook (2004) as aquatic or wetland plants. These plants, estimated as making up some 2% of the total southern Africa flora (Cook, 2004), include both hydrophytes and helophytes, defined by Glen *et al.* (1999) as follows:

- **hydrophytes** (also called obligate wetland plants): plants that are physiologically bound to water where at least part of the generative cycle takes place in the water or on the surface – this category can be further subdivided into s*ubmerged* and *emergent components;* and
- **helophytes** (sometimes called facultative wetland plants): essentially terrestrial plants of which the photosynthetically active parts tolerate long periods of submergence or floating on water.

The latter group includes another group of plants – the halophytes, or salt tolerant plants. Although these often occur in wetlands, USACE (2006) caution that they can be misleading wetland indicators, as they can dominate areas that are highly saline but lack wetland hydrology.

4.2 Available information regarding the distribution of wetland plants in South Africa

A number of publications exist with a focus on the distribution and/or habitat types of wetland plants in different regions of South Africa. These include:

- a guide to important water plants of KwaZulu-Natal, with a focus on aquatic (i.e. submerged and/or floating) rather than general wetland plants (Musil, 1973);
- various field guides to aquatic plants, which include major species but by no means attempt to provide complete listings (e.g. Ellery, 1997);
- a guide to aquatic plants in South Africa, with a focus on the most common plants found on the margins of and within impoundments (Gerber *et al.,* 2004);
- guides to the identification of invasive aquatic and wetland plants (e.g. Henderson and Cilliers, 2002);
- lists of plant species that occur in specific wetlands that have been identified during the course of different studies – these are often collected as a result of Environmental Impact Assessment studies, but in the Western Cape include work such as that

presented in the present WHI project by Corry (2010) for fynbos wetlands in the Western Cape, and well as detailed mapping and classification of wetland and vegetation type for wetlands in core areas in the Cape Floristic Region, as represented by the CAPE Fine Scale Planning data (Helme, 2008);

- various databases that list plant species in terms of habitat and known distributions – such databases include the SASFlora database that has been compiled and managed by COASTEC, and is referred to again later in this section;
- guides to aquatic plants (mosses and vascular plants) including work by Glen *et al.* (1999) and Cook (2004); and finally
- various lists of plant species that are believed to be indicative of various wetland conditions in different rainfall regions of South Africa – these include lists and guides to wetland plants included in DWAF (2005), with an emphasis on plants that indicate wetland conditions in the KwaZulu-Natal area.

The above publications are aimed primarily at the provision of a list of wetland plants that can be used as indicator species of general wetland conditions. Temporary/cryptic wetlands form a special case, in that the particular conditions that usually give rise to the establishment of a diagnostic wetland flora are either absent from the wetlands altogether, or present for such limited periods of time that there is only a small window of opportunity for the establishment of true aquatic and/or wetland plants in these habitats. A similar situation exists for the establishment of soil markers, which are another common indicator of wetland conditions. Such markers are often absent altogether from so-called temporary wetlands; this issue is discussed in more detail in Section 5.

4.3 Wetland plants that occur in temporary wetlands

In terms of the wetland flora, the kinds of plants that can be expected to occur in temporary wetlands can be divided into two broad categories:

- aquatic annuals which, like the invertebrates described in Section 3, have adaptations that allow them to survive long periods of desiccation, either in seed or bulb form, and which grow rapidly once the wetlands are sufficiently saturated or inundated to encourage plant growth. Such plants would all fall within the hydrophyte category described in Section 4.1; and
- helophytes, which grow in or along the margins of temporary wetlands during their dry seasons, and are able to survive the relatively short periods of inundation or saturation that correspond with the wet season. In practice, such plants are often limited to the edges of seasonally to ephemerally inundated wetlands, and the lower-

lying portions of many such wetlands are in fact characteristically bare of vegetation during the dry season.

Since evapo-concentration is usually a characteristic of temporary wetlands, particularly those that are isolated from channels through which water can drain, salt-loving halophytes are also often associated with these habitats.

4.4 The use of wetland plants in the identification of temporary wetlands in the dry season

The use of wetland plants as a key means for identifying the presence of wetland habitat in a particular area is well-established internationally. DWAF (2005) outlines a method for the use of hydrophytic vegetation as an indicator in wetland delineation. This method, which also forms part of the wetland assessment and delineation protocol of USACE (2006), is based largely on the identification of facultative and obligate wetland plants, with >50% cover by facultative and/or obligate wetland plants in either woody or herbaceous vegetation layers being taken as a clear indicator of at least temporarily hydric conditions; the presence of some facultative or obligate wetland plants, but at low rates of cover (<50%), is taken to suggest but not confirm hydric conditions.

Such an approach is of limited value, however, in assessing only infrequently and ephemerally inundated temporary wetlands in their dry condition. Plants in such wetlands may include annual macrophytes and algae during rare periods of inundation but under more normal, drier, circumstances may consist essentially of terrestrial species, often ruderal ones. The following three possible approaches have been suggested as a way around this problem during dry season assessments.

- Using abiotic indicators such as water level, soil characteristics and the presence of dead plant material (e.g. dried algae) as indicators of inundated conditions – these aspects are discussed in more detail in Section 5.
- Artificially germinating wetland seeds and bulbs in laboratory conditions – this aspect was included to a minor degree in the experiments described in Section 3. While laboratory inundation proved a successful and useful tool for assessing the invertebrate fauna of temporary wetlands during the dry season, it proved difficult both to germinate and then timeously to identify wetland plant seedlings under laboratory conditions. Thus although this method may lend itself to long-term testing and experimentation regarding life history patterns in these wetlands, it is unlikely to

be of value in providing a rapid means of assessing wetland character and/or condition.

- Identifying perennial wetland plant species that would provide clear evidence of wetland inundation or saturation during the wet season, as well as identifying wetland plant “markers” that would provide evidence that wetland conditions might occur during wetter periods, and which might be interpreted with higher levels of confidence if other wetland indicators are present (e.g. aquatic invertebrate propagules).

The rest of this section focuses primarily on the last approach.

4.5 Plants that might be indicative of temporary wetland conditions

At the outset of this project it was recognized that strong seasonal differences are likely to exist between the plant communities that characterize wetland habitats in different rainfall and geographic areas of South Africa. Limited budget for this component of the project means that at best, annotated plant lists for wetlands in different major areas can be developed to inform our understanding of the likelihood and characteristics of wetland habitats occurring within a particular area or site. The information provided in this section should thus be seen as complementing other plant indicator data, rather than being definitive.

Table A8.1 (Appendix 8) provides a list of plants that occur in seasonally inundated to saturated wetland habitats in the Western Cape, as collected and compiled by Corry (2010). The presence of these plants at a dry season site can be used as an indicator of wetland conditions – however, until this database is populated with information as to whether plant species are considered obligate or facultative wetland species at one part of their life cycle, they cannot be used as definitive wetland indicators. It should also be noted that some plants may require wetland conditions for one part of their life cycle, but may be able to remain *in situ* long after such wetland conditions have disappeared from an area, providing false indications of present wetland extent. *Palmiet prionium* is one such wetland plant, the extent of which can sometimes reflect past levels of inundation, rather than present wetland extent.

Table A8.2 (Appendix 8) summarises major grass species that are viewed by local experts as indicative of the temporary wetland pans in KwaZulu-Natal and the Free State. The presence of individuals belonging to these species should be viewed as a likely indication of wetland conditions.

The presence on a site of the perennial plant species listed in the above tables as either facultative or obligate wetland plants can be used as a ‘red flag’ indicating that the site is likely to support wetland communities, at least occasionally. On the other hand, the absence of annual wetland hydrophytes from a site in the dry season should clearly not be taken as an indication that wetland conditions do not occur at the site. The presence of *perennial* hydrophytes is likely to indicate wetland habitat which will usually be accompanied by other common wetland indicators such as markers of soil saturation; such wetlands would not be “cryptic”.

Both of these species lists should be subject to ongoing refinement, including annotation as to regional differences in plant habitat and tendency towards obligate or facultative occurrence in different wetland zones.

5. THE USE OF ABIOTIC INDICATORS IN THE IDENTIFICATION AND/OR CHARACTERIZATION OF TEMPORARY AND OTHER CRYPTIC WETLANDS

5.1 Overview

The previous sections of this report have focused on plants and animals that can be used in the dry season as indicators of episodically to seasonally inundated or saturated wetlands. In addition to these, a range of other factors can provide valuable insights into the presence and even the type of cryptic wetlands assessed outside of the wet season. These have been divided into topographical indicators, and indicators of inundation and saturation, and should be used in conjunction with the other approaches to wetland identification and characterization already outlined. It should be noted in this regard that many of these indicators have been included in DWAF's wetland delineation manual (DWAF, 2005). In the case of temporary cryptic wetlands, where identification and broad characterization of wetlands are the key issues, many of these indicators provide valuable clues as to the type of wetland under discussion and its recent hydrology, as well as to the usefulness of undertaking more time-consuming analyses such as the artificial incubation of inundated soil samples for particular wetlands.

5.2 Topographic indicators

The position in the landscape and local topography of a site can provide valuable indicators as to the type of wetland most likely to occur in a particular area. DWAF (2005) provides a simplified terrain unit indicator, based on the definitions of McVicar (1977), which refer to crest, scarp, midslope, footslope and valley bottom positions, in each of which wetlands might occur. At a local level, however, the topography of a site indicates the likelihood of its being inundated or simply saturated during the wet season; neither is likely to occur on terrain that is steeply sloping and/or convex rather than flat or concave.

The National Wetland Classification system (SANBI, 2009) has revised the above terrain units, using the landscape settings outlined in Figure 5.1. The likelihood of a cryptic wetland being inundated versus saturated during wet season conditions can be determined on the basis of setting, with inundation most likely in depressions on hilltop crests, on hill-slope flats, on plains and in valley bottoms. Saturation rather than inundation is more likely to occur if a wetland is located on a slope. Clearly, topographic indicators can provide a useful dry season indication of wetland type, but they cannot be assumed to confirm the presence or absence of a cryptic wetland unless water is actually present.

