EFFECTS OF HABITAT TRANSFORMATION ON TEMPORARY WETLANDS IN THE SOUTH-WESTERN CAPE, SOUTH AFRICA

by

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DECLARATION

This thesis reports original research carried out in the Department of Zoology, University of Cape Town, between 2007 and 2012. It has not been submitted in whole or in part for a degree at any other university. The data presented here are my own. A subset of the data from chapters 2 and 3 was also used by M. Mlambo for a separate and unrelated analysis of diversity patterns of macroinvertebrate assemblages in temporary wetlands (M.Sc dissertation, UCT). Any assistance I have received is fully acknowledged.

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Several publications are affiliated with the data collected during this thesis. The data from chapters 2 and 3 contributed to the following publications:

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ABSTRACT

The widespread loss of temporary wetlands to human activities has been well established, but virtually no information exists on how the human transformation of landscapes has altered the ecological character of those wetlands that remain. This thesis investigates environmental conditions and invertebrate assemblages in temporary wetlands in relation to the extent of habitat transformation in the adjacent landscape, using a broad (across multiple landscapes) and a fine (within-landscape) scale of analysis.

For the broad-scale studies, I hypothesized firstly that habitat transformation around wetlands is associated with changes in physico-chemical conditions and biotope characteristics in wetlands, and secondly, that invertebrate assemblages are resilient to these human-induced environmental changes because they are well adapted to naturally disturbed environments. Ninety isolated depression wetlands situated on coastal plains of the south-western Cape were sampled for environmental variables, macroinvertebrates and microcrustaceans. Linear regression models were used to relate environmental and invertebrate components to the surrounding extent of habitat transformation. Wetland physico-chemistry was significantly related to the extent of habitat transformation around wetlands, but there was no evidence for an effect of habitat transformation on the biotope characteristics or hydro-morphometry of wetlands. Relationships between invertebrate assemblage composition and gradients of habitat transformation were weak, but provided some indication that habitat transformation exerted an influence on invertebrate assemblages. Natural variations in environmental conditions, particularly physico-chemistry, had a strong influence on aquatic invertebrates.

It was expected that at a fine scale of analysis, where natural influence is considerably reduced, the effects of habitat transformation on temporary wetlands would be better elucidated than at the broad scale. Using repeated sampling of a set of 12 wetlands within a single landscape (~1km^2), the hypothesis was tested that the loss of indigenous vegetation around wetlands (in this case due to alien vegetation invasion) is associated with changes in wetland physico-chemistry and, given the strong influence of physico-chemistry observed at the broad scale, this was expected to mediate changes in the composition of invertebrate assemblages. Both physico-chemistry and biotope characteristics in wetlands displayed highly significant relationships with alien vegetation invasion and in turn appeared to mediate highly significant changes in the composition of invertebrate assemblages, thus generally upholding the hypothesis. Unlike at the broad scale, results for the fine-scale study present strong evidence that human alteration of temporary wetland environments *via* transformation of surrounding habitats can significantly influence invertebrate assemblage structure. These results contribute to wetland ecological theory by providing evidence that temporary wetland invertebrates are not necessarily resilient habitat generalists (as originally hypothesized) and are affected by human activities in the landscape.

From an applied perspective, the generally weak relationships between invertebrate assemblages and habitat transformation at the broad scale indicate that an invertebrate index for the biological assessment of temporary wetlands in the study region is not a feasible option. It is suggested that conservation of relatively narrow (~100 m) buffer strips of indigenous vegetation around temporary wetlands of the region would provide an effective step towards maintaining natural physico-chemical conditions within wetlands.

Frontispiece: Isolated temporary depression wetlands on the Cape Flats, Cape Town. The top left picture depicts a site extensively surrounded by indigenous fynbos vegetation, whilst top right is a site encroached by informal housing. The bottom picture shows a wetland surrounded by a mix of indigenous fynbos vegetation and invasive pioneer grasses within 100 m, with extensive urban transformation of the landscape beyond this.

CHAPTER 1

GENERAL INTRODUCTION

1.1. Wetland conservation: past and present

Wetlands are conspicuous features in the landscape and are now well recognized for their ecological importance and services they provide to human society. They may perform various hydrological functions such as purification of catchment surface water, floodwater attenuation, groundwater recharge and erosion control (Richardson 1994, Costanza *et al.* 1998, Mitsch and Gosselink 2000, Mitsch *et al.* 2005, Zedler and Kercher 2005, Brauman *et al.* 2007). Wetlands are a critical store of biological diversity and present unique habitats within terrestrial landscapes (Ramsar COP 7 1999, Williams *et al.* 2004, Dudgeon *et al.* 2006, Verhoeven *et al.* 2006). Furthermore, wetlands are regarded as highly productive systems and often have economic and social values (Thibodeau 1981, Leitch and Shabman 1988, Turner 1991, Gren *et al.* 1994, Costanza *et al.* 1998, Woodward and Wui 2001, Schuyt 2005, Brander *et al.* 2006).

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

A major turning point for wetland conservation worldwide was *The Convention on Wetlands of International Importance especially as Waterfowl Habitat* held in Ramsar, Iran, in 1971 (now commonly referred to as the "Ramsar Convention"). The broad aims of the Ramsar Convention are to halt the worldwide loss of wetlands and to ensure effective conservation of those that remain through wise use and management. Signatories are bound to incorporate wetland conservation into state policy and to ensure active measures are taken to meet the requirements of both the convention and the various COP ("Convention of the Parties") reports since then (DWAF 2004). By January 2011 there were 158 contracting parties to the Convention; South Africa was the fifth signatory. Furthermore, certain countries are actively addressing Ramsar obligations and their own need to sustain water resources through revolutionary water laws that aim to ensure availability of good quality fresh water with emphasis on aquatic ecosystems remaining intact (e.g. South Africa: National Water Act, 1998; USA: Clean Water Act, 1977; Australia: National Water Quality Management Strategy, 1992).

1.2. The term "wetland" as used in this thesis

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

1.3. Temporary wetlands

Wetlands may be further sub-divided into permanent and temporary wetlands, a distinction based on hydrological regime. Temporary wetlands may be defined as "bodies of water that experience a recurrent dry phase of varying length that is sometimes predictable in its time of onset and duration” (Williams 1997). This definition distinguishes them from permanent wetlands, which may on occasion dry out, but do not possess faunas tolerant of water loss. Thus, a key factor distinguishing temporary from permanent wetlands is the cyclical nature of the drought regime in temporary wetlands. Although permanent wetlands may undergo occasional drying out, the fauna is not adapted to survive such events and there will be significant mortality (Williams 2006).

The temporary wetland biota is best known in terms of its higher plants and metazoans, although studies are beginning to document the role of less conspicuous taxa such as bacteria, protists and fungi (Williams 2006). With the exception of water mites (Acarina), it is the insects and crustaceans that dominate the fauna of temporary wetlands (Williams 1997, Williams 2006). From a conservation viewpoint, however, these taxa have received less attention than amphibians, which are often associated with specific types of temporary wetlands and may be very rare (Semlitsch and Bodie 1998, Williams 2006). It is generally acknowledged that temporary wetlands present more variable habitats than permanent wetlands in terms of their physical and chemical environments (Wiggins *et al.* 1980, Williams 1997, Tarr *et al.* 2005, Williams 2006). This inherent variability in environmental conditions is regarded as the key factor driving biotic differences between temporary wetlands and other types of waterbodies (Williams 1996, 1997, Urban 2004, Waterkeyn *et al.* 2008). Within the realm of temporary wetlands, the relative roles of different environmental factors in structuring biotic assemblages is not well understood, due primarily to a lack of quantitative studies (Batzer *et al.* 2004). The few studies that have been performed in this regard have predominantly focussed on aquatic invertebrates. Hydroperiod (the length of the aquatic phase), and in particular whether the pattern of disappearance of water is predictable or unpredictable, is now generally regarded as the primary influence on temporary wetland invertebrate assemblages (Wiggins *et al.* 1980, Williams 1997, Tarr *et al.* 2005, Williams 2006, De Roeck 2008, Waterkeyn *et al.* 2008). Linked to the disappearance of water are associated physico-chemical changes, which in turn elicit changes in the composition of invertebrate assemblages (see review by Williams 1996). Figure 1.1 presents a summary of potential environmental influences on aquatic invertebrates in temporary wetlands (following Williams 1997). With the possible exception of hydroperiod, the relative importance of the environmental variables in Figure 1.1 is not well understood.

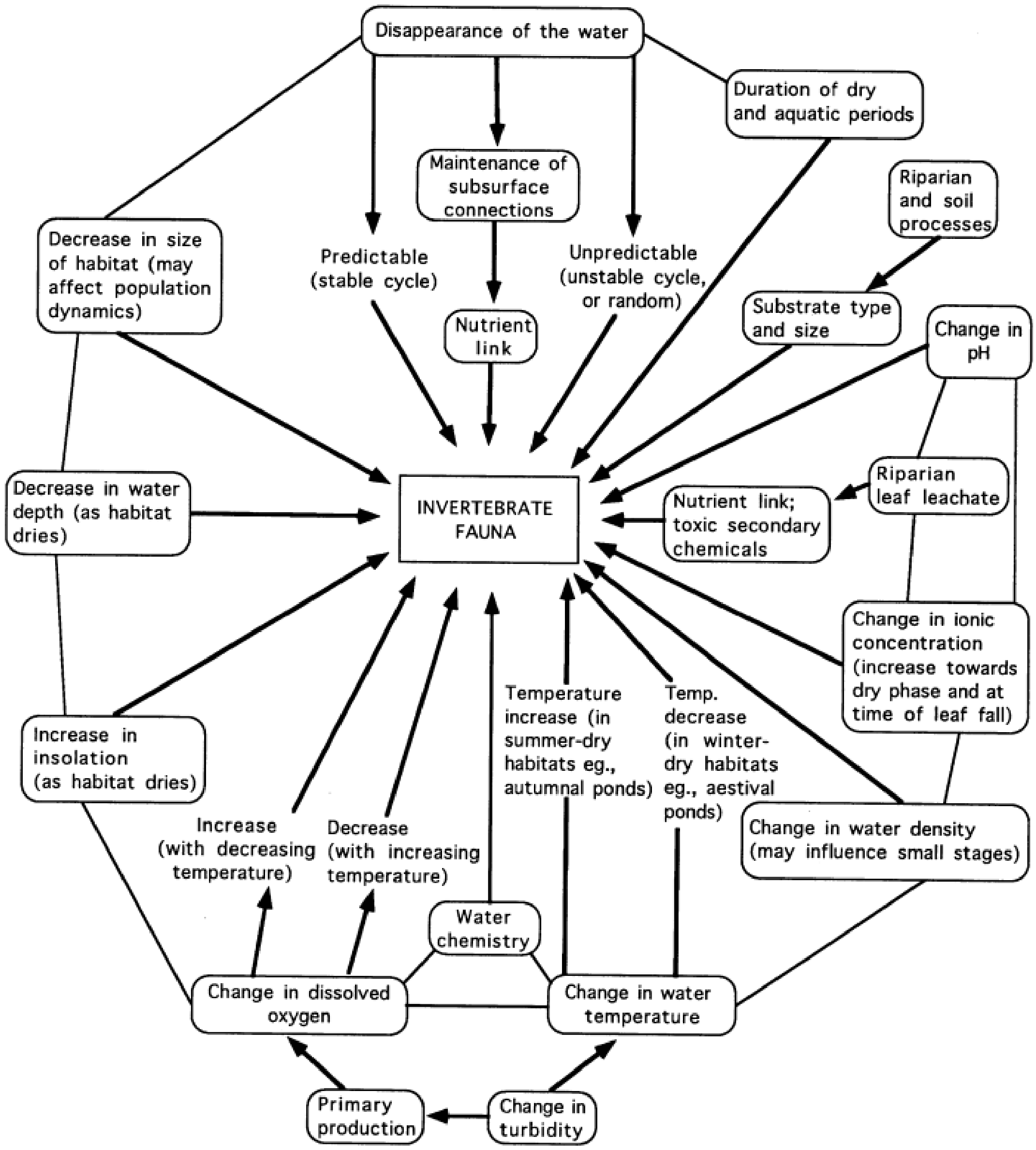


Figure 1.1. Summary of the physical and chemical factors that potentially influence the invertebrate faunas of temporary ponds (an arrow indicates that the "boxed" factor has been shown, or is likely, to have an effect on invertebrates - sometimes indirectly through another boxed factor; interactions between factors are shown by the lines joining the factor boxes). Taken from Williams (1997).