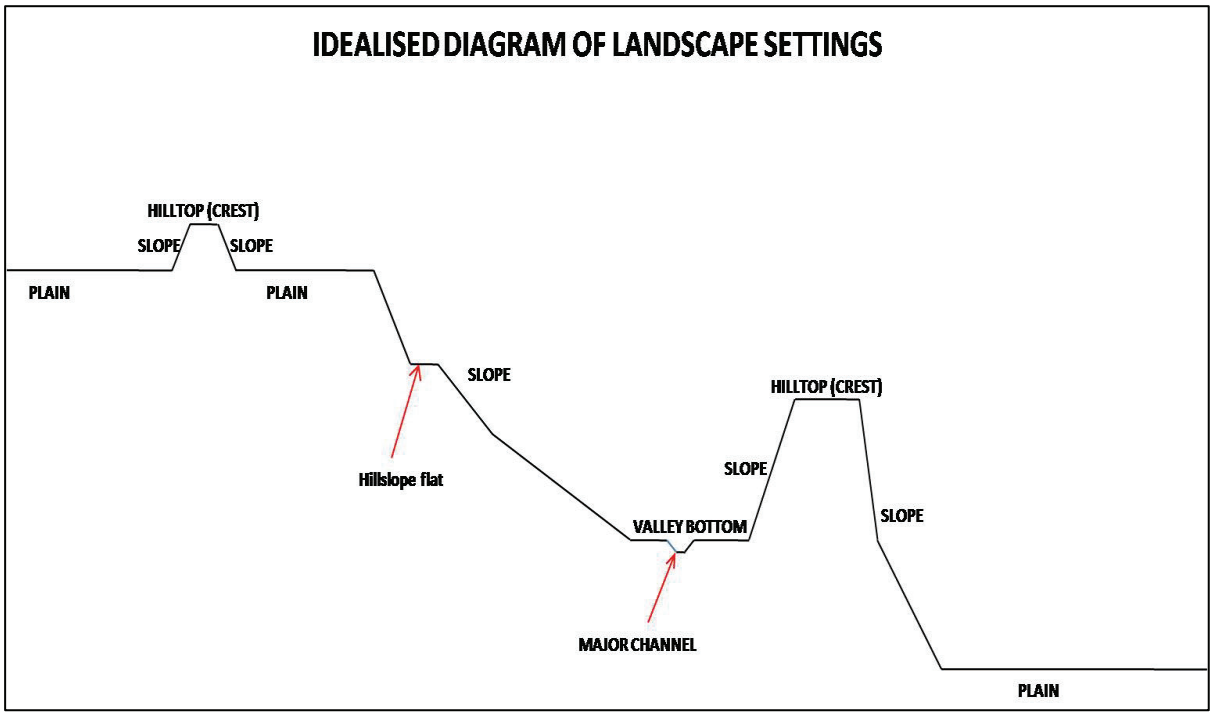

Figure 5.1: Landscape setting indicator, illustrating different landscapes in which inundated wetlands are likely to occur (after SANBI, 2009).

5.3 Soil wetness Indicators

Of all abiotic indicators, soil wetness is usually the least useful for identifying cryptic wetlands, since the soils are by definition not exposed to the specific conditions under which such indicators are formed. Nevertheless, for the sake of completion, soil wetness indicators that may at times be useful in the identification and/or characterization of these wetlands include the following.

- The presence of mottles: DWAF (2005) notes the presence of numerous mottles as indicative of seasonal saturation, while temporary or permanent saturation would both be associated with less abundant mottles. Cryptic wetlands do not usually exhibit mottling, though, often because the soils have naturally low levels of iron, so the absence of mottles does not necessarily indicate the absence of a wetland.
- The depth of the water table: where wetlands are primarily groundwater-fed, the depth of the water table in the dry season can provide limited information regarding the likelihood of occurrence of a temporary wetland in the area. Thus, where the dry season water table lies less than 50 cm from the surface, it is reasonable to assume that this will break surface in the wet season to form a wetland. While seasonal differences in water tables often exceed 1 m, this indicator should be used with caution, since wetlands formed as a result of a seasonally fluctuating water table are usually indicated by the presence of other more reliable soil wetness indicators.

5.4 Other useful abiotic indicators for dry season assessments

In addition to the standard soil wetness indicators listed above, a number of other indicators, specific to dry season assessments of potential wetland sites, are listed below. While the absence of such indicators from a site cannot be used as evidence of the lack of wetland at that site, their presence provides useful insight.

- The presence of a shallow clay or other impervious layer (e.g. rock) within 50 cm of the surface indicates conditions that might promote surface ponding and hence give rise to seasonal saturation or inundation, depending on the quantity of water and the position of the site in the landscape.
- The presence of deep polygonal cracks on the surfaces of relatively thick clayey substrata indicates previous saturation and expansion of clay material, followed by drying and shrinkage.
- The presence of thin, curled polygons of inorganic fines which collect on the surface of the substratum.

- A thin “muck” layer on the upper surface of a site, often overlaying sandy soils (USACE, 2006; Job, 2009). USACE (2006) describes muck as a “highly decomposed organic material, which has undergone slow decomposition to the point where recognition of individual plant parts is not possible. The layer is usually dark and has a greasy feel”. Muck disappears quickly in the absence of the hydrological conditions that led to its formation so USACE (2006) for the USA, and Job (2009) for the Western Cape, consider only thin layers of muck (<2 cm in depth) as being indicative of functional wetlands, since thicker layers may have been deposited in former but not extant wetlands. It should be noted that disturbances such as fire and recent ploughing are likely to remove muck from the surface of the landscape.
- The presence of sediment deposits on plant stems, leaves, rocks and other objects can provide valuable indicators of the minimum level of wet season inundation in cryptic wetlands. USACE (2006) defines such deposits as “thin layers or coatings of fine grained mineral material (e.g. silt or clay), sometimes mixed with other detritus, remaining after surface water recedes”. Such deposits usually indicate that water has stood for sufficient time to allow settlement of fine sediments. These deposits usually indicate minimum levels of inundation and can be extrapolated across areas of lower elevation in a cryptic wetland site (USACE, 2006). Deposits such as these may remain visible for some time after the wetland has dried out but will eventually be removed by precipitation or subsequent inundation.
- Biotic crusts, comprising the dried remains of free-floating filamentous algae, blue-greens (cyanobacteria) and benthic microflora including fungi, lichens and diatoms left on or near the wetland soil surface after it has dried out (USACE, 2006). Benthic crusts form in and around the margins of drying wetland pans, and are more common in sparsely vegetated pans than in vegetated areas, thus assisting in the identification of some cryptic pans. They indicate inundated conditions, rather than mere saturation. Biotic crusts on the wetland soil surface characteristically split into polygons, with upturned, sometimes curling edges, as the wetland dries out, and are usually a different colour from the underlying soil. USACE (2006) notes that the presence of rough or pedicellate (i.e. with multiple stalk-like protrusions) crusts, by contrast, do not indicate a history of standing water.
- Algal markers: previously inundated wetlands that supported free floating algae may also be identified by the presence of mats of dried algal remnants on low growing vegetation, as the ponds dry out. Many dry temporary wetlands in South Africa are covered with a layer of what looks like pale grey felt but is in fact the dead remains of *Cladophora*, a filamentous alga.

- Water marks – discolorations on rocks, poles, trees or other fixed objects can also provide useful evidence of the depth of some previous inundation, and can be extrapolated across other low points within a site.
- The presence of the shells, exoskeletons or bodies of aquatic invertebrates in surface sediments; these markers should be used with caution, as they may remain *in situ* for some time, indicating the presence of a previous wetland that no longer exists.

5.5 Summary of the use of abiotic indices in dry season wetland assessments

The various parameters briefly highlighted in this section are likely to provide useful indications of both the presence of a cryptic wetland, when assessed during the dry season, and, more specifically, of hydroperiod. Obtaining an indication of hydroperiod is useful even when assessing non-cryptic wetlands in the dry season, when distinct evidence of saturation or inundation may not be readily available. Although there is no real substitute for the information regarding wetland type that is available during a wet season assessment, the window of opportunity for such assessments is often very narrow and may not even occur every year for some systems in arid areas, and thus the availability of other indices is of immense value.

6. INDICATORS OF WETLAND PRESENCE AND TYPE IN THEIR DRY CONDITION

6.1 Summary

This report has outlined a number of approaches for the identification of temporary wetlands in their dry condition. Some of these approaches also lend themselves to wetland categorization and an enhanced understanding of the wet and dry season dynamics of both cryptic and non-cryptic wetlands.

This section attempts to show how the various indicators described in this report can be used as indicators of wetland type, character and function. If we have an idea of the reference conditions pertaining to each wetland to be assessed, such indicators also provide an indirect and non-quantitative means of assessing wetland condition or "health" resulting from changes in hydrological, physical or chemical characteristics or system drivers. For example, where the reference state for a particular wetland is assumed to be inundation in the wet season and complete desiccation in the dry season, and dry season indicators suggest that the wetland remains moist throughout the dry season, conclusions can be drawn about implications of such changes in hydroperiod for biodiversity and /or for the provision of ecosystem goods and services, at least on a qualitative basis.

Table 6.1 lists the indicators referred to in this report, and summarizes specific information that their presence and sometimes their absence can provide about wetland type, character and function. Based on the information provided in the table, a number of conclusions can be drawn about the use of these indicators in assessment of temporary and other cryptic wetlands during the dry season. These have been summarized as follows.

1. No one indicator provides adequate information about wetland presence, type, hydroperiod, biodiversity, function and principle ecological and hydrological drivers to be useful on its own – particularly with regard to actual or suspected cryptic and/or temporary wetlands. In fact, assessment of a suite of indicators is required, to build up even a conceptual understanding of wetland ecosystem structure and function – this comment bears out recommendations made by both USACE (2006) and Job (2009), regarding the need for a multi-dimensional approach to wetland assessment

(and in the case of these studies in particular, to wetland delineation and characterization).

2. The absence of an indicator does not necessarily equate to the absence of a wetland.
3. The confidence associated with linking the conditions outlined in Table 6.1 to each indicator is almost invariably low – this confidence can be improved substantially by corroboration with a number of other indicators.
4. Indicators for the presence of wetland conditions may be associated with a higher level of confidence than indicators of wetland character (e.g. seasonally inundated or seasonally saturated) and/or biodiversity.
5. Seasonally/ephemerally inundated wetlands may be identifiable to a higher level of confidence than seasonally saturated systems, as a result of specific indicators for these conditions (e.g. algae and the presence of aquatic invertebrate communities).
6. Detailed delineation of cryptic wetlands is unlikely to be achievable with any useful degree of confidence based on a dry season assessment only, although landform might be used in conjunction with other indicators to produce approximate estimates of wetland extent.
7. Water chemistry (e.g. nutrient concentrations and loading) is not easy to assess on the basis of dry season assessments, unless substantial macrophytes and algal material persist into the dry season.
8. Although some links have been made between crustacean taxa and various water qualities, hydrological and physical aspects, these require further investigation under controlled conditions, and are based at present on broad correlational data only.
9. Hydroperiod appears to be reflected most accurately by aquatic invertebrate communities – although such an approach would be applicable for seasonally inundated systems only.
10. Subtleties in hydroperiod appear to be of great importance in determining wetland crustacean community structure and hence are of biodiversity significance. The extent to which wetland soils actually dry out in the dry season apparently has the capacity to affect invertebrate ecosystem structure – for this reason, it is arguably an aspect that should be included even when wet season assessments can be carried out, as it adds significantly to the understanding of existing thresholds determining wetland character, and thus allows estimates of trajectories of wetland change to be made, particularly with respect to changes in hydroperiod.

In conclusion, this study has focused on measurements of wetland structure. Based on these, coarse estimates of function can be made. It is noted that once such estimates

have been informed by even a conceptual understanding of the major drivers and threshold conditions determining present wetland structure, other assessment protocols may be more easily applicable to the assessment of these systems. We consider assessment tools such as WET-EcosystemServices (Kotze *et al.,* 2008) and WET-Health (Macfarlane *et al.,* 2008) to be complementary to the assessment strategies outlined in this report, which are essentially enabling devices to improve conceptual understanding of these wetlands to a point where other metrics may reasonably be applied.

It is recognized that, as in all wetland assessments, the time taken for the elucidation of results is critical in determining the feasibility of applying any metric or suite of assessment protocols. With the exception of assessments of invertebrates from artificially inundated sediments, all of the assessment "tools" presented here are essentially structured observations that could be carried out during any routine dry season site visit. The collection of sediment samples for moisture analysis requires one day of laboratory time for drying of samples, as well as technician time in repeated weighing of soil samples. Incubation of sediment to assess wetland biota is more time-consuming, however, with preliminary results only possible between 20 and 35 days after inundation and, in terms of the protocol outlined in this study, inundation itself only occurring some three to four weeks after sample collection, to allow for drying.