Whether environmental factors are actually important at all in structuring invertebrate assemblages in temporary wetlands has been questioned. Batzer *et al.* (2004) proposed that temporary wetland invertebrates are adapted to fluctuating environments and thus are naturally resilient to the moderate levels of environmental variability typically found among individual wetlands within a landscape or even across a region. Their hypothesis argues that although assemblage composition will differ among wetlands, it is not likely to be strongly associated with environmental gradients and Batzer *et al.'s* data for a set of 66 temporary wetlands across the Minnesota region (USA) confirmed this expectation. A debate now exists concerning the resilience of temporary wetland invertebrates to environmental change, with some authors (Mahoney *et al.* 1990, Eitam *et al.* 2004, Vanschoenwinkel *et al.* 2007, De Roeck 2008, Waterkeyn *et al.* 2008, Bilton *et al.* 2009, Bagella *et al.* 2010) reporting a stronger influence of among-wetland environmental variation on the composition of invertebrate assemblages than has been reported by Batzer *et al.* (2004) and several other studies (Wissinger *et al.* 1999, Battle and Golladay 2001, Spencer *et al.* 2002, Studinski and Grubbs 2007, Ganguly and Smock 2010). The debate is especially relevant when considering the influence of anthropogenic activities as a disturbance agent on temporary wetland invertebrates, because assemblage response to disturbance effects is likely to be weak if invertebrates are naturally resilient to environmental changes due to evolutionary adaptation (Angeler and Moreno 2007). This controversy forms the theoretical context of this thesis, in which one of the core aims is to advance understanding of the resilience of temporary wetland invertebrate assemblages to both natural and human-induced environmental gradients.

1.4. Temporary wetland conservation

Temporary wetland ecosystems have until recently been a story of neglect, both from a scientific perspective and from an applied conservation perspective, having suffered widespread loss due to human activities (Williams *et al.* 2001, Oertli *et al.* 2005, Williams 2006, Zacharias *et al.* 2007). More recently these systems are becoming recognised as unique environments that should not be overlooked in conservation planning initiatives, particularly because of their contribution to regional biodiversity through offering a network of species metapopulations across landscapes (Gibbs 1993, Semlitsch and Bodie 1998, Nicolet 2001, Williams *et al.* 2001, De Meester *et al.* 2005, Williams 2006, Gómez-Rodríguez *et al.* 2009). Although generally considered less diverse than permanent wetlands, they tend to harbour unique species that are not found in other environments (Williams 2006). Small temporary wetlands present interesting model systems to investigate a variety of ecological theories such as those relating to the response of biotic communities to disturbance (Wiggins *et al.* 1980, Blaustein and Schwartz 2001, De Meester *et al.* 2005, Williams 2006, Angeler and Moreno 2007). They are generally small and abundant, and have well defined borders and reasonably simple food webs (Blaustein and Schwartz 2001). It is only relatively recently, however, that such advantages for study have become recognised in mainstream ecology and there exists a paucity of knowledge on the influential factors governing life in these systems (Williams 2006).

1.5. Temporary wetlands in transformed landscapes

Landscapes have become intensively altered by human activities across vast areas of our planet and human transformation of the landscape has become a key driver of ecological systems worldwide (e.g. Meffe and Caroll 1997, Vitousek *et al.* 1997, Löfman and Kouki 2001, Naveh 2007). Information on the effects of terrestrial habitat transformation on wetland ecosystems is scarce, particularly so for small temporary wetlands, which are often the most common wetland type in temperate, semi-arid and arid regions (Semlitsch and Bodie 1998, Angeler and Moreno 2007). Temporary wetlands in low-lying areas (e.g. coastal plains) are highly threatened by human transformations of surrounding natural habitats, most notably from agriculture and urban development and due to their often small size, the loss of many of these wetlands has historically not received much attention (Williams *et al.* 2001, Oertli *et al.* 2005, Williams 2006, Zacharias *et al.* 2007). Recognition of the wide-scale loss of small temporary wetlands is now well established (Semlitsch and Bodie 1998, Williams 2006, Zacharias and Zamparas 2010), although the ecological effects of human alteration of the landscape on those wetlands that still remain is poorly understood.

This thesis aims to provide better understanding of how temporary wetlands in the south-western Cape mediterranean-climate region of South Africa have become altered by changing landscapes. The focus of this landscape change is on habitat transformation in nearby landscapes (< 500 m of wetlands) induced by human activities. The ecological components of temporary wetlands that are investigated in relation to surrounding habitat transformation are, firstly, environmental conditions (physico-chemistry, biotope characteristics and hydro-morphometry), and secondly, invertebrate assemblages (macroinvertebrates and microcrustaceans). Further introduction to these components is provided in chapters 2 – 4.

1.6. Isolated depression wetlands

This thesis addresses a specific type of temporary wetland that is abundant in the south-western Cape, namely "isolated depression wetlands". Isolated depression wetlands are described in the South African wetland classification system of Ewart-Smith *et al.* (2006) as: "Basin-shaped areas with a closed elevation contour that allows for the accumulation of water and are not connected *via* a surface inlet or outlet to the drainage network. For example, they receive water by direct precipitation, groundwater or as limited runoff from the surrounding catchment but no channelled surface inflows or outflows are evident.” Isolated depression wetlands are usually basin-shaped, increasing in depth from the perimeter to the centre. These wetlands are hydrologically isolated from other water sources in terms of surface flows, but may be connected by localised groundwater flows. SANBI (2009) provides further details on the classification of South African wetlands, although that document was not available when planning this project. Saline depressions were not included, as these are naturally different from freshwater wetlands (Williams *et al.* 1990, Williams 1998) and would warrant a separate study.

Isolated depression wetlands formed the ideal wetland type for the investigations described in this thesis due to their closed nature. Their isolation helps to control for external processes related to the import of substances (e.g. nutrient input) and organisms (e.g. aquatic invertebrates) from other nearby waterbodies, as would be characteristic of hydrologically connected wetlands such as floodplains (De Meester *et al.* 2005, Williams 2006). Isolated depressions are also by far the most abundant wetland type in the south-western Cape (Silberbauer and King 1991a) and their abundance makes locating appropriate study sites relatively easily. The aim of the reconnaissance described in chapters 2 and 3 was to identify clusters of wetlands that occurred in close proximity (to control for variation of geographic factors), but that were differentially impacted by human land-use activities. In this regard, isolated depressions were an ideal choice as one area of low-lying coastal plain (e.g. the Cape Flats adjacent to Cape Town) may contain a large number of depressions in close proximity, but human land-use activities may also vary considerably among these wetlands. The study region has a mediterranean climate, with long dry summers, and this dictates that the majority of depressional wetlands in the area are naturally seasonal, filling up in autumn and remaining inundated till late spring. Thus the pattern of wetland inundation is reasonably predictable and can be described as “intermittent” rather than “episodic” (*sensu* Comín and Williams 1994, cited by Williams 2006). For the purposes of standardisation and to be consistent with the majority of the literature (for review see Williams 2006), the broad term “temporary” is used from herein to describe the wetlands being investigated. Many depression wetlands in the region have been dammed by farmers to increase the availability of water for livestock or irrigation, however only natural (i.e. non-dammed), winter-inundated temporary depression wetlands are investigated in this thesis.

1.7. Thesis outline

This work aims to improve our understanding of how human-induced changes in the landscape, through transformation of habitats, have impacted the ecology of temporary wetlands in the south-western Cape. To address this broad objective, two field studies were undertaken during the course of this thesis. The first was a broad-scale survey of temporary wetlands across the south-western Cape, which involved the once-off sampling of 90 wetlands during the wet season of 2007. The data collected from this survey is presented in chapters 2 and 3. The second field study focussed on a set of 12 wetlands within a single landscape and involved repeated sampling over two wet seasons (2008 and 2009). The results of this study are presented in chapter 4. Although it was hoped that an experimental approach could be incorporated into this thesis, the stressor of interest (habitat transformation) and the response (wetland ecosystems) were very difficult to manipulate at relevant spatial and temporal scales. Thus, it was decided to take a comparative field-based approach, which had the benefit of maintaining the full complexity of the ecosystems under study, but this complexity also presented confounding influences that had to be addressed during both the design and analysis phases of each study. A brief outline of each chapter follows below.

Chapter 2 presents a broad-scale survey of environmental characteristics in temporary wetlands of the south-western Cape in relation to the extent of habitat transformation in surrounding landscapes. Physico-chemical conditions, biotope characteristics and hydro-morphometry are the focal environmental components in this regard. Overall levels of habitat transformation in the landscape are proxied by the remaining areal cover of indigenous vegetation within 100 and 500 m of wetlands. Environmental conditions in wetlands are also assessed in relation to the areal cover of alien invasive vegetation, agriculture and urban land around wetlands. Two central questions are addressed:

1) Are the environmental conditions within temporary wetlands associated with the extent and type of habitat transformation surrounding these wetlands?
2) Which specific physico-chemical, biotope and hydro-morphometry variables appear to be affected by human transformation of surrounding landscapes?

The hypothesis is tested that the degree of habitat transformation around wetlands is associated with in-wetland physico-chemical conditions, which in turn mediates changes in the structural complexity of vegetation biotopes. An exploratory approach is taken to determine the individual variables most affected by habitat transformation (question 2).

Chapter 3 investigates the same set of wetlands explored in chapter 2, but focuses on invertebrate assemblages, relating them to gradients of habitat transformation in adjacent landscapes. It was also of interest to assess whether environmental changes in wetlands associated with habitat transformation (as explored in chapter 2) appeared to mediate changes in the composition of invertebrate assemblages. Relationships between invertebrate families and metrics (as summaries of assemblage composition) and gradients of disturbance (represented by habitat transformation and an index of human disturbance) are explored to assess the feasibility of a biotic index for temporary wetlands of the region using aquatic invertebrates. This chapter has three broad aims:

1) Are invertebrate assemblages in temporary wetlands significantly associated with levels of habitat transformation adjacent to these wetlands?
2) What is the relative influence of natural and anthropogenic factors in determining the composition of invertebrate assemblages?
3) Do temporary wetland invertebrates show potential as indicators of anthropogenic disturbance in and around wetlands (for use in a biotic index)?

I hypothesize that aquatic invertebrates of temporary wetlands are resilient to environmental disturbances in wetlands caused by adjacent habitat transformation and thus do not constitute effective indicators of human activities in the landscape.

Chapter 4 addresses the effects of habitat transformation on temporary wetland ecosystems at a fine scale (within a single landscape), where natural variation is expected to be considerably reduced. A set of isolated depression wetlands is investigated for their environmental conditions and invertebrate assemblages in relation to a gradient of habitat transformation induced primarily by a single agent, namely alien invasive vegetation. Wetlands were repeatedly sampled over the course of two wet seasons to assess the consistency of patterns with habitat transformation. Invertebrate families and metrics are once again incorporated to assess the feasibility of a biotic index of disturbance, in this case for a single set of wetlands and not across the region. The aim of this study is to elucidate the effects of a single agent of habitat transformation (alien vegetation) on temporary wetlands environments and invertebrate assemblages using a small-scale analysis. The hypothesis is tested that the loss of indigenous vegetation around wetlands (due to alien vegetation invasion) is associated with changes in physico-chemical conditions in wetlands, which in turn mediates changes in the composition of invertebrate assemblages. With reduced influence from natural variation, it is further hypothesized that environmental and invertebrate response patterns to habitat transformation are clearer at the fine scale of this study than for the broad-scale studies in chapters 2 and 3.

Chapter 5 summarises and explores my overall findings, placing my research within the context of current ecological knowledge. Some implications for theoretical and applied aspects of temporary wetland ecology are discussed in this chapter.

CHAPTER 2

THE INFLUENCE OF HABITAT TRANSFORMATION ON ENVIRONMENTAL CONDITIONS IN TEMPORARY WETLANDS

ABSTRACT

The adverse effects of human landscape transformation on aquatic environments have been widely documented for rivers and lakes. Temporary wetlands typically dominate the wet season landscape of temperate, semi-arid and arid regions, yet other than their direct loss to development and agriculture, little information exists on how remaining temporary wetlands have been altered by anthropogenic conversion of surrounding landscapes. This is particularly so for small temporary wetlands, which typically dominate the wet season landscape of temperate, semi-arid and arid regions. This study investigates relationships between the extent and type of habitat transformation around temporary wetlands and environmental characteristics within these wetlands. A set of 90 isolated depression wetlands (seasonally inundated) occurring on coastal plains of the south-western Cape mediterranean-climate region of South Africa was sampled during the winter/spring wet season of 2007 for variables relating to physico-chemical conditions, biotope characteristics and hydro-morphometry. Wetlands were sampled across habitat transformation gradients according to the areal cover of agriculture, urban development and alien invasive vegetation within 100 and 500 m radii of each wetland edge. The hypothesis is tested that the degree of habitat transformation around wetlands is associated with in-wetland physico-chemical conditions, which in turn mediates changes in the structural complexity of vegetation biotopes. Multivariate multiple regression analyses (distance-based redundancy analysis, dbRDA) indicated significant associations between wetland physico-chemical conditions and surrounding habitat transformation (overall transformation within 100 and 500 m, alien vegetation cover within 100 and 500 m, urban cover within 100 m), although the amount of variation explained was very low (ranging between ~2 and ~5.5%) relative to that explained by purely spatio-temporal factors (ranging between ~35.5 and ~43%). These physico-chemical relationships with habitat transformation were slightly stronger at the 100 m than 500 m scale. The nature of the relationships between each type of transformation in the landscape and individual environmental variables within wetlands were further explored with univariate multiple regressions. Neither multivariate nor univariate analyses were able to establish clear evidence for an influence of surrounding habitat transformation on the biotope characteristics or hydro-morphometry of wetlands. These data suggest that conservation of relatively narrow (~100 m) buffer strips around temporary wetlands in the region is likely to be effective in the maintenance of natural conditions in terms of physico-chemical water quality. Studies at smaller spatial scales and with increased temporal replication are likely to better elucidate habitat transformation influences within localised clusters of temporary wetlands, given that a strong spatio-temporal influence on environmental conditions in wetlands was observed at the broad scale of this study.