The actual identification of crustaceans during laboratory incubations is carried out at a coarse level, which would require relatively little training for a skilled technician to achieve. However, if biodiversity assessment is a key criterion of a dry season assessment, then identification of specimens to genus or species level would be desirable, and for this, input from an experienced aquatic invertebrate taxonomist would usually be required. Despite the additional time entailed in the laboratory assessments, it is argued that this approach, which lends itself to fine-tuning over time, does provide valuable additional information that can add substantially to the identification of a particular area as wetland, and its characterization.

Finally, we wish to stress that although considerable information can be gleaned about wetland function, structure and character through assessment of the suite of indicators outlined here, the assessment remains at best a surrogate for repeated sampling of a system in its wetted condition. Nevertheless, even where wet season assessments have been possible, dry season assessments add an important dimension to the understanding of wetland function, by indicating threshold hydrological, chemical and

physical conditions that in many cases constitute actual threshold conditions for the survival of particular species in that habitat.

6.2 Suggestions for further research

This project provides a useful platform from which to conduct further studies, which will increase scientific understanding of life history patterns and drivers of the invertebrate fauna of temporary wetlands. The potential usefulness of various crustaceans, as well as diatoms and algae, as bio-indicators of environmental conditions (e.g. heavy metal pollution, nutrient enrichment, anthropogenic salinity, toxicity) has been illustrated by several studies (ostracods: Ruiz *et al.,* 1995; copepods and cladocerans: Rinderhagen *et al.,* 2000; algae and diatoms: Charles, 1996; Schoeman, 1976; 1979; see also DWAF, 2004; Dallas and Day, 1994; Harding *et al.,* 2005). Similar hatching experiments to those described in this study may well provide further insight into the use of these organisms as bio-indicators.

It is known that for certain species of fairy shrimp, eggs from a single batch do not all hatch after the first inundation. Some will hatch only after multiple inundations, while the majority will hatch after being wet and dried only once (Davies and Day, 1998). Multiple inundations were not carried out in this study, but similar experiments to those conducted in this study and incorporating multiple inundations could prove valuable for understanding more about the biology of these organisms. Additionally, further investigation into the effects of drying of soil samples from wetlands impacted by longer than natural hydroperiods is suggested, since only a basic preliminary assessment was achieved in the present study.

Our knowledge of the plants most characteristic of temporary wetlands is poor. The plant species lists should therefore be subject to ongoing refinement resulting from studies of the habitat requirements of wetland plants.

Most importantly, we need to investigate regional differences in responses of invertebrates to *in vitro* incubation in order to obtain the greatest amount of information possible from incubation experiments. While the techniques themselves are probably adequate for propagules across the southern African region, optimal conditions of temperature and salinity are likely to differ from area to area, particularly when comparing propagules from summer and winter rainfall areas.

Given the information we now have, it should be possible to investigate more thoroughly the possibility of using propagules as a reliable means of estimating the environmental condition or integrity of temporary wetlands, even in the dry season.

6.3 Conclusions

Considerable progress has been made in our understanding of the biology of temporary wetland organisms. Such organisms, together with other biophysical indicators, can provide useful information on the presence of cryptic wetlands during the dry season.

Table 6.1: Summary of major physical, chemical and biological indicators available for assessment during the dry season and providing information on particular aspects of wetland condition

Indicator	Condition indicated	Complementary indicators	Confidence
Biotic indicators			
Invertebrates			
• Invertebrates hatched out from dry season sediments under laboratory conditions			
Overall invertebrate assemblage	Crustacean assemblage a surrogate for wet season component – can show expanded faunal component, including sequential colonization effects; insect and other invertebrate components unlikely to be represented in dry season sediment samples. If site known to include wetlands but crustacean component absent from hatched samples – then either hydroperiod is too long or wetland not seasonally inundated, but rather saturated Presence of aquatic invertebrates indicates wetland now or in past subject to seasonal inundation This may be the only indicator of small, cryptic wetlands on rocky substrata with no plants and virtually no soil.	Dry season soil moisture Presence of shells/ exoskeletons of aquatic invertebrates	High
Crustacean component			
Anostraca	Abundant when dry season soils very dry (<6%) Potentially intolerant of high free ammonia concentrations (>0.1 mg/L), high EC (>900 mS/m), summer moisture	Soil moisture Abiotic indicators	Low – data correlative only
Conchostraca	Abundant when dry season soils very dry (<6%) Absent when dry season soils >30% moisture		
Cladocera	Abundant when dry season soils very dry (<30%) Tolerant of wide range of nutrient availability, turbidity and EC		
Ostracoda	Abundant when dry season soils very dry (<30%) Tolerant of wide range of nutrient availability, turbidity and EC		
Copepoda	Abundant when dry season soils very dry (<30%) Tolerant of wide range of nutrient availability, turbidity and EC		
• Presence of old cases, exoskeletons, shells of aquatic invertebrates in sediments	Indicative that periodic inundation of the site has taken place in the past – not reliable indicator of present hydroperiod unless other factors present	Abiotic and invertebrate indicators	Low

Indicator	Condition indicated	Complementary indicators	Confidence
Macrophytes			
• Presence of perennial or annual hydrophytes – growing or clearly identifiable dried plant remnants during the dry season	Wetland conditions definitely present – plant species habitat requirements (e.g. inundation etc.) will determine wetland type (e.g. seasonally inundated) and (low confidence) range of habitats	Invertebrate and abiotic indicators	High
• Presence of facultative wetland species	Wetlands may be present – in drier climates, presence of facultative wetland species has a higher likelihood of being linked to wetland conditions	Invertebrate and abiotic indicators	Low to medium
• Absence of both dryland and wetland plants from site	Presence of a wetland cannot be ruled out on this basis alone; in absence of invertebrate, soil or other markers, presence of seasonally inundated wetland unlikely, but small wetlands in rocky substrata may have none of these	Signs of recent fire? Abiotic indicators Invertebrate indicators	Low
• Presence of halophytes	Indicate saline soils in and around wetlands – but may also indicate non-wetland saline soils, especially in mesic areas	Other abiotic and/or biotic indicators essential	Low
Algae			
• Algae developing in incubated samples	May simply represent opportunistic propagation of air-borne spores – identification to genus/species level may improve confidence	Abiotic and biotic indicators	Low
• Presence of dried algal remnants	Indicative of wet season water levels – indicates seasonal/periodic inundation	Soil moisture Invertebrates	High
Abiotic indicators			
• Topography	Indicates potential for accumulation of water in wet season – must be interpreted with other indicators	Abiotic and biotic	Low
• Soil wetness	Presence of gleying, mottling: if present as per DWAF (2005) then indicates wetland type (permanent/seasonal etc.) Absence of above, coupled with sandy soil, and/or arid climate and/or perched wetland conditions: cryptic wetland cannot be ruled out Dry season soil moisture data: ➢ >30% and presence of other indicators of wetland conditions:	Biotic and abiotic	High Low

Indicator	Condition indicated	Complementary indicators	Confidence
	wetland may not support crustacean fauna; lower species diversity ➢ <30% and presence of other indicators of wetland conditions: wetland may support crustacean fauna and potentially linked to higher species diversity/endemism		
	Dry season water table <0.5 m from surface OR impermeable layer <0.5 m from surface: indicates wetland presence but not hydroperiod (inundated or not) Dry season water table >0.5 m from surface OR impermeable layer >0.5 m from surface: no strong conclusions can be drawn	Biotic and abiotic – including soil moisture	Medium Low
• Muck layer	Thin layer (<2 cm deep): Presence: wetland conditions in recent past/present Absence: inconclusive Thick layer (<2 cm deep): wetland conditions in past		 Medium Medium
• Sediment deposits on plants and/or rocks	Presence: indicates minimum levels of inundation – wetland assumed to be seasonally inundated Absence: inconclusive		Medium Low
• Biotic crusts	Presence: indicates minimum levels of inundation – wetland assumed to be seasonally inundated Absence: inconclusive		High Low
• Water marks	Presence: indicates minimum levels of inundation – wetland assumed to be seasonally inundated Absence: inconclusive		Medium Low

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8. GLOSSARY

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

Aestivation: a state in which animals completely lack measurable activity during hot and/or dry periods

Anoxic: lacking in oxygen

Biotic: pertaining to living organisms (*cf.* abiotic)

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

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

Chironomidae: non-biting midges

Cladocera: water fleas such as *Daphnia*

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

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

Cryptic: hidden

Delineation: the process of marking out of the extent of

Diapause: a period of suspended activity broken by an appropriate environmental cue

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

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

Halophyte: a salt tolerant plant

Heleoplankton: floating vegetation

Helophyte: a marsh plant

Hydromorphic: of soil, with properties (e.g. mottling, greyness) imparted by wet conditions

Hydrophilic: water-loving

Hydrophyte: a water plant

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

Interstitial: of animals, living between grains of sand

Invertebrate: an animal without a backbone

Larva: the free-living immature stage of an animal that is unlike the adult

Macrophyte: a large plant; in wetland studies usually a large plant growing in shallow water or waterlogged soils

Morphology: structure

Nauplius: the first larval stage of some crustaceans

NTU: nephelometric turbidity units – the standard unit of turbidity

Pan: a shallow, usually large (>1ha), temporary water body

Perennial: permanent; persisting from year to year

Phyllopoda: essentially the same as Branchiopoda (*q.v.*)

Plankton: aquatic organisms, usually very small, which drift passively with the surrounding water

Podsol: a soil with an organic mat and a thin organic-mineral layer, above a light gray leached layer resting on a dark horizon

Podzolization: the process of podsol formation

Propagule: any structure (e.g. an egg or a spore) from which a new individual can be produced

Quiescence: inactivity

Rotifera: minute ciliated aquatic animals

Tardigrada: minute aquatic animals that are known for their ability to enter diapause for lengthy periods

Temporary zone: of wetlands, the zone that is alternately inundated and exposed

Temporary: of wetlands, those in which water is not permanently present

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

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

APPENDIX 1

DISTRIBUTION AND HABITATS OF SOME MAJOR MACROPHYTES OF TEMPORARY WETLANDS IN SOUTHERN AFRICA

NOTE: Although many of these species have been recorded from temporarily inundated wetlands, most are not confined to such biotopes. Numbered references listed at end of table.