2.1. INTRODUCTION

2.1.1. Background and topic of investigation

Pronounced negative effects of human land-use activities on freshwater ecosystem structure and function have been well documented for rivers and lakes (Carpenter *et al.* 1998, Leavitt *et al.* 2006, Pham *et al.* 2008), but only recently have wetland ecosystems come under the conservation spotlight due to recognition of their ecological functions and goods and services they provide to both humans and natural ecosystems (Gibbs 1993, Costanza *et al.* 1998, Mitsch and Gosselink 2000, Williams 2006). Recent research indicates that small isolated wetlands, including temporary wetlands, play an important role in maintaining regional biodiversity due to the unique and diverse assemblages of species they harbour, and perhaps most importantly, the connectivity they maintain among species metapopulations (Gibbs 1993, Semlitsch and Bodie 1998, Nicolet 2001, Williams *et al.* 2001, De Meester *et al.* 2005, Williams 2006, Gómez-Rodríguez *et al.* 2009).

This study presents the results of a broad-scale survey of environmental conditions within temporary isolated depression wetlands of the south-western Cape mediterranean-climate region of South Africa. In-wetland environmental characteristics are presented in relation to gradients of surrounding terrestrial habitat transformation induced by human activities. The term “habitat transformation” is used throughout this thesis with reference to the loss of natural terrestrial vegetation habitat around wetlands due to human land-use practices. Overall levels of habitat transformation are proxied in this study using the areal cover of remaining natural vegetation habitat around wetlands (see section 2.2.3).

Physico-chemical conditions (e.g. pH, nutrients, conductivity), biotope characteristics (e.g. cover of open water, complex- and simple-structured biotopes) and hydro-morphometry (e.g. hydroperiod, surface area, depth) have all been identified as potential determinants of biotic assemblage composition in wetlands and other freshwater ecosystems (e.g. Harrison 1962, Pedersen and Perkins 1986, Richards *et al.* 1993, Clenaghan *et al.* 1998, De Szalay and Resh 2000, Schell *et al.* 2001, Blinn *et al.* 2004, Batzer *et al.* 2006). More specifically, De Roeck (2008) found that these three sets of factors all exerted a significant structuring effect on invertebrate assemblage composition in temporary isolated depression wetlands of the south-western Cape. Alteration of these factors through anthropogenic disturbance thus has potential to mediate ecosystem changes in these wetlands through bottom-up effects on higher trophic levels, such as aquatic invertebrates and amphibians. Relationships between these in-wetland environmental factors and surrounding levels of habitat transformation were therefore chosen as a relevant focus for this study.

2.1.2. Literature review

The limited numbers of related studies in the literature have focussed on permanent wetland types. In these permanent habitats, various authors have reported significant effects of habitat transformation on an array of individual physico-chemical variables including turbidity, pH, nutrients, conductivity and dissolved oxygen (Crosbie and Chow-Fraser 1999, Bruland *et al.* 2003, Faulkner 2004, Houlahan and Findlay 2004, Declerck *et al.* 2006, Skagen *et al.* 2008, Akasaka *et al.* 2010). In terms of biotope characteristics in permanent wetlands, several authors have observed a trend towards a decreasing structural complexity of vegetation habitats with increasing levels of habitat transformation around wetlands (Lougheed *et al.* 2001, Lougheed and Chow-Fraser 2002, Declerck *et al.* 2006, Lougheed *et al.* 2008). The hydro-morphometry of wetlands can also be influenced by land use. For instance, the hardening of catchments and increased stormwater inputs associated with urbanisation may result in the deepening of wetlands and corresponding size increases (Azous and Horner 1997, Reinelt *et al.* 1998). Conversely, networks of drainage ditches associated with agricultural practices may result in lowered groundwater tables and reduction in wetland depth and size (Bruland *et al.* 2003). Alien invasive vegetation in the region has also been shown to reduce groundwater levels and thus reduce the amount of surface water available to aquatic ecosystems (Enright 2000, Le Maitre *et al.* 2000, Görgens and van Wilgen 2004, Richardson and van Wilgen 2004), but such effects have not been documented for wetlands specifically.

At the time of this review, very few studies could be found that specifically addressed relationships between terrestrial habitat transformation and environmental conditions within temporary wetlands. Carrino-Kyker and Swanson (2007) found a significant positive relationship between agricultural land use and conductivity levels in a study of thirty temporary pools in northern Ohio, USA. Brooks *et al.* (2002) studied four ephemeral forest pools in Massachusetts, USA, and reported higher pH and conductivity, and lower concentrations of dissolved oxygen, for two of the pools occurring in urban areas compared with the two pools situated in undisturbed areas. Rhazi *et al.* (2001) found higher levels of nutrients (nitrogen and phosphorus) in wetlands surrounded by agricultural fields than for those in natural areas for a set of ten temporary wetlands in Morocco. No studies have addressed the impact of habitat transformation on biotope characteristics or hydro-morphometry in temporary wetlands. It appears that no universally consistent impacts of habitat transformation on physico-chemical conditions within temporary wetlands have been established thus far, and no information exists on the effects on biotope characteristics or hydro-morphometry.

2.1.3. Study approach and research contribution

In this study a broad approach has been taken and a considerably larger number of sites was sampled (90 wetlands, Fig. 2.1) than for the abovementioned studies on temporary wetlands. The aim was to sample wetland sites across gradients of habitat transformation. Gradients were defined in terms of the amount of surrounding habitat converted to agriculture, urban area, or invaded by alien vegetation. These are the three major agents of habitat transformation in the Western Cape (Rebelo 1992, Lombard *et al.* 1997, Rouget *et al.* 2003) and are all particularly prevalent on the low-lying coastal plains of the region (Heijnis *et al.* 1999), thus threatening isolated depression wetlands. The assessment of alien invasive vegetation as a type of habitat transformation around wetlands was a key element of the approach to this investigation. As mentioned earlier (pg. 13), the negative effects of invasive vegetation on the quantity of groundwater available to aquatic systems in the region has been well documented (Enright 2000, Le Maitre *et al.* 2000, Görgens and van Wilgen 2004, Richardson and van Wilgen 2004). No empirical studies could be found that address the influence of invasive vegetation on surface water quality (e.g. physico-chemistry) of aquatic systems (rivers or wetlands) in the region however. This study therefore appears to be the first of its kind to address relationships between alien invasive vegetation and environmental conditions in aquatic ecosystems of the south-western Cape. Furthermore, no studies could be found worldwide which address the influence of terrestrial habitat transformation by alien invasive vegetation on wetland environments (although there are numerous studies on the effects of invasive aquatic plants).

This is also the first broad-scale study to address the effect of anthropogenic transformation of landscapes on temporary wetland environments in the mediterranean-climate region of South Africa. The study region falls within the broader Cape Floristic Region (CFR, encompassing both winter and summer rainfall areas), which has been intensively studied for its phenomenal diversity of terrestrial biotic assemblages (Rebelo and Siegfried 1992, Cowling *et al.* 2003, Rouget *et al.* 2003). Aquatic habitats, and in particular wetlands, have received scant attention in the CFR (Amis *et al.* 2009). An exception is the PhD thesis of De Roeck (2008) on the ecology of temporary wetlands in the Western Cape, which provides important baseline data on the abiotic and biotic characteristics of these wetlands, but does not address human impacts. The current study builds on the work of De Roeck (2008) by placing the wetlands of the region in the context of human activities in the landscape.

2.1.4. Objectives and hypotheses

This study poses two primary questions:

(1) Are the environmental conditions within temporary wetlands associated with the extent and type of habitat transformation surrounding these wetlands? Environmental conditions are represented here by multivariate sets of variables describing physico-chemistry, biotope characteristics and hydro-morphometry of wetlands.
(2) Which specific physico-chemical, biotope and hydro-morphometry variables appear to be affected by human transformation of surrounding landscapes? This question is conditional on question 1.

Development of hypotheses:

Physico-chemistry

Despite the paucity of information on the principal drivers of physico-chemical conditions in temporary isolated depression wetlands, certain key factors have emerged from the literature, which include local geological substrate (soil properties), morphology of the wetland basin, surrounding landscape topography, surrounding terrestrial vegetation type and local climate (for reviews see Colburn 2004, Magnusson and Williams 2006, Williams 2006). I hypothesize, given that one or more of these driving factors are expected to be significantly altered by human habitat transformation (e.g. soil physico-chemical properties may be affected by the type of land use), that physico-chemical conditions in temporary wetlands will in turn show significant association with changes in these driving variables and thus are expected to be affected by surrounding habitat transformation. External factors such as movement of groundwater and surface water runoff may also affect physico-chemical conditions in temporary wetlands (Williams 2006, Carrino-Kyker and Swanson 2007). Given the flat nature of the plains on which the studied wetlands occur, and their small size and isolated nature, they are expected to drain only a localised area and to be minimally affected by activities in the broader catchment. This assumption is lent support by the findings of Davies *et al*. (2008) who compared the catchment areas associated with five waterbody types (ditches, ponds, rivers, lakes and streams) in an agricultural area of lowland England and found that isolated ponds (< 2ha in size) drained very localised catchment areas, reporting an average drainage catchment size of just 18ha.

In terms of the second study question, the specific physico-chemical effects on aquatic ecosystems associated with habitat transformation varies regionally and with different types of land use. Nutrients, pH, conductivity and turbidity have been variously reported as being positively associated with the loss of natural habitat around aquatic resources, whilst dissolved oxygen has been reported to be negatively associated (Crosbie and Chow-Fraser 1999, Bruland *et al.* 2003, Faulkner 2004, Houlahan and Findlay 2004, Declerck *et al.* 2006, Skagen *et al.* 2008). Whether these trends hold true for the temporary wetlands of the south-western Cape is difficult to predict given the lack of previous work in the region. Specific relationships between each type of habitat transformation and each measured physico-chemical variable in this study are not speculated upon here. Instead an exploratory approach is adopted to generate further hypotheses regarding effects of habitat transformation on wetland physico-chemistry in the region.

Biotope characteristics

Vegetation forms the key structural habitats (biotopes) in lentic waterbodies and has been shown to mediate trophic interactions between different biotic assemblages (Jeppesen *et al.* 1998, Scheffer 1998). The relative abundance of aquatic vegetation *versus* phytoplankton and macroalgae is dependent on light availability in the water column and physico-chemical characteristics of the sediment and water column (Declerck *et al.* 2006). Intensive land-use practices may affect these characteristics in numerous ways such as by sedimentation, by alteration of surrounding soil physico-chemistry and by input of nutrients into the system, to mention just a few. As previously noted, various studies have reported a general reduction in the structural complexity of vegetation biotopes in permanent wetlands with increasing levels of surrounding habitat transformation. Following these findings, I hypothesize that physico-chemical changes in wetlands due to transformation of surrounding habitats will mediate changes in the biotope characteristics of temporary wetlands. More specifically, the cover of complex-structured vegetation within the temporary wetlands of this study is expected to be negatively associated with the transformation of surrounding natural habitats, whilst cover of the simple-structured and un-vegetated biotopes are expected to show positive associations in this regard.

Hydro-morphometry

The potential effects of habitat transformation on temporary wetland hydro-morphometry are difficult to hypothesize using existing literature for permanent wetlands (see section 2.1.2), because the small isolated systems of this study exhibit far more natural variability in depth and surface area over short time scales than do large permanent wetlands. Given the complete lack of studies on associations between habitat transformation and temporary wetland hydro-morphometry, an exploratory approach is taken in this study when investigating this question. Hydro-morphometry may affect wetland physico-chemistry, biotope characteristics and invertebrate assemblages, as indicated by the findings of De Roeck (2008) for temporary wetlands in the south-western Cape region. It was thus considered important to assess whether the hydro-morphometry of these wetlands is related to habitat transformation overall, and also to different types of habitat transformation, despite having no clear expectations in this regard.