Table A1.1: Distribution and habitats of some major macrophytes of temporary wetlands in southern Africa

FAMILY	SPECIES	HABITS AND TEMPORARY HABITATS	RECORDED OCCURRENCE IN TEMPORARY WATERS	BIOGEOGRAPHICAL DISTRIBUTION	REF
Characeae	*Nitella dregeana*	Submerged aquatic: alpine tarns	Drakensberg	Endemic to southern Africa	5
Isoetales	*Isoetes capensis*	Submerged/emergent: seasonal marshes	S-w Cape	Endemic (endangered)	1,4
	I. schweinfurthii	Submerged/emergent: pools and pans	Free State	Drier western parts of SA to Namibia and Botswana	1,4
Marsileaceae	*Marsilea macrocarpa*	Submerged/emergent: pools in river beds; sporocarps resistant to desiccation	Kalahari	South Africa (excluding s-w Cape) to central Africa	3,4,8
Apogonetonaceae	*Aponogeton angustifolius*	Floating-leaved aquatic: pools and pans	S-w Cape	Endemic	2,3,10
	Aponogeton azureus	Floating-leaved aquatic	NW Namibia	Narrow endemic	10

FAMILY	SPECIES	HABITS AND TEMPORARY HABITATS	RECORDED OCCURRENCE IN TEMPORARY WATERS	BIOGEOGRAPHICAL DISTRIBUTION	REF
	Aponogeton desertorum	Floating aquatic: rock pools	S Zaire to southern Africa	E Cape to Namibia and Botswana	2,3,10
	Aponogeton distachyos	Floating-leaved aquatic: pools and pans	S-w Cape	Endemic	2,3,10
Apogonetonaceae	*Aponogeton junceus*	Emergent aquatic: pools, rivers and high-altitude tarns	Kalahari; Drakensberg	Widespread in southern Africa	2,3,5,8, 10
	Aponogeton rehmannii	Emergent aquatic: pools, not high-altitude	Kenya to South Africa	Widespread in southern Africa	10
Cyperaceae	*Eleocharis limosa*	Emergent: pools	Free State	South and east of SA to Namibia, Madagascar	
	Isolepis cernua (= *Scirpus cernuus* in 3)	Emergent: pools	Free State	S-w Cape to eastern parts of SA	2,3,10
	Isolepis fluitans	Emergent: pools and tarns	Free State, Drakensberg	Southern and tropical Africa except s-w Cape and Namibia	1,3,5,10
	Ficinia nodosa	Emergent: seasonal marshes	S-w Cape	S-w Cape to KwaZulu-Natal and southern continents	27
	Schoenoplectus sp.	Emergent: varied	S-w Cape	Widespread	10
	Bolboschoenus maritimus	Emergent	S-w Cape	Northern hemisphere and Africa	10
Hydracharaceae	*Lagarosiphon muscoides*	Submerged aquatic: pans, alpine tarns	Drakensberg	E Cape to tropical Africa (widespread)	2,3,5,10
Juncaceae	*Juncus kraussii*	Emergent: seasonal marshes	S-w Cape	S-w Cape to KwaZulu-Natal; southern hemisphere	2,7,10
	J. oxycarpus	Emergent : subalpine tarns	Drakensberg	S-w Cape to Zimbabwe	2,5,10

FAMILY	SPECIES	HABITS AND TEMPORARY HABITATS	RECORDED OCCURRENCE IN TEMPORARY WATERS	BIOGEOGRAPHICAL DISTRIBUTION	REF
Najadaceae	*Najas graminea*	Submerged aquatics: pans; tolerates brackish water		Knysna and north into southern Africa	2,3,10
Poaceae	*Agrostis lachnantha* (= *A. huttoniae)*	Tall, tufted grass: subalpine tarns	Drakensberg	Widespread in Africa	1,5,6,10
	A. subulifolia	Tufted grass: subalpine tarns	Drakensberg	Rare Afromontane endemic	1,5,6
	Aristida adscencionis	Tall, tufted grass: pans	Pro-Namib	Widespread in southern Africa and the tropics	2,6,8
Poaceae	*Cynodon dactylon*	Sward-forming grass: pans and marshes	Caprivi; Etosha; Caribbean	Widespread: s-w Cape to tropical Africa and cosmopolitan	2,6,9
	Eragrostis planiculmis	Erect, tufted grass: pans and tarns	Drakensberg	Karoo and Namibia north to tropical Africa and India	5,6,10
	Imperata cylindrica	Very tall, erect grass: floodplain pans	Caprivi; North America	S-w Cape to Old World tropics	2,6,9,10
	Ischaemum afrum	Tall, erect grass (ruderal weed): pans	Former Transvaal lowlands	KwaZulu-Natal, former Transvaal to tropics worldwide	6,9,10
	Merxmuellera cincta	Tall, reed-like grass: seasonal marshes	S-w Cape	Fynbos endemic	2,6,7,10
	Miscanthus junceus = *Miscanthidium junceum*	Tall, tufted grass: floodplain pans	Caprivi	Savanna and grasslands of southern Africa	6,9,10
	Odyssea paucinervis	Mat-forming grass: pan edges	Etosha	Drier western parts of southern Africa to Congo River	6,9,10
	Phragmites australis = *Phragmites communis*	Very large reed	Widespread; Caribbean	Widespread in tropics	10

FAMILY	SPECIES	HABITS AND TEMPORARY HABITATS	RECORDED OCCURRENCE IN TEMPORARY WATERS	BIOGEOGRAPHICAL DISTRIBUTION	REF
	Phragmites mauritanus	Very large reed	Widespread (not in W Cape)	Africa	10
	Setaria incrassata (= *S. woodii* in 9)	Erect, tufted grass: pans	Former Transvaal lowveld	Southern Africa, widespread in grassland and savanna	6,9,10
	Sporobolus consimilis	Short, tufted, grass: pans	Former Transvaal lowveld	Northern parts of southern Africa to tropical Africa	6,9,10
	S. coromandelianus	Ephemeral grass: pans	Kalahari; Caribbean	Drier western parts of southern Africa	6,8,10
	S. spicatus	Mat-forming grass: saline pans	Etosha	Northern Namibia and Botswana to tropical Africa, Mediterranean, India	6,9,10
Poaceae	*S. virginicus*	Mat-forming grass: seasonal marshes	S-w Cape; Caribbean	Cosmopolitan	2,7,9,10
Potamogetonaceae	*Potamogeton pusillus* (unlikely to be temporary)	Submerged or emergent aquatic	Pans; N. America	Cosmopolitan	2,3,10
Restionaceae	*Chondropetalum nudum*	Emergent: seasonal marshes	S-w Cape; Darling to Albertina)	Endemic	2,7
	Chondropetalum rectum	Emergent: damp clay	Peninsula to Agulhas	Endemic	2
		Emergent: seasonal marshes	S-w Cape	Endemic	2,7,10
Aizoaceae	*Trianthema triquetra*	Ephemeral in drying pans	Kalahari	Drier western parts of southern Africa	2,8
Amaranthaceae	*Calicorema capitata*	Emergent: loamy pans	Namib	E Cape to Namibia	1,2,8
Asteraceae	*Gnaphalium filagopsis* (=*Amphidoxa filaginea* in 3)	Emergent: pools	Free State	Central highlands of southern Africa, including Angola	1,3,10

FAMILY	SPECIES	HABITS AND TEMPORARY HABITATS	RECORDED OCCURRENCE IN TEMPORARY WATERS	BIOGEOGRAPHICAL DISTRIBUTION	REF
	Nicolasia costata	Emergent: floodplain pans	Caprivi	Namibia and Botswana	1,9
	Platycarpha carlinoides	Emergent: clay pans	Namibia	Widespread in drier western parts of southern Africa	1,2,8
	Plecostachys serpyllifolia (= *Helichrysum orbiculare* in 7)	Emergent: seasonal marshes	S-w Cape	S-w Cape to KwaZulu-Natal	2,7
	Senecio cryptolanatus	Emergent: alpine tarns	Drakensberg	Mountains of South Africa	2,5,10
Ceratophyllaceae	*Ceratophyllum demersum*	Submerged aquatic: pans	Widespread; Caribbean	E Cape and north: cosmopolitan	2,3,10
Amaranthaceae (= Chenopodiaceae)	*Salsola aphylla*	Emergent: pans	Karoo	Drier western parts of southern Africa to Namibia and Botswana	2,8,10
	Sarcocornia natalensis = *Arthrocnemum affine*	Perenniall dwarf halophyte	Saline wetlands	Angola to Madagascar	2,10
	Sarcocornia pillansi = *Arthrocnemum dunense* = *A. hottentoticum* = *A. namaquense*	Perenniall dwarf halophyte	Saline wetlands	Namibia to Mozambique	2,10
Crassulaceae	*Crassula expansa*	Submerged aquatic: pools	Free State	S Cape to former Transvaal, Namibia	2,3
	C. inanis	Submerged aquatic: tarns, pools	Free State, Drakensberg	S-w Cape to former Transvaal	2,3,5,10
Eriocaulaceae	*Eriocaulon abyssinica*	Submerged semiaquatic: bogs and pools	Free State	Southern Africa (except s-w Cape) to tropical Africa	1,3,10
Gentianaceae	*Nymphoides indica*	Aquatic, floating leaves: pans	Widespread; Caribbean	S Cape to Old World tropics	2,3,10

FAMILY	SPECIES	HABITS AND TEMPORARY HABITATS	RECORDED OCCURRENCE IN TEMPORARY WATERS	BIOGEOGRAPHICAL DISTRIBUTION	REF
	Orphium frutescens	Emergent: seasonal marshes	S-w Cape	Endemic	2,7
Nymphaeaceae	*Nymphaea capensis*	Aquatic, floating leaves; perennating organs resist desiccation: pans	N. America	S-w Cape to tropical east Africa, Madagascar	2,3
Onagraceae	*Ludwigia adscendens* = *L. stolonifera*	Emergent: survives dry phase as reduced terrestrial form	Widespread in pans	Orange River to Iran and N America	1,2,3,10
Portulacaceae	*Samolus porosus*	Emergent: seasonal marshes	S-w Cape	S-w Cape to KwaZulu-Natal	2,7,10
Ranunculaceae	*Ranunculus meyeri*	Floating-leaved aquatic: tarns and pools	Drakensberg	South Africa (except s-w Cape)	1,5,10
Scrophulariaceae	*Cyncium tubulosum* (= Rhamphicarpa tubulosa in 9)	Emergent: floodplain pans	Caprivi	E Cape and Namibia to tropical Africa	1,2,9
	Lindernia conferta	Floating-leaved aquatic: pools	Drakensberg, Free State, Zimbabwe	Central highlands of southern Africa	1,3,10
	Chamaegigas intrepida = *Lindernia intrepida*	Floating-leaved aquatic: potholes in granite	Namibia	Endemic	3,10
	Limosella grandiflora (= *L. capensis* in 3, 5)	Submerged aquatic: pools, tarns, seasonal marshes	Free State, s-w Cape, Drakensberg,	Widespread, s-w Cape to KwaZulu-Natal, former Transvaal and Namibia	2,3,5,10
	L. africana	Floating-leaved aquatic: seasonal marshes	S-w Cape	S-w Cape to tropical Africa	2,10
	Sopubia simplex	Emergent: floodplains; semi-parasitic	Caprivi	Knysna and eastwards to tropical Africa	2,9
Typhaceae	*Typha capensis* = *Typha latifolia capense*	Large reed	Widespread; Uganda to S. Africa	Africa	10

FAMILY	SPECIES	HABITS AND TEMPORARY HABITATS	RECORDED OCCURRENCE IN TEMPORARY WATERS	BIOGEOGRAPHICAL DISTRIBUTION	REF
	Typha domingensis = *Typha australis*	Large reed	Widespread	Pantropical	10

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APPENDIX 2

INVERTEBRATE SPECIES KNOWN TO INHABIT TEMPORARY WATERS IN SOUTHERN AFRICA

Note: This list includes species that are likely to colonize from adjacent permanent water bodies during periods when temporary wetlands are inundated.

Numbers in brackets refer to references citing distributions.

BOT	Botswana
EC	Eastern Cape
FS	Free State
GT	Gauteng, South Africa
KZN	KwaZulu-Natal
LES	Lesotho
MP	Mpumalanga, South Africa
NC	Northern Cape
NSC	Skeleton Coast, Namibia
NNN	Namib-Naukluft Park
NAM	Namibia
SA	South Africa
SnA	Southern Africa
WC	Western Cape
ZIM	Zimbabwe

COELENTERATA	
HYDROZOA	
HYDROIDEA	
Hydra sp.	NSC (5)
PLATYHELMINTHES	
TURBELLARIA	
RHABDOCOELA	
Mesostoma	NSC(5), WC (pers), BOT (6, 10)
Syrinx kolasarum	BOT (10)
Mesostoma thamagai	BOT (10)
Caliadne isoldae	BOT (10)

Gieysztoria faubeli	BOT (10)
Numerous unnamed species	widespread (pers)
NEMATODA	
Numerous unnamed species	widespread (pers)
ROTIFERA	
Branchionus calyciflorus dorcas	NSC (5)
Numerous unnamed species	widespread (pers)
CRUSTACEA	
BRANCHIOPODA	
NOTOSTRACA	
Triops granarius	throughout arid southern Africa (13) FS (15), NAM (NSC, NNN: 5), WC (pers)
ANOSTRACA	
Artemia salina	NAM (pers) WC (pers, 11)
Branchinella ondonguae	SnA dry savanna, BOT, NAM (7, 8)
Branchinella ornata	SnA dry savanna, BOT (7, 8)
Branchipodopsis barnardi	SA (7, 8)
Branchipodopsis browni	SA arid west (7, 8)
Branchipodopsis dayae	SA (7, 8)
Branchipodopsis drakensbergensis	SA eastern escarpment (7, 8)
Branchipodopsis drepane	NAM (7, 8)
Branchipodopsis hodgsoni	EC (7, 8)
Branchipodopsis hutchinsoni	SA (7, 8)
Branchipodopsis kalaharensis	BOT (7, 8)
Branchipodopsis kaokoensis	NAM (7, 8), NSC (5)
Branchipodopsis karroensis	SA (7, 8)
Branchipodopsis natalensis	SA eastern escarpment (7, 8)
Branchipodopsis scambus	SA (7, 8)
Branchipodopsis simplex	NAM (7, 8)
Branchipodopsis tridens	FS (15), NAM (NNN: 5), BOT, ZIM, SA arid west (7, 8)
Branchipodopsis underbergensis	SA eastern escarpment (7, 8)
Branchipodopsis wolfi	NAM, BOT, SA (7, 8)

Metabranchipus sp.	tropical (7, 8)
Phallocryptus spinosa (= *Branchinella spinosa*)	BOT (7, 8)
Pumilibranchipus deserti	NAM (7, 8)
Rhinobranchipus martensi	EC (7, 8)
Streptocephalus bidentatus	SA, ZIM, subtropical (7, 8)
Streptocephalus bourquinii	SA subtropical (7, 8)
Streptocephalus cafer	NNN (5), NAM, BOT, ZIM, SA (7, 8)
Streptocephalus cirratus	SA highveld (7, 8)
Streptocephalus cladophorus	NAM, ZIM, SA dry savanna (7, 8)
Streptocephalus dendrophorus	SA subtropical (7, 8)
Streptocephalus dendyi	WC (11), EC (7, 8)
Streptocephalus dregei	SA E Cape inland (7, 8)
Streptocephalus gracilis	EC (7, 8)
Streptocephalus indistinctus	FS (15), NAM, BOT, ZIM dry savanna (7, 8)
Streptocephalus kaokoensis	NAM (7, 8)
Streptocephalus macrourus	FS NAM, BOT, dry savanna (7, 8)
Streptocephalus namibiensis	NAM, BOT, SA dry savanna (7, 8)
Streptocephalus ovamboensis	NAM (NSC: 5), BOT, SA arid west (7, 8)
Streptocephalus papillatus	SA arid west (7, 8)
Streptocephalus proboscideus	NAM, BOT, SA dry savanna (7, 8)
Streptocephalus *propinquus*	SA (7, 8)
Streptocephalus purcelli	WC (7, 8, 11)
Streptocephalus spinicaudatus	EC inland (7, 8)
Streptocephalus trifidus	ZIM (7, 8)
Streptocephalus vitreus	ZIM (7, 8)
Streptocephalus wirminghausi	ZIM (7, 8)
Streptocephalus zuluensis	ZIM, SA subtropical (7, 8)
CONCHOSTRACA	
Caenestheriella cf. *australis*	FS (15), NNN (5) (2)
Cyclestheria hislopi	BOT (3), NAM, ZIM (2)

Cyzicus australis	widespread in dry SnA (2)
Eocyzicus obliquus	central SA (2)
Eulimnadia cf. *africana*	NSC (5), n SA, ZIM, BOT (3), NAM (2)
Eulimnadia alluaudi	Kalahari (2)
Eulimnadia dentatus	EC (2)
Eulimnadia dentatus	NAM (2)
Leptestheria brevirostris	NAM, BOT (2, 3)
Lepthestheria rubidgei	NAM (NNN: 5), WC (pers), NC, EC, GT, BOT (3), LES (2)
Lepthestheria cf. *striatoconcha*	NAM (NSC: 5), GT (2)
Leptestheriella calcarata	BOT (3), NC (2)
Leptestheriella cf. inermis	NAM (NNN: 5), NC (2)
Leptestheriella setosa	Kalahari (2)
Lyncaeus bicarinatus	FS, NAM (2)
Lyncaeus lobatsianus	BOT (2, 3)
Lyncaeus pachydactylus	GT (2)
Lyncaeus triangularis	EC (2)_
Lyncaeus truncatus	NAM, KZN (2)
CLADOCERA	
Alona karua	NAM (5)
Alona sp. (*rectangula* group)	NAM (5)
Alona sp.	WC (11)
Alonella exigua	WC (11)
Bosmina longirostris	WC (11)
Ceriodaphnai dubia	NAM (5), WC (11)
Ceriodaphnia cf. *megops*	WC (11)
Ceriodaphnia cf. *laticaudata*	WC (11)
Ceriodaphnia reticulata	WC (pers)
Ceriodaphnia rigaudii	FS (15)
Ceriodaphnia reticulata	WC (11)
Ctenodaphnia sp.	NAM (5)
Chydorus ‘sphaericus’	WC (11)
Daphnia atkinsoni	WC (11)
Daphnia carinata	WC (11)
Daphnia daphniopsis	WC (11)

Daphnia 'pulex'	WC (11)
Daphnia 'similis'	WC (11)
Dunhevedia crassa	WC (11)
Eurycercus cf. *lamellatus*	WC (11)
Karualona karua	WC (11)
Leberis n. sp.	BOT (10)
Leydigia macrodonta	WC (pers)
Macrothrix capensis	WC (11)
Macrothrix cf. *gouldi*	NAM (5)
Macrothrix triserialis	NAM (5)
Megafenestrata aurita	WC (11)
Moina belli	NAM (5)
Moina brachiata	WC (11)
Moina dubia	NAM (5)
Moina cf. *hartwigi*	NAM (5)
Moina micrura	FS (15), NNN (5)
Moina 'mongolica'	WC (11)
Moina reticulata	NAM (5)
Scapholeberis kingi	WC (pers, 11)
Simocephalus australiensis	WC (pers)
Simocephalus exspinosus	WC (11)
Simocephalus vetulus	FS (15), WC (11)
COPEPODA	
Acanthocyclops vernalis	WC (pers)
Eucyclops (Afrocyclops) gibsoni	NAM (5)
Eucyclops serrulatus	WC (11)
Lovenula africana	BOT (14)
Lovenula excellens	MPUM (14)
Lovenula falcifera	FS (15), GT, NAM (14)
Lovenula simplex	WC (11, 14, pers)
Harpactacoida	widespread, many species
Mesocyclops major	WC (11)
Mesocyclops oblongatus	NAM (5)
Metadiaptomus capensis	WC (11, pers)
Metadiaptomus purcelli	*WC* (11, pers)
Metadiaptomus colonialis	SA, NAM, ZIM (14)

Metadiaptomus transvaalensis	FS (15), MPUM, ZIM, BOT (14)
Metadiaptomus meridianus	NAM, SA (14)
Metadiaptomus gauthieri	NAM (14)
Microcyclops crassipes	WC (11)
Microcyclops inopinatus	NAM (5)
Paracyclops poppei	WC (11)
Paradiaptomus hameri	WC (11)
Paradiaptomus lamellatus	WC (11, pers)
Paradiaptomus natalensis	KZN, EC, NAM (14)
Paradiaptomus similis	NAM (14)
Paradiaptomus schulzei	FS (15), NAM (5)
Paradiaptomus peninsularis	WC (14)
Paradiaptomus hameri	WC (14)
Paradiaptomus warreni	Drakensberg (14)

OSTRACODA

Amphibolocypris sp.	BOT (10)
Apatelecypris schulzei	NAM (5)
‘*Cyclocypris’ pusilla*	WC (pers)
Cypricercus episphaena	WC (pers)
Cypricercus inermis	widespread (10)
Cypricercus inermis	BOT (10)
Eucypris cf. *trigona*	NAM (5)
Eundacypris superba	NAM (12)
Globocypris trisetosa	widespread (12)
Gomphocythere cf. *expansa*	WC (pers)
Hemicypris reticulata	widespread (10)
Heterocypris cf. *congenera*	NAM (5)
Heterocypris cf. *giesbrechti*	NAM (5)
Heterocypris sp.	BOT (10)
Homocypris conoidea	WC (pers)
Isocypris perangusta	NAM (5)
Korannacythere (4 sp)	Drakensberg (12)
Leucocythere helenae	EC (12)
Megalocypris durbani	EC, Drakensberg (12)
Megalocypris hispida	WC (12, pers)
Megalocypris princeps	WC (12)

	Ovambocythere milani	NAM (12)
	Parastenocypris declivis	WC (pers)
	Parastenocypris pardalis	WC (pers)
	Pseudocypris sp.	widespread SA, BOT, Nam (12)
	Physocypria capensis	WC (pers)
	Plesiocypridopsis inaequivalva	NAM (12)
	Potamocypris mastigophora	NAM (12)
	Potamocypris sp.	BOT (10)
	Ramotha sp.	widespread in SA, ZIM, NAM (12)
	Sarscypridopsis gregaria	widespread (10)
	Sarscypridopsis cf. pygmaea	NAM (12)
	Sarscypridopsis spinifera	WC (pers)
	Sarscypridopsis striolata	WC (pers)
	Sarscypridopsis trigonella	WC (pers)
	Sclerocypris clavularis	FS (15), BOT (10)
	Sclerocypris coomansi	NAM (12)
	Sclerocypris dayae	NAM (12)
	Sclerocypris dedeckkeri	NAM (12)
	Strandesia sp.	BOT (10)
	Strandesia cf. *vinciguerrae*	NAM (12)
	Zonocypris cordata	WC (pers)
ARACHNIDA		
ACARINA	‘Hydracarina’: many species	widespread (pers)
	Aquanothrus montanus (Oribatidae)	BOT (10)
INSECTA		
EPHEMEROPTERA		
	Cloeon sp.	NAM (NSC, NNN: 5), BOT (10), WC (11)
ODONATA		
	Crocothemis sp.	WC (11)
	Diplocodes sp.	WC (11)
	Enallagma sp..	NAM (NSC: 5), WC (11)
	Nannothemis sp.	NAM (NNN, NSC: 5)
	Pantala flavescens	BOT, widespread (10)
	Paragomphus sp.	NAM (5)
	Peltothemis sp.	NAM (NNN, NSC: 5)