2.2. METHODS

2.2.1. Study area and site selection

The south-western Cape is the only area in sub-Saharan Africa with a mediterranean climate, typically encompassing cool, wet winters and warm, dry summers. Although lacking strictly defined borders, for the purposes of this study the south-western Cape region is considered to be the area of land extending from Cape Agulhus in the south, to St Helena Bay in the north (Fig. 2.1). The region extends inland towards the Karoo and encompasses the Cape Fold Mountains, although only the coastal forelands are dealt with in this study. The natural vegetation of the south-western Cape is characterised by an evergreen, sclerophyllous shrub-dominated vegetation type known as fynbos and hence the region is classified within the Fynbos Biome (Rebelo *et al.* 2006). Despite its name, the Fynbos Biome actually comprises three quite different, naturally fragmented vegetation types (fynbos, renosterveld and strandveld), all of which occur within the region of this study. Fynbos vegetation (*sensu* Rebelo *et al.* 2006) is an evergreen, serotinous shrubland characterised by the presence of Restionaceae, Ericaceae, Proteaceae. Renosterveld (literally translating to "rhinoceros vegetation") is also an evergreen, fire-prone shrubland, but is instead dominated by small cuppressoid-leaved, evergreen asteraceous shrubs (principally "renosterbos" *Elytropappus rhinocerotis*) with an understorey of grasses (Poaceae) and a high biomass and diversity of geophytes (McDowell and Moll 1992, cited in Rebelo *et al.* 2006). Strandveld vegetation consists of communities of medium dense to closed shrublands dominated by broad-leaved shrubs with a conspicuous succulent element (Moll *et al.* 1984, cited in Rebelo *et al.* 2006). Unlike fynbos, strandveld is generally non-serotinous, lacking members of the Proteaceae, whilst Ericaceae are extremely rare (Rebelo *et al*. 2006).

Soils of the south-western Cape region are formed from a mosaic of geological substrates, but they are generally characterised by being well-leached and oligotrophic. Study wetlands occurred within five broad soil types, namely ferricrete, sandstone, shale, alkaline sands and acid sands (Rebelo *et al.* 2006). Isolated depression wetlands within the south-western Cape occur predominantly on low-lying coastal plains and reach maximum abundance in two particular areas. The first of these corresponds to the South-Western Coastal Plain (SWCP) aquatic ecoregion (Kleynhans *et al.* 2005), running from the Cape Flats in the south (adjacent to Cape Town, Figs 2.1 and 2.3) to St Helena Bay in the north (Figs 2.1 and 2.2). The second is the Agulhus Plain, situated west of Cape Agulhus at the southern tip of South Africa (Figs 2.1 and 2.4) and falling within the Southern Coastal Belt (SCB) aquatic ecoregion (Kleynhans *et al.* 2005). Within the SWCP area there is a gradient of decreasing rainfall from Cape Town northwards along the west coast. The Cape Flats, a low-lying sandy isthmus adjacent to the city of Cape Town, separates the Cape Peninsula from the Hottentots-Holland mountain range (Fig. 2.3). Mean annual precipitation on the Cape Flats is 575 mm (Rebelo *et al.* 2006), decreasing steadily northwards, with mean annual precipitation at the northern end of the SWCP being approximately 325 mm (Rebelo *et al.* 2006, as recorded for the town of Hopefield). Mean annual precipitation for the Agulhus Plain is 545 mm, falling predominantly within the winter months (Rebelo *et al.* 2006). The region thus presents similar climatic conditions to those on the Cape Flats.

During this study, a total of 90 isolated temporary depression wetlands, all occurring on coastal plains of the south-western Cape, were sampled. The majority of wetlands were situated on the SWCP (78 sites), whilst 12 sites were sampled on the Agulhus Plain. The small number of sites on the Agulhus Plain was due to the lack of comparable sites, a result of the geological and vegetational patchiness of the area. Sampling was conducted in 2007 during the late winter and early spring months (late-July to early-October), a period of the year when wetlands in the study region are generally expected to have reached maximum inundation. The timing of sampling was largely determined by the aim of collecting representative aquatic invertebrate assemblages (see chapter 3) during mid- to late-successional phases in assemblage composition, when assemblages are expected to be at maximum diversity and abundance in accordance with the "index sampling period" as prescribed by Helgen (2002).

2.2.2. Incorporating wetland clusters

To facilitate the comparison of wetlands that would be the most similar in their natural (or least impaired) state, the goal of sampling was *a priori* to select wetlands that occurred in clusters containing sites that were the most comparable. The approach used in this study was to incorporate clusters based on the natural vegetation units in which wetland sites occurred, based on the premise that isolated temporary depression wetlands are expected to be affected by local climate, soils and vegetation due to their small size and lack of connection to any drainage network (Ewart-Smith *et al.* 2006, Davies *et al.* 2008). Rebelo *et al.* (2006) (in Mucina and Rutherford 2006) provide the most up to date and extensive vegetation classification system for the Fynbos Biome. The vegetation groups defined in their classification scheme are closely linked to local climate and soil properties, and were designated using the taxonomic composition of plant assemblages together with associated soils and climate. The vegetation groups were therefore used to define naturally comparable clusters of wetlands and were used as a proxy for local climatic and soil conditions, due to the intimate link between vegetation type and these abiotic factors in the study region (Rebelo *et al.* 2006). Five wetland clusters (i.e. wetlands occurring within five different vegetation groups) were sampled in the study region, namely Sand fynbos (n = 44), Western strandveld (n = 28), Shale renosterveld (n = 6), Ferricrete fynbos (n = 6) and Sandstone fynbos (n = 6). The Ferricrete fynbos and Sandstone fynbos clusters were situated on the Agulhus Plain. All other sites occurred on the SWCP. It should be noted that sites in Sand fynbos, Western strandveld and Sandstone fynbos were situated on sandy, well-drained soils and were predominantly groundwater-fed, whereas sites on the more impervious shale and ferricrete soils received most of their water from direct precipitation.

To help minimize the confounding effects of temporal variation, sites within each cluster were sampled over as short a time as possible given logistical constraints. This was generally achieved by sampling all sites in a cluster before moving onto sampling the next cluster. However, certain sites within Sand Fynbos and Western Strandveld were sampled significantly later in the wet season than the other sites in those clusters, since they were discovered only later in the season. To incorporate any potential confounding effects of temporal variation in this study, a quantitative covariable for time was included in analyses relating land use and wetland ecological conditions (see section 2.2.5). The period over which each cluster of wetlands was sampled is as follows (see Appendix 1): Sand fynbos – 62 days; Western strandveld – 75 days; Shale renosterveld – 3 days; Ferricrete fynbos – 4 days; and Sandstone fynbos - 3 days.

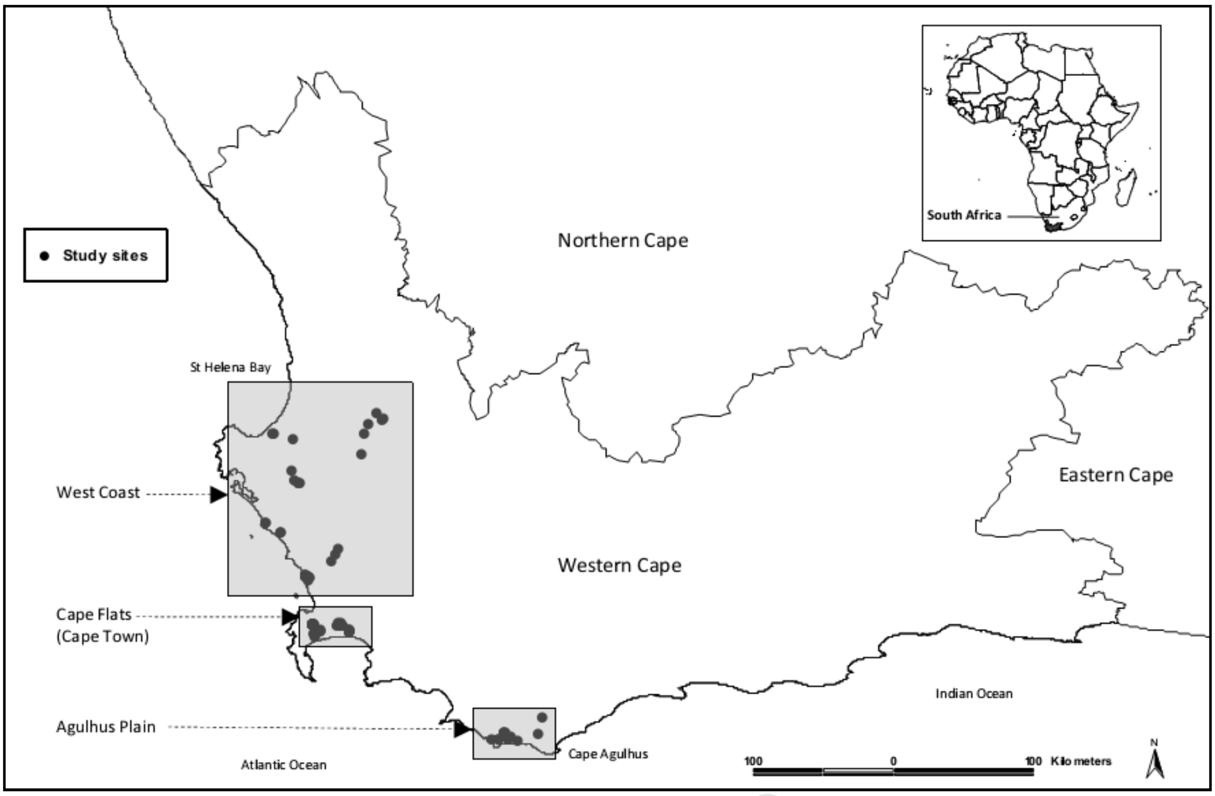


Figure 2.1. Study region showing sites sampled during the 2007 wet season (n = 90), with three broad clusters of wetlands indicated by the shaded blocks. Study sites were concentrated on the coastal plains of the south-western Cape mediterranean-climate region of South Africa, bounded approximately by Cape Agulhus in the south and St Helena Bay in the north.

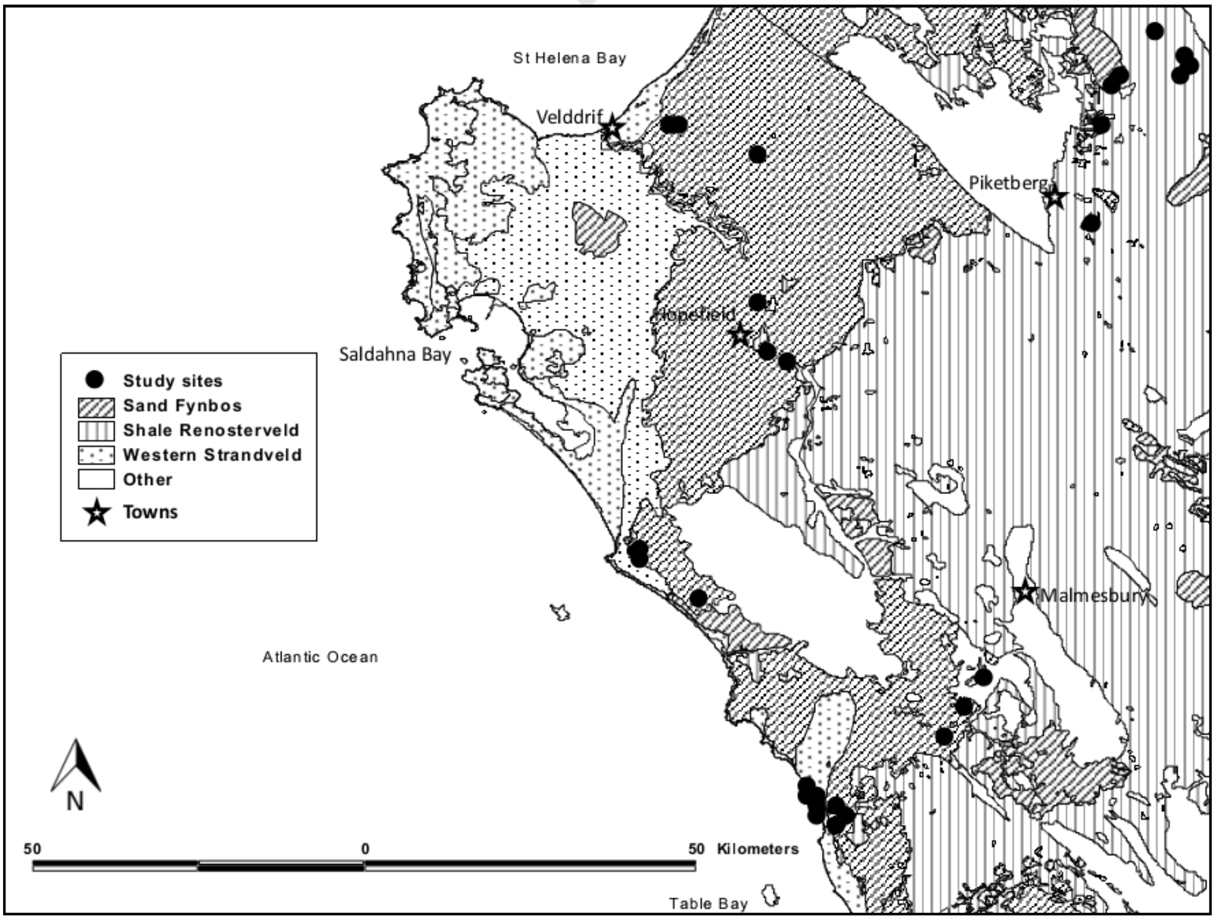


Figure 2.2. Closer view of the West Coast sampling sites, with vegetation types indicated (Rebelo *et al.* 2006).