Progomphus sp.	NAM (NSC: 5)
HEMIPTERA	
NOTONECTIDAE	
Anisops amaryllis	WC (11)
Anisops debilis/balcis	WC (11)
Anispos hypatia	WC (11)
Anisops sardea	NAM (5), WC (11)
Buonoa sp.	NAM (NSC: 5)
Notonecta lactitans	WC (11)
NAUCORIDAE	
Pelocoris sp.	NAM (5)
CORIXIDAE	
Micronecta scutellaris	WC (11)
Micronecta youngiana	BOT (10)
Rhamphocorixa sp.	NAM (NSC: 5)
Sigara cf. *contortuplicata*	NAM (5)
Sigara meridionalis	WC (11)
Sigara pectoralis	WC (11)
Sigara wahlbergi	WC (11)
PLEIDAE	
Plea sp.	WC (pers)
TRICHOPTERA	
Oxyethira velocipes	WC (11)
LEPIDOPTERA	
Pyralidae sp.	WC (11)
COLEOPTERA	
GYRINIDAE	
Dineutus subspinosus	NAM (5)
HYDROPHILIDAE	
Amphiops sp.	WC (11)
Berosus cuspidatus	BOT (10)
Berosus sp.	NAM (5), WC (11)
Caelostoma rufitarse	NAM (5)
Derallus sp.	WC (11)
Hydrochus sp.	WC (11)
Laccobius sp.	WC (11)

Tropisternus sp.	NAM (5)
DYTISCIDAE	
Bidessus sp.	WC (11)
Cybister tripunctatus africanus	NAM (5)
Herophydrus sp.	NAM (5)
Hydroglyphus lineolatus	NAM (5)
Hydroglyphus infirmus	NAM (NSC: 5), BOT (10)
Hydroglyphus zanzibarensis	NAM (NSC: 5)
Hydroporus sp.	WC (11)
Laccophilus simplicistriatus	NAM (5)
Yolina brincki	NAM (NSC: 5)
HALIPLIDAE	
Haliplus sp.	WC (11)
HYDRAENIDAE	
Hydraena sp.	WC (11)
Ochthebius sp.	NAM (5), WC (11)
DRYOPIDAE	
Heterocerus sp.	NAM (5)
PTILIDAE	
Unknown sp.	WC (11)
STAPHYLINIDAE	
Unknown sp.	WC (pers: salt pans)
DIPTERA	
CHIRONOMIDAE	
Ablabesmyia sp.	WC (11)
Chaetocladius n. sp.	WC (11)
Chironomus formosipennis	WC (11)
Chironomus imicola	most of Africa (9)
Chironomus pulcher	most of Africa (9)
Chironomus sp.	BOT (10)
Cladotanytarsus capensis	WC (11)
Corynoneura sp.	WC (11)
Dicrotendipes pilosimanus	WC (11)
Einfeldia n. sp.	WC (11)
Psectrocladius viridescens	WC (11)
Paramerina nigromarmorata	WC (11)

Procladius sp.	WC (11)
DIXIDAE	
Unknown sp.	WC (11)
CULICIDAE	
Anopheles rufipes	BOT (10)
Anopheles sp.	NAM (5)
Culex sp.	BOT (10), NAM (5), WC (11, pers)
Culiseta sp.	NAM (5), WC (11, pers)
Mimomyia sp.	WC (11)
PSYCHODIDAE	
Unnamed species	NAM (NSC: 5)
TABANIDAE	
Unnamed species	NAM (NSC: 5)
CERATOPOGONIDAE	
Atrichopogon sp.	WC (11)
Dasyhelea sp.	NAM (5)
MOLLUSCA	
GASTROPODA	
PROSOBRANCHIA	
Tomichia spp.	SA, WC (11, pers)
PULMONATA	
Bulinus forskali	C and S Africa (1, 4)
Bulinus globosus	SnA (1, 4)
Bulinus reticulatus	SnA (1, 4)
Bulinus tropicus	E Africa to S Africa (1, 4)
Ceratophallus natalensis	SnA (1, 4), WC (11, pers)
Ferrissia spp.	SnA (1, 4)
Lanistes ovum	Angola (1, 4)
Lymnaea truncatula	widespread in Africa (1, 4)
Pila ovata	tropical Africa (1, 4)
Physa acuta	WC (11, pers)
BIVALVIA	
Unionidae and *Spathopsis*	widespread (1)

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APPENDIX 3

BACKGROUND INFORMATION ON THE LIFE HISTORIES AND OTHER CHARACTERISTICS OF MAJOR GROUPS OF INVERTEBRATES THAT HATCHED OUT DURING LABORATORY TRIALS

A3.1 Ostracods (Crustacea, Ostracoda: seed shrimps)

Ostracods form an important part of the zoobenthos in African inland waters. Many species can survive harsh environmental conditions. Most species are relatively unselective scavengers. Ostracods are small- (<1 mm) to medium-sized (8 mm) bivalved crustaceans. Their bodies are completely enclosed by the carapace, which consists of two lateral valves. There are eight larval instars, the ninth instar being the adult. The animal no longer moults after it reaches the adult stage and matures: in other words, growth is determinate. All Cypridoidea, the superfamily to which the ostracods in this study belong, can produce drought-resistant eggs able to survive in desiccated form, sometimes for many decades (see Martens, 2001 for further details).

Megalocypris princeps (Sars 1898) is a remarkable ostracod. The subfamily to which it belongs is almost entirely endemic to Africa and the individuals of most species of the subfamily are very large. *Megalocypris princeps* is the largest non-marine ostracod in the world, reaching a length of 8 mm. It is typical of temporary vleis in the Western Cape province of South Africa.

A3.2 Phyllopods (Crustacea: Class Branchiopoda: clam and fairy shrimps)

A3.2.1 Conchostracans (clam shrimps)

The Conchostraca, commonly known as clam shrimps, are small (<20 mm), primitive freshwater crustaceans. They occur on all the continents except for Antarctica. In general conchostracans are found in temporary rainwater or snow-melt pools that regularly dry up completely or partially. Most conchostracans are laterally compressed, with a bivalved carapace that completely encloses the body and limbs. They reach between 3 mm and 18 mm in length, depending on species. The antennae are large and are used for swimming and burrowing. The trunk is generally composed of many segments, each with a pair of flattened phyllopodous limbs used for locomotion, feeding

and respiration. Conchostracans are mainly benthic animals. Most burrow into the surface layers of the substratum, where they lie with their ventral surfaces pointed upwards, and feed non-selectively on detritus and algae in suspension. They produce feeding currents by beating their thoracic limbs. Some species, including some of the leptestheriids (the type found in our samples), bumble about on the bottom, often permanently *in copulo,* the male holding the female in front of and at right angles to himself.

Conchostracans breed continuously throughout the adult stage. Resting eggs within resistant cysts are usually dropped when the female moults. These cysts can survive extremely unfavourable conditions. The time of hatching is often capricious and triggered by specific environmental conditions. The hatchlings are free-swimming metanauplius larvae with three pairs of appendages and a median eye. There are about five naupliar stages. From the moment of hatching it is a race against time for the organism to reach the adult stage and produce the maximum number of cysts before the pools dry up through evaporation (see Brendonck, 2000 for more details).

The conchostracan in our samples was *Leptestheria rubidgei* (Baird 1862), which is widespread in South Africa.

A3.2.2 Anostracans (fairy and brine shrimps)

Anostracans are small (in southern Africa, <30 mm in length), primitive crustaceans, most of which live in fresh water, although the common brine shrimp, *Artemia*, lives in extremely saline inland salt pans. Fairy shrimps occur on all continents bar Antarctica and are found only in temporary pools that regularly dry up. Anostracans are mid-water animals, normally swimming on their backs and filtering particulate matter, often algae, from the water or scooping organic material from the surface. Their flattened limbs provide propulsion for swimming, currents for feeding, and a large surface area for respiration. Reproduction is usually sexual, the eggs developing to metanauplii (second-stage larvae) within the egg sac of the female. The metanauplii are retained within the ‘egg’ shell, more properly called a cyst, which is covered by a hard, resistant coat before being laid. The larvae within their cysts are able to survive desiccation for many months or years. The cysts of all southern African species appear to require desiccation before they will hatch (see Brendonck, 2000 for more details).

The fairy shrimps in our samples belonged to the genus *Streptocephalus.* Only adult male anostracans can be identified to species level, and none of those from the incubation experiments grew to adulthood, so we cannot be sure of the species. Both *S. dendyi* and *S. purcelli* are known from the wetlands we sampled.

A3.3 Cladocerans (Crustacea: Class Branchiopoda: Order Cladocera)

The Cladocera are commonly known as 'water fleas'. Most species are found in fresh water at more or less neutral pH values. They vary in length from <1 mm to nearly 5 mm. The head is not covered by a carapace. The antennae are large and biramous and are the primary organs of locomotion. The body is covered by a folded, unhinged carapace consisting of two valves. The five or six pairs of leaf-like thoracic limbs are greatly modified for food gathering. Most species are filter-feeders, feeding being aided by the beating of the thoracic legs. Other species are benthic scavengers. Dorsally the carapace forms a brood chamber into which eggs are laid. Reproduction is often parthenogenetic. Unfertilized eggs are laid in the brood chamber and after a few days the juveniles, which look like small adults, are released into the surrounding water. After a further two or three days, the young females produce their own eggs. Sexual reproduction generally occurs during times of stress. Males are produced and mating results in the production of fertilized ephippial eggs, which are surrounded by a hard, resistant shell and constitute the life stage that is able to resist desiccation (see Seaman *et al.,* 2000 for details).

APPENDIX 4

PARTICLE SIZES OF SOILS

Table A4.1: Particle-size analysis of soils from sites sampled both in this project and by Corry (2009; see Corry, 2009, for details)

Sample No.	Clay %	Silt %	Sand %
KOE 02	0.0	2.0	98.0
KOE 05	0.0	2.0	98.0
SOU 01	0.2	3.8	92.0
LOT 11	0.6	2.8	96.6
KEN 13	1.0	1.0	98.0
BAD 01	1.4	4.0	94.6
DRE 02	1.8	1.8	96.4
YZE 02	1.8	1.8	96.4
DIE 04	3.4	4.0	92.6
DRE 01	25.6	7.0	68.0

APPENDIX 5

SUMMARISED RANGES OF ENVIRONMENTAL VARIABLES

Table A5.1: Summarized ranges of environmental variables collected during the wet season for sites at which maximum abundances of different taxa occurred and where absences of various taxa were recorded

Taxa		Soil moisture (%)	Organic Content (g/kg)	D.O (mg/L)	pH	E.C (mS/m)	Turbidity (NTU)	Orthophosphate (mg/L)	Nitrate + Nitrite (mg/L)	Total Ammonia (mg/L)
Anostraca	Max. abundance	<6	<70	4-8	8-9	600-900	15-50	0-0.420	0-0.004	0-0.05
	Absence	>27	-	>8	<6.5	>915	>50	>0.420	>0.005	>0.85
Conchostraca	Max. abundance	<20	<70	4-8	8-9	400-900	15-50	0-0.300	0-0.01	0-0.05
	Absence	>20	-	-	<7.3	<50	>50	-	-	-
Cladocera	Max. abundance	<20	<60	2-8	8-9	18-900	15-50	0-0.450	0-0.01	0-0.2
	Absence	-	-	-	-	-	>55	-	-	-
Ostracoda	Max. abundance	<30	20-70	2-8	7-8	18-400	0-10	0-0.450	0-0.01	0-0.2
	Absence	-	-	-	<6.5	-	>130	-	-	-
Copepoda	Max. abundance	4-45	<70	2-9	7-8	19-400	0-10	0-0.450	0-0.015	0-0.05

Note: A dash (-) indicates that representatives from taxonomic groups were recorded across the entire measured range of specific variables.