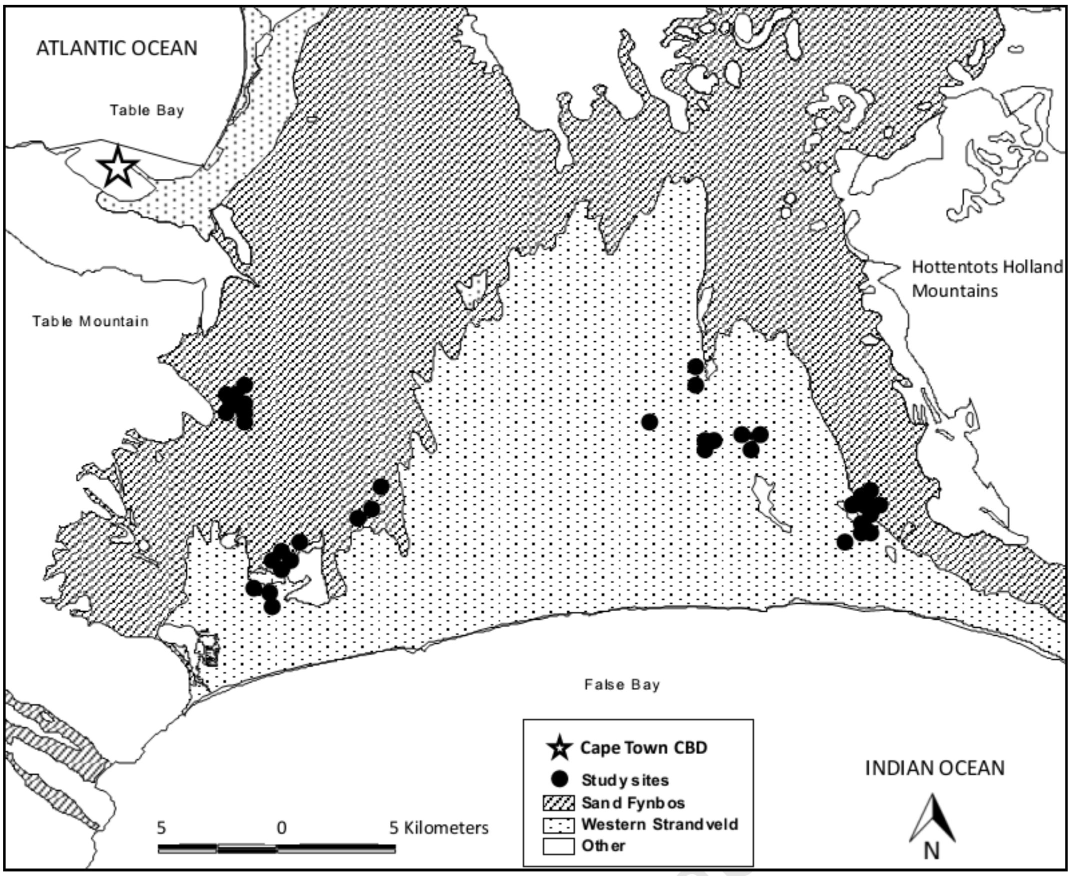


Figure 2.3. Closer view of the Cape Flats sampling sites, with vegetation types indicated (Rebelo *et al.* 2006).

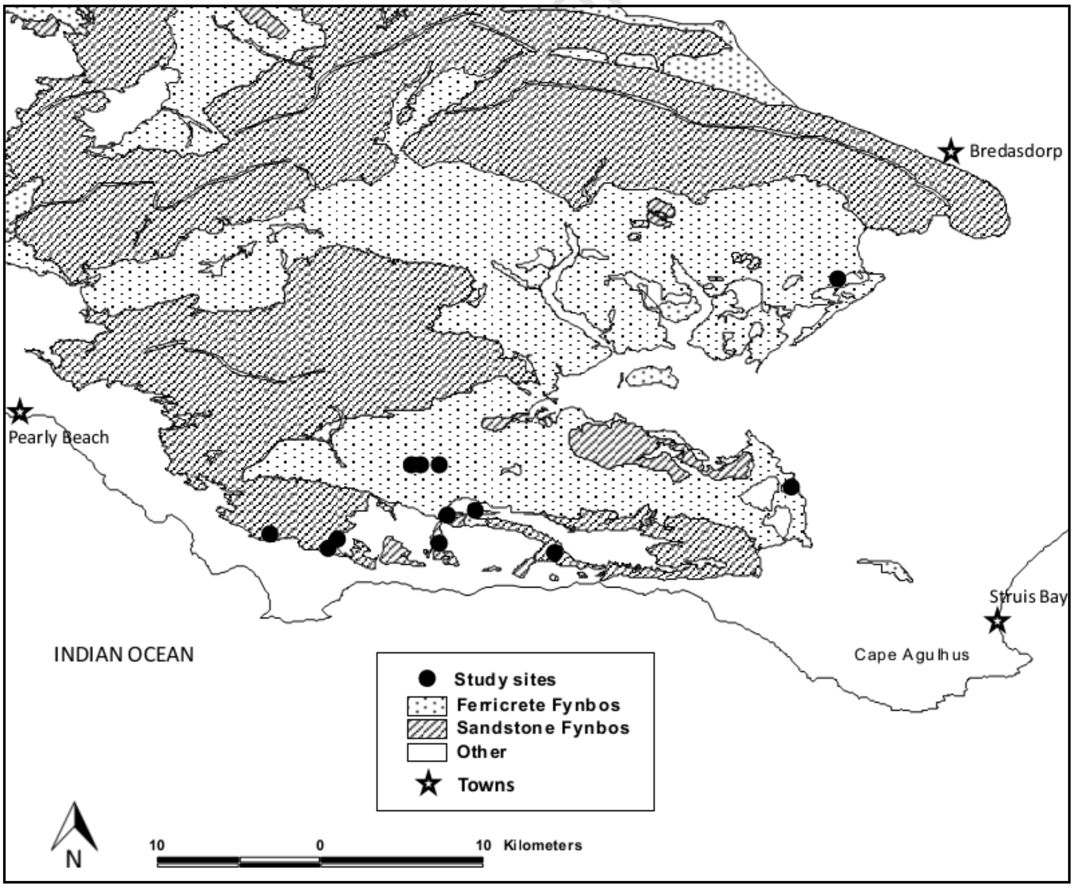


Figure 2.4. Closer view of the Agulhus Plain sampling sites, with vegetation types indicated (Rebelo *et al.* 2006).

2.2.3. Assessing habitat transformation

Agricultural practices encountered during this study were mostly wheat cultivation and pasture land for livestock grazing. Urban land was defined as any surface which had become altered due to urban development and in this study was mostly represented by residential areas (including informal settlements), although commercial offices and industrial areas were also encountered. Vacant land within urban areas that was invaded by alien vegetation was classified within the “alien invasive vegetation” category and not the “urban land” category. This was following the objective of distinguishing alien vegetation from urban influences even within areas that would broadly be classified as “urban” in national land cover (NLC) maps for South Africa (e.g. NLC 2000, NLC 2009). Alien invasive vegetation was of two predominant forms, namely grassy pioneer species dominated by Kikuyu grass (*Pennisetum clandestinum*, of East African origin) and shrubs of the genus *Acacia* from Australia (dominated by Port Jackson, *Acacia saligna*).

For each cluster, wetlands were selected for sampling so as to include least impaired sites (surrounded by predominantly indigenous vegetation within 500 m of the wetland), intermediate sites (moderate levels of conversion of natural vegetation within 500 m), and highly transformed sites (surrounded predominantly by transformed land within 500 m). Special care was taken to select sites based on surrounding terrestrial habitat and not on environmental conditions within wetlands. The types of habitat transformation varied among the clusters: Sand fynbos was affected by alien vegetation invasion, agriculture and urbanisation; Western strandveld by alien vegetation invasion and urbanisation; Shale renosterveld and Ferricrete fynbos by agriculture only; and Sandstone fynbos by alien vegetation only. For each cluster, intermediate and highly transformed sites were selected for each type of habitat transformation so that gradients of habitat conversion could be analysed according to both the transformation type and the intensity of conversion. Ideally one would sample a balanced number of least impaired, intermediate and highly transformed sites, but in fact the number of suitable sites available for comparison depended on the cluster. For example, in the Shale renosterveld cluster agricultural conversion of the landscape is so dominant that only one least impaired and one intermediate site could be found in the area, whereas wetlands completely surrounded by wheat fields were reasonably abundant. On the other hand, a relatively balanced and plentiful number of least impaired, intermediate and impacted sites was encountered in the Sand fynbos and Western strandveld clusters. Although Ferricrete fynbos and Sandstone fynbos contained only six sites in each cluster, the number of sites across different levels of habitat conversion was relatively well balanced.

The protocol for quantifying habitat transformation at each wetland involved an assessment of the cover of natural vegetation (untransformed land), alien vegetation, agriculture and urban land within 100 and 500 m of each wetland. The areal cover of each of these four habitat categories was estimated for circular areas that corresponded to 100 and 500 m radii from the edge of each wetland (i.e. approximate circular areas of 0.03 and 0.8 km^2). The 100 and 500 m radii were chosen because these scales could be relatively accurately assessed on the ground without using GIS data. For both scales of assessment (100 and 500 m radii), the cover of each habitat type was estimated and assigned to one of four ordinal cover categories: 0 - none; 1 – sparse cover (< 33%); 2 – moderate cover (33 - 66%); 3 – extensive cover (> 66%). Habitat cover within 100 m of each wetland was assessed purely on the ground (in the field). Certain wetlands were difficult to survey for a 500 m radius on foot, due to bush encroachment (usually alien invasive shrubs) or inaccessible private land (e.g. residential and commercial areas); for these sites high resolution satellite imagery (using Google Earth software, accessed 2007) was combined with ground survey information to score the ordinal categories of habitat cover within 500 m of wetlands. The 100 m scale was included in addition to 500 m, in order to ascertain whether land cover immediately adjacent to wetlands might have an effect on wetlands and to assess at which scale relationships appear to be stronger. This has relevance to the issue of buffer zones and whether small strips of remnant indigenous vegetation can provide significant buffering against the effects of land uses beyond these buffer zones. Despite the usefulness of GIS layers for mapping land cover transformations at broad scales (> 500 m) around wetlands (see Houlahan and Findlay 2004, Declerck *et al*. 2006), no high resolution GIS map of alien invasive vegetation cover for the region existed at the time of this study, although certain parts of the Agulhus Plain had been mapped at a fine scale (Rouget *et al.* 2003). Due to the prevalence of invasive vegetation as an agent of habitat transformation in the south-western Cape, it was decided to keep to a scale of assessment that allowed relatively accurate field-based quantification of each type of land cover around wetlands.

2.2.4. Sampling environmental variables

The geographical position and altitude at the centre point of each wetland were recorded using a Garmin eTrex Vista handheld GPS device (point accuracy of 3 m).

Hydro-morphometry

Various hydro-morphometrical aspects were measured at each wetland. Maximum depth (cm) was measured with a meter stick (approximately 0.5 cm accuracy) and was used as a proxy for hydroperiod. In order to make sure no permanently inundated wetlands were included in the dataset, only sites with maximum depth < 2 m were sampled. Most of the deeper sites were re-visited in summer to confirm that they had dried up. Length (m) and breadth (m) measurements of the wetland area inundated by surface water were made using a 100 m measuring tape, and for larger wetlands, GPS points were taken to estimate length and breadth. Total surface area (m^2) was estimated using the standard formula for an ellipse: Area = $\pi \times r_v \times r_h$, where r_v is the vertical radius and r_h is the horizontal radius. The equivalent here to r_v is half the width of the wetland and r_h is half the length.

Biotope characteristics

Biotope cover within each wetland was assessed in accordance with the aims of relating the structural complexity of habitats in wetlands to surrounding land cover, and also to quantify the proportion of the major biotope types available to aquatic invertebrates (see chapter 3). Emphasis lay in assessing the structure of vegetation habitats and therefore macrophyte assemblage composition was not assessed taxonomically. The biotope types assessed in each wetland were: a) complex vegetation (generally submerged, inter-woven, rooted or non-rooted with fine dissected leaves, including species such as *Isolepis rubicunda*, *Potamogeton pectinatus*, *Chara glomerata* and *Paspalum vaginatum*); b) simple vegetation (typically rooted and emerging from the water surface, reed- or sedge-like vegetation, including species such as *Typha capensis*, *Phragmites australis*, *Bolboschoenus maritimus* and *Juncus kraussii*); c) open water (no vegetation, deeper than 30 cm); and d) benthic un-vegetated habitat (no vegetation, shallower than 30 cm). The percentage surface area covered by each of these four different biotopes in each wetland was recorded visually in the field. During field sampling it was noted that a maximum of three biotopes existed in any one wetland simultaneously and thus although all four biotope types were encountered among wetlands during field sampling, only three or fewer were represented within each wetland.