APPENDIX 6

ENVIRONMENTAL DATA FOR EACH SAMPLING SITE

Table A6.1: Environmental data measured in the wet phase, together with soil moisture content of dry samples when they were collected

Site	% Mean Soil Moisture	pH	EC (mS/m)	NTU	Dissolved oxygen (mg/L)	Mean Organic Component (mg/kg)	NO3 + NO2 (ug/L)	NO2 (ug/L)	NO3 (ug/L)	PO4 (ug/L)	NH4 (ug/L)
BAD 01	0.974	8.14	43	17.25	5.1	20.117	2.57	0.79	1.78	13.674	33.44
PIK 11	1.128	7.59	24.9	706.00	6.4	31.811	47.65	2.60	45.05	579.561	130.81
DRE 02	1.411	7.75	83.8	130.00	7.0	6.332	0.71	0.00	0.71	4.458	2.24
YZE 02	1.593	8.40	914	35.00	6.6	50.915	0.18	0.00	0.18	256.122	73.27
DAR 03	1.728	7.38	312	42.60	2.3	56.833	1.06	0.02	1.04	413.105	850.18
DRE 01	2.060	7.37	19.7	4.08	8.0	51.713	3.95	0.03	3.92	4.036	19.63
KOE 05 *	2.758	8.71	771	0.82	15.0	14.432	0.99	0.02	0.96	2.251	34.44
DIE 04	4.413	7.32	57	5.35	2.6	67.895	1.04	0.01	1.03	301.252	19.00
KOE 02 *	5.064	8.15	644	-	5.9	20.503	0.37	0.01	0.36	43.866	64.42
SOU 01 *	12.677	9.09	1580	2.24	15.0	18.445	0.01	0.00	0.01	119.916	6.70
PIK 06	14.282	7.33	35.2	52.10	4.2	47.882	5.63	0.98	4.65	137.352	164.10
LOT 2B *	14.632	7.71	135.7	1.39	6.4	13.049	12.14	1.01	11.13	9.453	19.32
LOT 06	15.337	7.47	124.8	2.93	8.0	38.512	8241.59	199.47	8042.12	1276.727	1087.33
KEN 13 *	17.072	4.62	18	1.18	4.6	41.463	37.48	1.06	36.42	1.497	23.57

LOT 04	18.717	7.62	94.9	2.69	4.9	23.385	5.87	0.99	4.88	121.890	14.58
MFU 03 *	20.820	8.25	143.8	2.00	8.0	27.513	0.06	0.02	0.03	0	10.64
MFU 01	25.922	7.13	112.7	1.00	3.1	21.253	0.04	0.02	0.02	10.752	42.98
KEN 11 *	26.448	6.57	18.35	0.64	4.6	59.416	0.67	0.00	0.67	2.903	15.26
LOT 05 *	27.891	7.55	91.4	1.80	3.5	114.005	-	-	-	-	-
LOT 11 *	30.402	8.34	267	0.93	8.7	54.237	1.72	0.06	1.66	15.592	21.14
LOT 01	32.448	7.35	137.7	1.78	5.1	112.281	13.74	0.98	12.76	5.463	40.80
LOT 2A *	42.742	7.65	134.1	1.72	6.7	114.673	12.14	1.01	11.13	9.453	19.32

*Denotes sites for which EC and pH were measured in laboratory conditions to compare against natural conditions.

APPENDIX 7

pH AND CONDUCTIVITY VALUES

Table A7.1: Comparison of pH and conductivity values from data collected *in situ* during the wet season and in dry season inundation experiments

Site	Laboratory pH	Laboratory EC (mS/m)	Wet Season pH	Wet Season EC (mS/m)
LOT 2A	8.1	914	7.7	134
MFU 03	8.7	209	8.2	143
LOT 05	5.9	901	7.7	91
LOT 2B	8.5	1123	7.7	135
SOU 01	8.3	1126	9.2	1580
KOE 02	8.3	861	8.2	644
LOT 11	8.7	662	8.3	267
KOE 05	8.3	588	8.6	771
KEN 13	5.1	24	4.5	18
KEN 11	5.56	56	6.4	18

APPENDIX 8

MACROPHYTE SPECIES LIST

Table A8.1: Species list of macrophyte taxa from seasonal to temporary wetlands from the coastal forelands of the Western Cape (Corry, 2010). cf. stands for compare and indicates where species identification is not certain. * indicates a non-indigenous species and in some instances a non-indigenous genus. Naming authorities are provided in situations where without which, it would be difficult to determine the species.

Family	Species
Aizoaceae	*Carpobrotus edulis* *Drosanthemum hispicaulia* group *Galenia Africana* *Galenia* cf. *crystalline*
Alismataceae	*Alisma lanceolatum**
Alliaceae	*Nothoscordum gracile**
Amaranthaceae	*Amaranthus deflexus** *Atriplex muelleri** *Atriplex semibaccata** *Atriplex vestita* *Chenopodium album** *Chenopodium glaucum** *Nelsia paniculata**
Anacardiaceae	*Rhus glauca* *Rhus laevigata var laevigata* *Rhus laevigata var villosa* *Rhus lucida* *Schinus terebenthifolius**
Apiaceae	*Apium inundatum* *Arctopis echinatus* *Berula erecta* *Stoibrax capense*
Apocynaceae	*Asclepias fruiticosa**
Aponogetonaceae	*Aponogeton angustifolius* *Aponogeton distachyos* *Aponogeton fugax (ex A. ranunculifloris* Jacot Guill. & Marais) *Zantedeschia aethiopica*
Araliaceae	*Centella asiatica* (L.) Urb. *Hydrocotyle verticillata*
Asparagaceae	*Asparagus capensis* *Asparagus lignosus*
Asphodelaceae	*Bulbine annua* *Bulbinella elata* *Trachyandra filiformis* *Trachyandra revoluta*
Asteraceae	*Amellus asteriodes*

Family	Species
Asteraceae	*Arctotheca calendula* *Arctotis flaccida* *Artemesia afra* *Athanasia dentata* *Athanasia trifurcata* *Chrysanthemoides monilifera* *Chrysocoma coma aurea* *Cineraria geifolia* *Cirsium vulgare** *Conyza canadensis** *Conyza scabrida* *Cotula coronopifolia* *Cotula pusilla* *Cotula turbinata* *Cotula vulgaris* *Dimorphotheca fruiticosa* *Elytropappus rhinocerotis* *Felicia tenella* *Gnaphalium pauciflorum* *Helichrysum cf. moeserianum* *Helichrysum cymosum* (L.) D.Don *Helichrysum moeserianum (ex rutilanis)* *Helichrysum niveum (ex metalasioides)* *Helichrysum patulum* *Hippia fruitescens* *Hypochaeris radicata** *Metalasia densa* *Metalasia muricata* *Nidorella foetida* *Oncosiphon glabratum* *Oncosiphon grandiflorum* *Osmitopsis asteriscoides* *Othonna* cf. *parviflora* PJ Bergius *Picris echioides** *Plecostachys serpyllifolia* *Pseudognaphalium luteo-album** *Pseudognaphalium undulatum*
Asteraceae	*Rhynchopsidium sessiliflorum* *Senecio abruptus* *Senecio arenarius* *Senecio burchellii* *Senecio* cf. *inaequidens* *Senecio halimifolius* *Senecio littoreus* *Senecio pubigerus* L.

Family	Species
	Senecio rigidus *Senecio rosmarinifolius* *Seriphium plumosum* *Sisymbrium capense* *Sonchus asper** *Sonchus oleraceus** *Stoebe capitata* *Stoebe* cf. *fusca* *Stoebe plumosa* *Trichogyne verticillata* *Ursinia anthemoides* *Ursinia nana* ssp. *nana* *Ursinia tenuifolia* *Vellereophyton dealbatum* *Xanthium strumarium**
Boraginaceae	*Echium plantagineum** *Echium vulgare** *Myosotis arvensis**
Brassicaceae	*Heliophyla africana* (L) Marais *Sinapis alba** *Sinapis arvensis**
Bruniaceae	*Berzelia abrotanoides* *Berzelia lanuginosa*
Campanulaceae	*Roella incurva* A.DC. *Wahlenbergia tenella*
Cannaceae*	*Canna indica**
Caryophyllaceae	*Cerastium capense* *Sagina apetala** *Silene gallica** *Spergularia media**
Characeae	*Chara ecklonii* *Tolypella* cf. *nidifica var glomerata*
Convolvulaceae	*Falkia repens*
Crassulaceae	*Crassula* cf. *coccinea* *Crassula glomerata* *Crassula natans*
Cyperaceae	*Bulboschoenus maritimus* (L.) Palla *Carex aethiopica* Schkuhr. *Carex* cf. *acutiformis* Ehrh. *Carex clavata* Thunb. *Carpha glomerata* (Thunb.) Nees *Chrysitrix capensis* *Cladium mariscus* *Cyperus longus* L. *Cyperus marginatus* Thunb.

Family	Species
Cyperaceae	*Cyperus sphaerospermus* Schrad. *Cyperus textilis* *Eleocharis limosa* *Epischoenus gracilis* *Ficinia capitella* (Thunb.) Nees *Ficinia distans* CB Clarke *Ficinia elatior* Levyns *Ficinia indica* (Lam.) Pfeiffer *Ficinia nodosa* (Rottb.) Goetgh., Muasya and DA Simpson *Fuirena hirsuta* (P J Bergius) PL Forbes *Hellmuthia membranacea* *Isolepis cernua* (Vahl) Roem. and Schult *Isolepis diabolica* (Steud.) Schrad. *Isolepis hystrix* (Thunb.) Nees *Isolepis inconspicua* (Levyns) J Raynal *Isolepis levynsiana* *Isolepis ludwigii* (Steud.) Kunth *Isolepis marginata* (Thunb.) A Dietr. *Isolepis prolifer* R Br. *Isolepis rubicunda* Kunth *Isolepis sepulcralis* Steud. *Isolepis venustula* Kunth *Mariscus thunbergii* (Vahl) Schrad. *Neesenbeckia punctoria* *Schoenoplectus* cf. *roylei* *Schoenoplectus scirpoideus* *Schoenus nigricans* L. *Scirpoides thunbergii* (Schrad.) Sojak *Tetraria cuspidata* cf. *cuspidata* *Tetraria cuspidata* group fine form cf. *autumnalis* *Tetraria cuspidata* large form cf. *paludosa* Levyns *Trianoptiles capensis (*Steud.) Harv. *Trianoptiles solitaria* (CB Clarke) Levyns
Dennstaedtiaceae	*Histiopteris incisa* *Pteridium aquilinum*
Droseraceae	*Drosera* cf. *trinervia* *Droserca* cf. *cistiflora*
Ebenaceae	*Euclea racemosa*
Ericaceae	*Erica barbigeroides* *Erica capillaris* *Erica laeta* *Erica margaritaceae* *Erica muscosa* *Erica perspicua*