Physico-chemical variables

A number of *in situ* physico-chemical variables were measured in each of the biotopes within each wetland, producing three sets of *in situ* physico-chemical measures per wetland. For sites with only two biotopes, a double and a single set of physico-chemical readings were taken in the more and less abundant biotopes respectively. For sites where only one biotope covered the entire wetland, three replicate sets of physico-chemical readings were taken, with the aim of covering as much of the spatial extent of the wetland as possible among each set. All physico-chemical readings were taken at a standardized depth of 30 cm across all biotopes. Exceptions to this were for readings taken from the conductivity meter, which floats on the water surface, and for any readings taken in habitats < 30 cm deep.

Measurements were taken as follows: pH was measured using a Crison pH25 meter; dissolved oxygen was recorded using a Crison OXI45 oxygen meter; electrical conductivity was recorded using a Crison CM35 conductivity meter; and turbidity was measured using a Hach 2100P turbidimeter. Temperature was recorded on the pH, oxygen and conductivity meters, although for analytical purposes an average of the readings across all three instruments was used. Water column nutrient concentrations were measured at each site. Five 1L surface water samples were collected from each wetland, with the aim of covering the full spatial extent of each site, and pooled to form a bulk 5L sample. This pooled sample was then thoroughly mixed and a 200 ml sub-sample was taken for analysis of nutrients levels in the laboratory. Samples for nutrient analysis were stored immediately in the dark at 4 ^{o}C and upon return to the laboratory were frozen at -18 ^{o}C. All samples were analysed for nutrient concentrations within 30 days of collection from the field. NO_3^-+NO_2^-–N, PO_4^{3+}–P and NH_4^+–N concentrations were estimated using a Lachat Flow Injection Analyser, as follows: NH_4^+-N was measured using Lachat's QuikChem® Method 31-107-06-1, based on the Berthelot reaction in which indophenol blue is generated; NO_3^- and NO_2^- were estimated using Lachat's QuikChem® Method 31-107-04-1-E, in which NO_3^- is converted to NO_2^- and diazotized with sulfanilamide to form an azo dye; PO_4^{3+} was measured by forming an antimony-phospho-molybdate complex using QuikChem® Method 31-115-01-1. Approximate detection limits are: for PO_4^{3+} 15µg.L^{-1} P; for NO_3^- and NO_2^- 2.5µg.L^{-1} N; and for NH_4^+ 5µg.L^{-1} N. These variables are herein referred to in the text as "phosphates", "nitrates + nitrites" and "ammonium" respectively.

2.2.5. Data analysis

Data subsets

Separate subsets of the dataset were used to analyse relationships between each type of habitat transformation and environmental conditions in wetlands. These data subsets were composed of sites that were affected by only one type of habitat transformation (e.g. agriculture). This was done to exclude sites that were affected by habitat transformations other than the type of interest. Each separate dataset (i.e. for analysis of each separate type of transformation) was composed of least impaired sites (surrounded by extensive indigenous vegetation) and those sites that were impacted by varying degrees of habitat conversion for the given transformation type. For example, to analyse relationships between agriculture and environmental conditions in wetlands, a subset of the data was used which contained sites impacted by only agriculture, as well as sites with minimal or no impacts to allow comparison to least impaired conditions. Only least impaired sites occurring within the same wetland cluster (defined by vegetation type) as impacted sites were selected, to ensure comparison with naturally similar wetlands in the area. Thus each dataset contained a gradient of impact for each type of habitat transformation and this gradient formed the primary variable for detecting patterns between wetland conditions and surrounding habitat. It was not possible to analyse sites only impacted by alien vegetation or only urbanisation, as the two types of habitat transformation often overlapped (i.e. where there was urban land surrounding wetlands there was often invasive vegetation and *vice versa*). To help address this overlap, when assessing relationships between invasive vegetation cover and wetland conditions, the amount of urban land cover was specified as a covariable and *vice versa* when assessing the effects of urban land cover as the primary variable.

Two different but largely overlapping datasets were created for each habitat transformation type, corresponding to the 100 m and 500 m scales of analysis, because in certain cases sites used in analysis of impact at one scale were not applicable at the other. For example, a given site may have had some alien vegetation but no urban cover within 100 m of the site, yet may have been moderately or extensively surrounded by urban land at the broader 500 m scale and thus would not have been included as a site in the 100 m dataset, but would have been included in the “Urban 500 m” dataset. An exception to this was for analyses relating to the amount of natural vegetation cover around wetlands, as this criterion was applicable to all sites in the dataset and at both scales of analysis. The latter analyses essentially address relationships with overall levels of habitat conversion around wetlands as measured by the amount of remaining natural vegetation cover and thus do not attempt to separate the effects of different types of habitat transformation.

Analysis approach: multivariate versus univariate response data

Two broad types of environmental response data were analysed in relation to surrounding gradients of habitat transformation. First were analyses on sets of environmental variables (multivariate response) and second were analyses on individual environmental variables (univariate response). In terms of the former, three sets of variables describing wetland environments formed the focus of this study. These were physico-chemical conditions, biotope characteristics and hydro-morphometry. As introduced in section 2.1.1, these sets of variables are recognised as potentially important determinants of biotic assemblages in wetlands and other freshwater ecosystems. I wished to explore the influence of habitat transformation on each of these groups of variables as a whole (multivariate approach) in order to assess the effects of habitat transformation on these broad types of environmental conditions in wetlands, and not just individual variables (univariate approach). This multivariate approach was also relevant for hypothesizing further general effects of habitat transformation on the biotic assemblages inhabiting these wetlands (see chapters 3 and 4). Each of the three sets of environmental variables was analysed separately as a multivariate response by first normalizing the variables and then converting it to a Euclidean distance matrix, which was subsequently related to surrounding levels of habitat transformation. Each physico-chemical matrix consisted of the following variables: pH, conductivity, average temperature, turbidity, oxygen, nitrate + nitrites, phosphates and ammonium (Table 2.1). For the physico-chemical variables that were measured *in situ* for each biotope (pH, conductivity, average temperature, turbidity and oxygen) an average of the three readings per wetland was used in subsequent analyses because replication in this study was at the level of individual wetlands and not biotopes. Each matrix representing biotope characteristics consisted of the following variables: % complex vegetation, % simple vegetation, % open water and % benthic un-vegetated habitat (Table 2.1). Benthic un-vegetated habitat was uncommon in this study and due to the large number of zero values for this variable, it was only included in analyses using the full dataset (i.e. against overall habitat transformation within 100 and 500m) and not for those using the data subsets. The hydro-morphometry matrices were made up of the variables “maximum depth” and “total surface area” (Table 2.1). Univariate analyses were also performed in this study in order to assess the potential effects of habitat transformation on individual environmental variables in wetlands (see *Multivariate and univariate regression techniques*, below).

Analysis approach: Linear versus unimodal response gradients

Detrended Correspondence Analysis (DCA) of the gradient lengths in the physico-chemical, biotope and hydro-morphometry data indicated linear rather than unimodal distributions in the response data (gradients lengths were all < 3, Lepš and Šmilauer 2003). Therefore the data in this study were analysed using linear as opposed to unimodal models.

Analysis approach: Categorical (ANOVA) versus correlational (regression) tests

Given the ordinal (semi-quantitative) categories representing the cover of remaining natural habitat around wetlands, as well as the different types of habitat transformation, the two broad statistical approaches for relating these predictor categories to environmental response gradients were ANOVA and regression. The approach taken in this chapter, and throughout this thesis, follows from the findings of Somerfield *et al.* (2002) who showed that correlational (regression) models are more powerful than categorical (ANOVA) models in terms of detecting impacts in community ecology studies where gradients are hypothesized in the response data. They found greater statistical power for regression over ANOVA tests using both univariate and multivariate scenarios. Their analysis focussed on community impacts from pollution gradients associated with oilfields in the North Sea (using categories “near”, “mid” and “far” from drilling centres), but concluded that their findings were general in nature and applicable to any ecological investigation in which a gradient in response may be hypothesized (thus including environmental response gradients, the focus of the current study). Another advantage to the regression approach is that a number of covariables can be readily included in a model and interpretation of the effect of interest is relatively straightforward, whereas the incorporation of more than two covariables in ANOVA (i.e. using ANCOVA) leads to complex interactions in the model, which are difficult to interpret.

Multivariate and univariate regression techniques

The ordinal scores for each type of habitat transformation (integers 0-3) formed the individual predictor variables for all regression analyses. Multivariate regressions of Euclidean distance matrices on the habitat transformation predictor variables were performed using distance-based Redundancy Analysis (dbRDA, Legendre and Anderson 1999, McArdle and Anderson 2001). dbRDA is a non-parametric multivariate multiple regression procedure based on any given dissimilarity measure, in this case Euclidean distance. P values were tested by 9999 permutations of residuals under the reduced model. Traditional Redundancy Analysis (RDA, *sensu* Gittins 1985) assumes a Euclidean distance matrix for the multivariate response and thus could have also been used in this study. dbRDA was the preferred technique, however, as it allows for any choice of resemblance measure in the response matrix and this was relevant for multivariate analyses on invertebrate assemblages in the following chapters.

Univariate environmental response variables were related to the habitat transformation predictor variables using parametric multiple linear regression (MLR) models. The coefficient of partial determination (partial r^2) was also incorporated into model results by squaring the partial correlation coefficient (r) for the predictor variable of interest (Quinn and Keough 2002). The r^2 due to the covariables in each model was calculated by subtracting the partial r^2 due to the predictor variable of interest (i.e. the habitat transformation variable) from the full model r^2. These steps were taken so as to apportion the percentages of explained variation in response variables that were due to both the predictors of interest and also the covariables. The reliability of parametric univariate regression models was assessed by examination of partial residual plots, where heterogeneity in the spread of residuals, deviations from linearity and outliers were scrutinized (see the section below: *Visualizing patterns*). Potential outliers were quantitatively assessed using Cook's distances (Cook's D_i, *sensu* Cook and Weisberg 1982), where D_i values > 1 or D_i values considerably larger than the rest of the values would warrant an outlier (Quinn and Keough 2002).

For all analyses conducted in this study, environmental variables were $\log_{10}$ transformed where appropriate to improve normality and for the same purpose the percentage variables were arcsine square root transformed. The significance level (α) for all regression tests was 0.05, except for tests related to agriculture, as the smaller sample sizes of the two agricultural datasets (100 m: n = 24; 500 m: n = 21) indicated that the possible lack of power to detect effects could be countered by interpreting P values < 0.10 as offering some evidence against the null hypothesis. The issue of multiple testing should be noted here, as both the multivariate and univariate regressions run a family of simultaneous statistical tests and thus the chance of rejecting the null hypothesis by chance (Type I error) is a possibility for any given variable/matrix (Quinn and Keough 2002). However, the philosophy followed in the current study, and throughout this thesis, is not to use a multiple correction factor (such as Bonferroni) due to the corresponding increase in the probability of Type II errors, which are believed to be as important, if not more important, than Type I errors in the context of ecological studies (Nakagawa 2004, Houlahan *et al.* 2006). Furthermore, the Bonferroni procedure (including sequential adjustments) has been criticised (Perneger 1998, Moran 2003, Garamszegi 2006) as being inappropriately conservative since it is concerned with the general null hypothesis (that all null hypotheses are true simultaneously), which is seldom appropriate in a biological context; this is no exception in the current study. The approach taken here, as recommended by various other workers in ecology and biology (e.g. Bland and Altman 1995, Cabin and Mitchell 2000), is to explain clearly which statistical tests have been performed and why, and to interpret significant results ($P < 0.05$) with caution if there are only one or two significant variables out of a large group of tested variables.

Incorporating covariables

The partialling out of covariables was an important aspect of the regression procedure used in this study. The physico-chemistry, biotope and hydro-morphometry variables may be affected by factors that happen to co-vary with gradients of habitat transformation by chance, but were not a focus in this study. These covariables may confound patterns if they have a significant effect on the response variables of interest. These potential confounding factors are typical of field surveys of the kind conducted in this study, because only a limited number of factors can be controlled. Therefore their influence needs to be dealt with during the analysis phase by partialling out their effect and then assessing the amount of remaining variation that can be explained by the factor of interest (in this case gradients of habitat transformation). These kinds of statistical tests are known as conditional tests, due to their being conditioned upon the covariables. Multiple linear regression models used in this study (both multivariate and univariate) were conditioned upon covariables in order to assess the relationships between adjacent habitat transformation and environmental conditions in wetlands, given the effects of potentially confounding variables.

The list of covariables that could potentially influence environmental conditions in wetlands, but which were not of interest in this study, included the following measures for each wetland (see Table 2.1): longitude and latitude (decimal degrees); time (recorded as number of days since first sampling event); altitude (m); and vegetation type (coded as five dummy variables which defined the wetland clusters). Furthermore, for analyses involving the invasive vegetation datasets (both scales), the amount of urban land was specified as a potential covariable and *vice versa* when assessing patterns in the urban datasets due to the co-occurrence of these land cover types for a number of wetlands. To maximise parsimony, covariable subsets were pre-selected for each model using step-wise regression of each response variable/matrix on the full list of possible covariables (see Appendix 1). For multivariate analyses, the full list of covariables was first regressed against each of the physico-chemistry, biotope and hydro-morphometry response matrices for each particular dataset and at each scale of analysis (100 m and 500 m) using step-wise selection. Similarly for univariate analyses, individual environmental variables were first regressed against the full list of covariables using step-wise selection. Once the covariable subsets were selected for each response variable/matrix, the final regression model was run. Final models involved the regression of each response variable/matrix on the relevant predictor variable, whilst holding the pre-selected subset of covariables constant.

Table 2.1 provides a list of all the variables analysed in this study. The names used for these variables in Table 2.1 are herein referred to throughout the text of chapters 2 and 3.

Table 2.1. List of the environmental response variables, habitat transformation predictor variables and spatio-temporal covariables incorporated into the analyses of this study. The response variables were further divided into three sets, which were analysed as multivariate resemblance matrices based on the Euclidean distance measure (variables were first normalised).

Variable type	Variable scale	Category/set	Variable name	Description
Response variables	Quantitative (continuous)	Physico-chemistry	pH	Measured *in situ* for each biotope, average value used
			Conductivity	Measured *in situ* for each biotope, average value used
			Average temperature	Measured *in situ* for each biotope, average value used
			Turbidity	Measured *in situ* for each biotope, average value used
			Dissolved oxygen	Measured *in situ* for each biotope, average value used
			Nitrates + nitrites	Integrated sample from across the wetland
			Phosphates	Integrated sample from across the wetland
			Ammonium	Integrated sample from across the wetland
		Biotope characteristics	% Complex vegetation	% Areal cover of complex-structured vegetation biotope in wetland
			% Simple vegetation	% Areal cover of simple-structured vegetation biotope in wetland
			% Open water	% Areal cover of open water biotope in wetland
			% Benthic un-vegetated	% Areal cover of benthic un-vegetated biotope in wetland
		Hydro-morphometry	Maximum depth	Measured at the estimated deepest point in wetland
			Total surface area	Inundated area of wetland
Predictor variables	Semi-quantitative (ordinal)	Habitat transformation	Natural 100 m	Areal cover of indigenous vegetation within 100 m radius of wetland edge
			Natural 500 m	Areal cover of indigenous vegetation within 500 m radius of wetland edge
			Invaded 100 m	Areal cover of alien invasive vegetation within 100 m radius of wetland edge
			Invaded 500 m	Areal cover of alien invasive vegetation within 500 m radius of wetland edge
			Agriculture 100 m	Areal cover of agriculture within 100 m radius of wetland edge
			Agriculture 500 m	Areal cover of agriculture within 500 m radius of wetland edge
			Urban 100 m	Areal cover of urban surface within 100 m radius of wetland edge
			Urban 500 m	Areal cover of urban surface within 500 m radius of wetland edge
Covariables	Quantitative (continuous)	Spatio-temporal	Longitude	Taken at the wetland centre-point
			Latitude	Taken at the wetland centre-point
			Altitude	Taken at the wetland centre-point
			Time	Number of days since first sampling event
	Categorical	Spatio-temporal	Ferricrete fynbos	*Indigenous terrestrial vegetation type historically surrounding wetland
			Sand fynbos	*Indigenous terrestrial vegetation type historically surrounding wetland
			Sandstone fynbos	*Indigenous terrestrial vegetation type historically surrounding wetland
			Shale renosterveld	*Indigenous terrestrial vegetation type historically surrounding wetland
			Western strandveld	*Indigenous terrestrial vegetation type historically surrounding wetland

* *Sensu* Rebelo *et al.* (2006)

Visualizing patterns

Patterns in the multivariate environmental data (i.e. physico-chemistry, biotope characteristics and hydro-morphometry) were visually explored using Principal Components Analysis (PCA) ordination. Sites were coded on each PCA plot according to three factors of interest, namely surrounding overall levels of habitat transformation, the wetland cluster into which they were classified, and the broad latitudinal region in which they were situated (West Coast, Cape Flats and Agulhus Plain). These factors were incorporated in order to assess the variation in environmental conditions in wetlands in relation to habitat transformation gradients, as well as natural spatial factors. To limit unwieldy repetition of results, exploration of multivariate response matrices using PCA plots was not undertaken for those sets of environmental variables that were not significantly associated with overall levels of habitat transformation in the multivariate regressions.

Linear regression relationships between individual environmental response variables and the habitat transformation predictor variables were visualised using partial residual plots. These plots involve two sets of residuals, where the residuals on the vertical axis of the plot come from the ordinary least squares regression of the response variable against all the predictors except the one of interest. The residuals for the horizontal axis of the plot come from the ordinary least squares regression of the predictor variable of interest against all other predictors. This residual scatterplot shows the relationship between a given univariate response variable and a predictor variable of interest, holding the other predictor variables constant (hence a partial analysis). The slope of the line in such plots is equal to the partial regression slope (non-standardised) for that particular predictor variable in the full multiple regression model involving all the predictor variables. Partial residual plots have a distinct advantage over ordinary bivariate scatterplots in this study in that bivariate scatterplots simply regress Y on X ignoring any covariables, which would present potentially confounded patterns in the current study.

Another substantial benefit of using partial residual plots is the ability to visually assess inhomogeneity of variance, outliers and deviations from linearity in multiple regression models. These core assumptions of parametric linear regression models have traditionally been assessed by plotting the residuals against the independent variables or the predicted values and visually checking for wedge-shaped patterns, influential outliers or curvilinear relationships (Larsen and McCleary 1972, Quinn and Keough 2002). Partial residual plots, however, have been proposed as an effective means to visually check these same assumptions, whilst simultaneously visualising the partial relationship between the response variable and predictor of interest, holding the covariables constant (Larsen and McCleary 1972, Mansfield and Conerly 1987, Quinn and Keough 2002). Thus, partial residual plots are presented in this study to assess the strength and reliability of linear regression models. It should be noted that the ordinal predictor variables for habitat transformation do not produce continuous plots of partial residuals, but rather the points are arranged vertically within each category (integers 0, 1, 2, 3) and interpretation of such plots is similar to that for box plots when assessing ANOVA models. Box plots however cannot be used to represent a linear relationship whilst holding covariables constant and were thus not appropriate for this study.

Software used

Detrended Correspondence Analysis (DCA) ordinations were performed using CANOCO for Windows v4.5 (Ter Braak and Šmilauer 2002). PCA ordinations were performed using PRIMER v6 software (Clarke and Gorley 2006). The multivariate distance-based Redundancy Analysis (dbRDA) models were implemented using the DISTLM routine of the PERMANOVA+ software package (Anderson *et al.* 2008), which is an add-on to PRIMER v6. Univariate regression models and partial residual plots were performed using STATISTICA v10 software (Statsoft Inc. 2010, Tulsa, Oklahoma, USA).

2.3. RESULTS

The full lists of raw data for the spatio-temporal covariables, physico-chemistry, biotope characteristics, hydro-morphometry and habitat transformation scores are presented in Appendices 1-3. Summary statistics for all environmental variables are reported per wetland cluster in Appendix 4. These data were not a focus of the study and were not further analysed, but are provided for general reference.

2.3.1. Environmental responses to habitat transformation: multivariate patterns

Physico-chemical conditions

Table 2.2 presents the multivariate linear regression relationships between the physico-chemical response matrices and the habitat transformation predictor variables. Physico-chemical conditions in wetlands were significantly related to the cover of natural (indigenous) vegetation within 100 m (P = 0.002) and 500 m (P = 0.010), the cover of alien invasive vegetation within 100 m (P = 0.005) and 500 m (P = 0.005), and urban cover within 100 m of wetlands (P = 0.022). Despite these results being significant, only very little of the variation in physico-chemical conditions was explained by these land cover variables (ranging between 2.08 and 5.57%) in comparison to that explained by the spatio-temporal covariables (ranging between 35.57 and 43.09%). No significant relationships were found between physico-chemical conditions in wetlands and urban cover within 500 m or agricultural cover within 100 and 500 m.

Table 2.2. Non-parametric multivariate regression tests (dbRDA) for relationships between habitat transformation gradients and physico-chemical conditions in wetlands. Natural - indigenous vegetation; Invaded - alien invasive vegetation; Agriculture - agricultural land; Urban - urban area. The areal cover of these variables is represented within 100 and 500 m radii of each wetland edge. To maximise parsimony, covariable subsets were pre-selected for each model using step-wise regression of each response matrix on the full list of possible covariables (see Appendix 1). % Var - the percentage of variation in each Euclidean distance matrix (normalized physico-chemical variables) that is explained by each respective predictor variable or covariable set in each model; Time – number of days since the first sampling event; SF – Sand fynbos; SR – Shale renosterveld; FF – Ferricrete fynbos; Res. df – residual degrees of freedom for each model. Significant P values are presented in boldface (α = 0.05, with the exception of agriculture, where α = 0.10).

Predictor variable	Res. df	F	P	% Var	Covariables	% Var (covariables)
Natural 100 m	82	3.962	**0.002**	2.62	Time, longitude, latitude, altitude, SF, SR	43.09
Natural 500 m	82	3.106	**0.010**	2.08	Time, longitude, latitude, altitude, SF, SR	43.09
Invaded 100 m	65	3.529	**0.005**	3.26	Time, longitude, latitude, altitude	36.71
Invaded 500 m	66	3.441	**0.005**	3.18	Time, longitude, latitude, altitude	35.79
Agriculture 100 m	18	1.333	0.243	2.50	Time, longitude, latitude, SR	63.71
Agriculture 500 m	16	1.186	0.299	3.45	Time, FF, SR	50.04
Urban 100 m	31	2.927	**0.022**	5.57	Time, longitude, latitude, altitude	35.47
Urban 500 m	49	1.879	0.090	2.24	Time, latitude, altitude, Invaded 500 m	39.37

Principal components analysis (PCA) ordination was used to help visualize the variation in physico-chemical conditions among wetlands for all sites sampled in this study (i.e. the whole dataset, n = 90). The pattern with the variable “Natural 100 m” (Table 2.2) was focussed on, in part because this was the most significant result (P = 0.002), but also because the natural vegetation gradient across the whole dataset represents levels of habitat transformation in total and thus forms a useful proxy for adjacent habitat disturbance in general. Although this variable was significantly related to physico-chemical conditions (Table 2.2), the pattern is not obvious in the PCA (Fig. 2.5) and there appears to be no clear grouping according to the different levels of natural vegetation within 100 m. The PCA does not allow visualization of patterns with the effects of covariables partialled out (e.g. time and latitude), and thus may limit its usefulness in visualizing the effects of habitat transformation when covariables are involved in the analysis. In terms of physico-chemical constituents, sites showed better grouping according to the vegetation types in which they would naturally occur (i.e. the wetland clusters), but none of the vegetation types formed clusters that were clearly separated from the rest of the groups (Fig. 2.6). Variation within certain groups was large, particularly for the Sand fynbos vegetation type. At a broader level, sites were also classified according to the three latitudinal regions depicted in Figure 2.1 in order to see if sites grouped out more clearly at this broad level of categorisation. The southernmost region is the Agulhus Plain, the northernmost is the west coast area north of Cape Town, whilst the Cape Flats occurs roughly in between (Fig. 2.1). Physico-chemical conditions at this broader level of classification appear to be more distinguishable, with the majority of sites separating out according to latitudinal region, although there is some overlap of regions towards the centre of the ordination (Fig. 2.7).