Family	Species
	Erica subdivaricata *Erica verticilata* *Erica villosa*
Euphorbiaceae	*Euphorbia helioscopa** *Euphorbia terracina**
Fabaceae	*Acacia* cf. *pycnantha** *Acacia cyclops** *Acacia longifolia** *Acacia saligna** *Argyrolobium lunare* *Aspalathus angustifolia* *Aspalathus ciliarus* L. *Aspalathus hispida* *Aspalathus sericea* *Indigofera candolleana* Meisn. *Leersertia frutescens*
Fabaceae	*Liparia angustifolia* (Eckl. and Zeyh.) AL Schutte *Lotus subiflorus** *Medicago polymorpha** *Melilotus indica** *Otholobium* cf. *bracteolatum* *Paraserianthes lopantha** *Podalyria* cf. *hirsuta* (Aiton) Willd. *Psoralea glaucophylla* *Psoralea laxa* T.M.Salter *Psoralea monophylla* (L.) C.H.Stirt. *Psoralea pinnata L.* *Psoralea restioides* (Eckl. and Zeyh.) *Sesbania punicea** *Spartium junceum** *Trifolium angustifolium** *Vicia benghalensis** *Quercus ilex*
Fumariaceae	*Cysticapnos versicaria* *Fumaria* muralis**
Gentianaceae	*Chironia linoides* L. *Orphium fruitescens* *Sebea ambigua* *Sebea exacoides*
Geraniaceae	*Geranium* cf. *molle** *Geranium incanum* *Geranium purpureum** *Geranium rotundifolium** *Pelargonium cucullatum* *Pelargonium grossularioides*

Family	Species
	Pelargonium myrrhifolium
Gleicheniaceae	*Gleichenia polypodioides*
Haemodoraceae	*Wachendorfia* cf. *paniculata*
Haloragaceae	*Laurembergia repens*
Hyacinthaceae	*Albuca fragrans* *Ornithogalum* cf. *thyrsoides*
Hypoxidaceae	*Spiloxene aquatic* *Spiloxene canaliculata*
Hypoxidaceae	*Spiloxene capensis*
Iridaceae	*Aristea glauca* *Bobartia indica* *Geissorhiza aspera* *Hesperantha* cf. *juncifolia* *Ixia dubia* *Micranthus alopecuroides* *Moraea* cf. *flaccida* *Moraea ramosissima* *Romulea* cf. *tabularis* *Romulea* cf. *rosea* *Sparaxis bulbifera* *Watsonia angusta* Ker Gawl *Watsonia meriana* (L.) Mill.
Juncaceae	*Juncus bufonius* * L. *Juncus capensis* Thunb. *Juncus effusus* *Juncus exsertus* *Juncus kraussii* *Juncus lomatophyllus* *Juncus oxycarpus*
Juncaginaceae	*Triglochin bulbosa*
Lamiaceae	*Salvia africana-lutea*
Lauraceae	*Cassytha ciliolate*
Lentibulariaceae	*Utricularia bisquamata*
Linaceae	*Linum africanum*
Lobeliaceae	*Lobelia anceps* *Lobelia comosa* *Lobelia erinus* L. *Lobelia quadrisepala* (RD Good) E Wimm. *Monopsis debilis* *Monopsis lutea* (L.) Urb.
Lythraceae	*Lythrum hyssopifolium*
Malvaceae	*Lagunaria* patersonii**
Melianthaceae	*Melianthus major*
Menyanthaceae	*Nymphoides indica*

Family	Species
Molluginaceae	*Adenogramma glomerata**
	Hypertelis trachysperma
Myricaceae	*Morella cordifolia*
	Morella quercifolia
Myrtaceae	*Eucalyptus* conferruminata**
	Leptospermum laevigatum**
	Leptospermum scoparium**
	Syzygium cordatum (alien to and invasive within the Western Cape)
Orchidaceae	*Corycium* cf. *orobanchoides*
Oxalidaceae	*Oxalis* cf. *luteola*
	Oxalis natans
	Oxalis versicolor L.
Pinaceae	*Pinus* radiata**
Plantaginaceae	*Plantago lanceolata**
Plumbaginaceae	*Limonium equisetinum*
Poaceae	*Aira cupaniana**
	Avena fatua**
	Brachypodium flexum
	Briza maxima**
Poaceae	*Briza* minor**
	*Bromus diandrus**
	*Bromus hordeaceus**
	*Bromus pectinatus**
	Cortaderia selloana**
	Cynodon dactylon
Poaceae	*Digitaria debilis**
	Diplachne fusca
	Echinochloa crus-gali**
	Ehrharta calycina
	Ehrharta cf. *setacea*
	Ehrharta longifolia
	Ehrharta rupestris ssp. *dodii*
	Ehrharta villosa
	Eragrostis curvula
	Eragrostis plana
	Eragrostis sabulosa
	Hainardia cylindrica**
	Helictotrichon longum
	Imperatra cylindrica
	*Lagurus ovatus**
	*Lolium perenne**
	Merxmuellera cincta
	Paspalum distichum**
	Paspalum urvillei**
	Paspalum vaginatum**

Family	Species
Poaceae	*Pennisetum clandestinum**
	Pennisetum macrourum
	Pentaschistis pallid
	Pentaschistis tortuosa
	*Phalaris aquatica**
	Phragmites australis
	*Poa annua**
	*Polypogon monspeliensis**
	Polypogon strictus
	Puccinella cf. *fasciculata**
	Sporobolus africanus
	Sporobolus virginicus
	Stenotaphrum secundatum
	Themeda triandra
	Tribolium hispidum
	Tribolium obtusifolium
	Tribolium uniolae
Polygalaceae	*Muraltia* cf. *mitior*
	Muraltia heisteria (L.) DC
	Polygala ludwigiana
	Polygala nematocaulis Levyns
Polygonaceae	*Persicaria attenuata*
	Persicaria decipiens
	*Rumex acetosella** ssp. *angiocarpus*
	*Rumex crispus**
Portulacaceae	*Portulaca oleracea**
Potamogetonaceae	*Potamogeton pectinatus*
	Potamogeton pusillus
Primulaceae	*Anagallis arvensis**
	*Samolus valerandi**
Prioniaceae	*Prionium serratum*
Proteaceae	*Leucadendron spissifolium* ssp. *spissifolium*
	Leucodendron laxum
	Leucodendron linifolium
	Serruria aemula
Resedaceae	*Reseda lutea**
Restionaceae	*Anthocortus* cf. *laxiflorus*
	Calopsis paniculata
	Chondropetalum microcarpum
	Elegia asperiflora (Nees) Kunth
	Elegia filacea
	Elegia nuda
	Elegia rectum
	Elegia spathacea

Family	Species
	Elegia tectorum *Ischyrolepis capensis* (L.) Linder *Ischyrolepis cincinnata* *Ischyrolepis feminea* *Ischyrolepis paludosa* (Pillans) Linder *Platycaulos compressus* (Rottb.) Linder *Restio burchellii* *Restio filiformis* *Restio quinquefarius* *Restio tetragonus* *Staberoha banksii* *Thamnochortus fruticosus* Berg
Rosaceae	*Cliffortia ericifolia* *Cliffortia ferruginea* *Cliffortia obcordata* *Cliffortia phyllanthoides* *Cliffortia strobilifera* *Cliffortia subsetacea* (Eckl. and Zeyh.) Diels ex Bolus ex Wolley-Dod *Rubus* cf. *fruiticosus* or *R. pinnatus*
Rubiaceae	*Carpacoce spermacocea* (Rchb.f.) Sond. *Galium spurium* *Oldenlandia capensis*
Rutaceae	*Agathosma* cf. *serpyllacea* *Diosma oppositifolia* or *D. demissa*
Salicaceae	*Populus* canescens**
Santalaceae	*Thesium rariflorum*
Scrophulariaceae	*Dischisma arenarium* *Dischisma ciliatum*
Scrophulariaceae	*Halleria lucida*
Rubiaceae	*Anthospermum bergianum* *Manulea tomentosa* *Microdon polygaloides* *Nemesia affinis (ex versicolor)* *Pseudoselago* cf. *quadrangularis* *Pseudoselago spuria* (L.) Hilliard *Veronica anagallis-aquatica*
Solanaceae	*Lycium ferocissimum* *Solanum* cf. *lycopersicum** *Solanum retroflexum*
Stilbaceae	*Stilbe albiflora* E Mey.
Thymeleaceae	*Gnidia subulata* *Lachnaea capitata* (L.) Crantz *Lachnaea densiflora* *Lachnaea grandiflora*

Family	Species
	Lachnaea uniflora *Passerina corymbosa* *Passerina paludosa* *Struthiola dodecandra* (L.) Druce
Typhaceae	*Typha capensis* (Rohrb.) N.E. Br.
Vallerianaceae	*Valeriana capensis*
Verbenaceae	*Verbena* bonariensis**
Zannichellia	*Zanichellia palustris*

Table A8.2: Grass species indicative of wetland conditions in areas of KwaZulu-Natal and the Free State (data from DWAF, 2005 and unpublished data from Mr N Collins, Dept of Agriculture and the Environment, Free State)

A: Wetland species typical of a grassland biome in the KwaZulu-Natal area

Family	Species	Family	Family	Family	Family
Gramineae (Grasses)	*Andorpogon eucomis* *Andropogon appendicularis* *Aristida junciformis* *Arundinella nepalensis* *Eragrostis plana* *Eragrostis planiculmis* *Festuca caprina* *Hemarthria altissima* *Imperata cylindrical* *Ischaemum fasciculatum* *Leersia hexandra* *Miscanthus capensis* *Miscanthus junceus* *Paspalum dilatatum* *Paspalum distichum* *Paspalum urvillei* *Pennisetum thunbergii* *Phragmites australis* *Setaria sphacelata*	Cyperaceae (Sedges)	*Cyperus fastigiatus* *Cyperus latifolius* *Cyperus marginatus* *Cyperus sexangularis* *Eleocharis dregeana* *Eleocharis limosa* *Fuirena pubescence* *Kyllinga erecta* *Schoenoplectus brachycerus* *Schoenoplectus corymbosus* *Scleria welwitschii*	Typhaceae (Bulrushes) Potamogetonaceae (Pondweeds) Asphodelaceae (Red-hot pokers) Amaryllidaceae (Vlei lilies) Polygonaceae (Knotweeds)	*Typha capensis* *Potamogeton thunbergii* *Kniphofia species* *Kniphofia linearfolia* *Crinum species* *Crinum macowanii* *Persicaria attenuata*

B: Grass species occurring in the upland areas of the eastern seaboard that indicate wetland conditions

Family	Species	Family	Family
Gramineae (Grasses)	*Agrostis eriantha* *Agrostis lachnantha* *Andropogon appendiculatus* *Andropogon eucomis* *Arundinella nepelensis* *Brachiaria eruciformis* *Diplachne fusca* *Echinochloa crus-galli* *Echinochloa jubata* *Eragrotis lappula* *Eragrotis plana* *Eragrotis planiculmis* *Festuca caprina* *Fingerhuthia sesleriiformis* *Helictotrichon turgidulum* *Hemarthria altissima* *Imperata cylindrica* *Ischaemum fasciculatum* *Koeleria capensis* *Leersia mexandra* *Merxmuellera macowanii*	Gramineae (Grasses)	*Miscanthus capensis* *Miscanthus junceus* *Panicum coloratum* *Panicum hymeniochilum* *Panicum repens* *Panicum schinzii* *Paspalum dilatatum* *Paspalum distichum* *Paspalum scrobiculatum* *Paspalum urvillei* *Pennisetum macrourum* *Pennisetum natelense* *Pennisetum sphacelatum* *Pennisetum unisetum* *Phalarus arundinacea* *Phragmites australis* *Phragmites mauritianus* *Setaria sphacelata* *Stiburus alopecuriodes*

C: Grasses of temporary wetlands in the Free State

Family	Species
Gramineae (Grasses)	*Eragrostis bicolor* *Imperata cylindrica* *Leptochloa fusca* *Setaria sphacelata* *Sporobolus albicans* *Sporobolus ioclados*