Biotope characteristics

Table 2.3 presents the multivariate linear regression relationships between biotope characteristics and the habitat transformation variables. The multivariate matrices representing biotope characteristics in wetlands generally showed a poor response to levels of habitat transformation in the surrounding landscape. Relationships with overall levels of transformation within 100 and 500 m of wetlands displayed P values that were very close to the prescribed 5% significance level (P = 0.075 and P = 0.056 for “Natural 100 m” and “Natural 500 m” respectively), thus offering some evidence against the null hypothesis that biotope characteristics are unrelated to habitat transformation. The percentage of explained variation due to the “Natural 100 m” and “Natural 500 m” variables was very low however (2.26 and 2.46% respectively). Biotope characteristics were unrelated to surrounding cover of alien invasive vegetation and agriculture. There was a stark contrast in the strength of relationships for urban cover within 100 m *versus* 500 m (P = 0.848 and P = 0.010 for the “Urban 100 m” and “Urban 500 m” variables respectively). The percentage variation explained by the “Urban 500 m” predictor variable (7.33%) was also considerably higher than for the other variables in Table 2.3, indicating a stronger relationship for this variable relative to the others. As observed for the physico-chemical results, the amounts of explained variation in the biotope matrices due to the habitat transformation variables (ranging between 0.25 and 7.33%) were all low compared to that explained by the spatio-temporal covariables (ranging between 14.60 and 30.76%). Due to the lack of significant results, and for the sake of brevity, PCA plots representing biotope characteristics were not further explored.

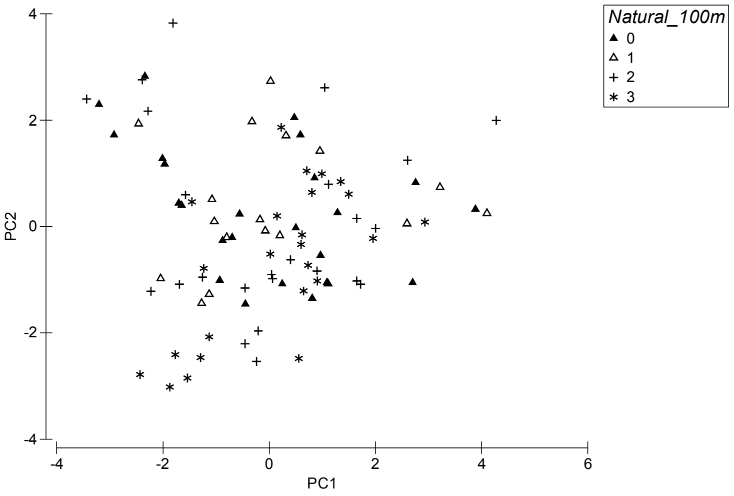


Figure 2.5. PCA of the physico-chemical variables (normalized) for all study sites (n = 90), displaying the first two principal component axes. Sites are coded according to the areal cover of natural (indigenous) vegetation within a 100 m radius of each wetland edge: 0 - none; 1 – sparse cover (< 33%); 2 – moderate cover (33-66%); 3 – extensive cover (> 66%, see Appendix 3).

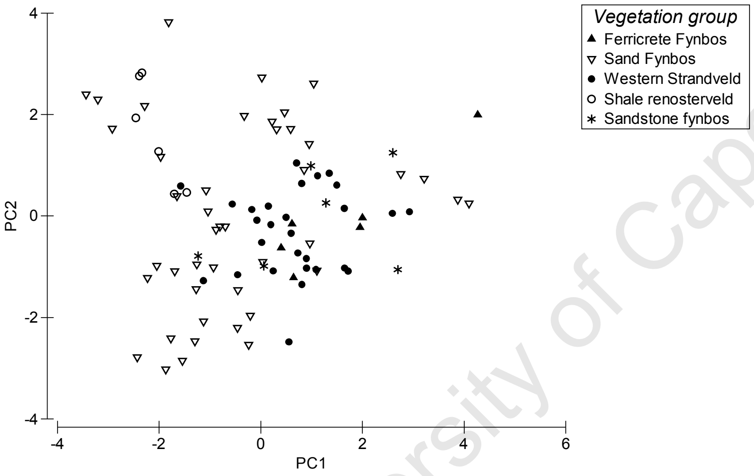


Figure 2.6. PCA of the physico-chemical variables (normalized) for all study sites (n = 90), displaying the first two principal component axes. Sites are coded according to the vegetation types in which they would naturally occur (see Appendix 1).

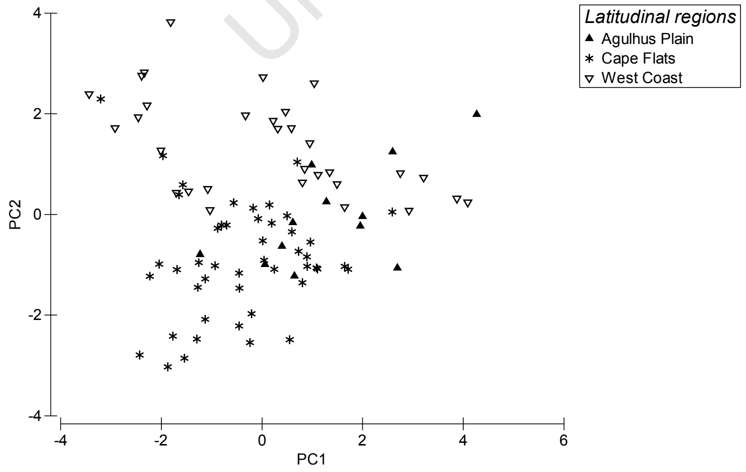


Figure 2.7. PCA of the physico-chemical variables (normalized) for all study sites (n = 90), displaying the first two principal component axes. Sites are coded according to the three broad latitudinal regions covered in this study (see Fig. 2.1).

Table 2.3. Non-parametric multivariate regression tests (dbRDA) for relationships between habitat transformation gradients and biotope characteristics in wetlands. Natural - indigenous vegetation; Invaded - alien invasive vegetation; Agriculture - agricultural land; Urban - urban area. The areal cover of these variables is represented within 100 and 500 m radii of each wetland edge. To maximise parsimony, covariable subsets were pre-selected for each model using step-wise regression of each response matrix on the full list of possible covariables (see Appendix 1). % Var - the percentage of variation in each Euclidean distance matrix (normalized physico-chemical variables) that is explained by each respective predictor variable or covariable set in each model; Time – number of days since the first sampling event; WS – Western strandveld; FF – Ferricrete fynbos; SF – Sand fynbos; Res. df – residual degrees of freedom for each model. Significant P values are presented in boldface (α = 0.05, with the exception of agriculture, where α = 0.10).

Predictor variable	Res. df	F	P	% Var	Covariables	% Var (covariables)
Natural 100 m	84	2.320	0.075	2.26	Latitude, time, altitude, WS	16.00
Natural 500 m	84	2.535	0.056	2.46	Latitude, time, altitude, WS	16.00
Invaded 100 m	67	1.427	0.230	1.78	Latitude, WS	14.60
Invaded 500 m	68	0.198	0.856	0.25	Latitude, WS	14.62
Agriculture 100 m	20	1.080	0.360	3.74	Latitude, FF	27.11
Agriculture 500 m	18	0.369	0.767	1.57	Longitude	21.91
Urban 100 m	31	0.182	0.848	0.40	Longitude, time, SF, Invaded 100 m	30.76
Urban 500 m	50	5.102	**0.010**	7.33	Latitude, time, SF	20.84

Hydro-morphometry

Table 2.4 presents the multivariate linear regression relationships between the multivariate matrices representing hydro-morphometry and the habitat transformation variables. The hydro-morphometry of wetlands was remarkably unresponsive to the surrounding habitat transformation gradients. All the predictor variables explained < 1% of variation in the multivariate matrices representing hydro-morphometry, with the exception of the “Invaded 500 m” predictor variable (2.83% explained variation). No significant relationships were detected between hydro-morphometry and surrounding levels of habitat transformation. Following the same reasoning outlined for biotope characteristics (see section: *Biotope characteristics*), PCA plots for hydro-morphometry were not further explored.

Table 2.4. Non-parametric multivariate regression tests (dbRDA) for relationships between habitat transformation gradients and the hydro-morphometry of wetlands. Natural - indigenous vegetation; Invaded - alien invasive vegetation; Agriculture - agricultural land; Urban - urban area. The areal cover of these variables is represented within 100 and 500 m radii of each wetland edge. To maximise parsimony, covariable subsets were pre-selected for each model using step-wise regression of each response matrix on the full list of possible covariables (see Appendix 1). % Var - the percentage of variation in each Euclidean distance matrix (normalized physico-chemical variables) that is explained by each respective predictor variable or covariable set in each model; Time – number of days since the first sampling event; SF – Sand fynbos; WS – Western strandveld; Res. df – residual degrees of freedom for each model. Significant P values are presented in boldface (α = 0.05, with the exception of agriculture, where α = 0.10).

Predictor variable	Res. df	F	P	% Variation	Covariables	% Variation (covariables)
Natural 100 m	86	0.150	0.851	0.15	Longitude, SF	11.44
Natural 500 m	86	0.433	0.642	0.44	Longitude, SF	11.44
Invaded 100 m	68	0.634	0.534	0.87	WS	5.68
Invaded 500 m	69	2.150	0.125	2.83	WS	6.47
Agriculture 100 m	21	0.111	0.892	0.44	Time	15.22
Agriculture 500 m	19	0.080	0.928	0.42	No covariables	NA
Urban 100 m	34	0.234	0.774	0.62	Altitude	9.30
Urban 500 m	51	0.623	0.508	0.98	Latitude, Invaded 500 m	19.01

2.3.2. Environmental responses to habitat transformation: univariate patterns

Table 2.5 presents the univariate multiple linear regression relationships (a – r) between each of the environmental response variables measured in this study and the ordinal variables representing habitat transformation around wetlands (predictor variables), given the spatio-temporal covariables in each model. To prevent unwieldy presentation of results, only the significant relationships are presented here. The physico-chemical variables pH, phosphates, oxygen and turbidity were negatively related to indigenous vegetation cover within both 100 and 500 m radii of wetlands. With the exception of turbidity, the same variables were positively related to invasive vegetation cover within 100 m of wetlands. Only dissolved oxygen concentrations were significantly related (positive slope) to invasive vegetation cover within 500 m. Phosphate concentrations in wetlands were positively related to agricultural cover within 100 m, whilst ammonium concentrations and % open water biotope were negatively related to agricultural cover within 500 m. pH and % simple-structured vegetation biotope were positively related to urban cover within 100 and 500 m of wetlands respectively. These relationships were however generally weak, as inferred from the low amounts of explained variation in the response variables due to the habitat transformation predictor variables (partial r^2 values mostly < 0.20 i.e. 20%, and none were >0.30). As was the trend observed for the multivariate regressions, the percentages of explained variation due to the spatio-temporal covariables (see “r^2 - Covariables”) were for the most part considerably higher than that explained by the habitat transformation predictor variables (see “Partial r^2 – predictor”). Neither of the two variables representing the hydro-morphometry of wetlands (“maximum depth” and “total surface area”) was significantly related to the habitat transformation predictor variables.

The partial residual plots of Figure 2.8 offer visual representation of the regression relationships reported above, holding the covariables constant. Apparent in most of the plots is the considerable amount of vertical (Y axis) scatter in the residual points, which accounts for the low partial r^2 values observed in Table 2.5 and shows that relationships were generally weak. The plots also allow identification of outliers or groups of high leverage points. The pattern for pH appears to be highly leveraged by five very low pH sites occurring in one particular area on the Cape Flats at Kenilworth, as evident at the bottom of plots “a“, “e”, “i" and “q". To test their influence, a *post hoc* analysis was run without these sites and revealed that the partial relationships between pH and natural vegetation cover within 100 and 500 m remained significant at $\alpha = 0.05$, but were substantially weaker (“Natural 100 m”: $t_{80} = -2.124$, $P = 0.037$, partial $r^2 = 0.053$; “Natural 500 m”: $t_{80} = -1.994$, $P = 0.049$, partial $r^2 = 0.047$). Partial relationships between pH and invasive vegetation cover within 100 m, and between pH and urban cover within 100 m, were rendered non-significant by exclusion of these sites from the models (“Invaded 100 m”: $t_{61} = 1.303$, $P = 0.198$, partial $r^2 = 0.027$; “Urban 100 m”: $t_{29} = -0.187$, $P = 0.853$, partial $r^2 = 0.001$), indicating a strong influence of these sites in the regressions.

Relationships between phosphate concentrations and habitat transformation (plots “b”, “g”, “j” and “m”) showed high phosphate values associated with several of the extensively transformed wetlands, which may have influenced the reliability of these trends. Examination of Cook’s distances for these models did not however indicate that any of these high phosphate values had undue leverage on the trends (the maximum Cook’s D_i value was 0.228). Dissolved oxygen concentrations showed similar patterns as for phosphates, although only one outlier was clearly apparent in these plots (see top left of plots “c” and “f”, top right of plot “k”, and top middle of plot “l”). Cook’s distances once again indicated that no points had particularly undue leverage in these models (maximum Cook’s D_i value was 0.373). Turbidity displayed weak linear trends with the “Natural 100 m” and “Natural 500 m” variables (plots “d” and “h” respectively), with low gradient slopes and considerable spread in the residual points on either side of the regression line. The positive relationship between turbidity levels and urban cover within 100 m (plot “p”) was clearer however, and showed less scatter among points and a steeper gradient in the regression line. The remainder of the relationships depicted in Figure 2.8 (see plots “n”, “o” and “r”) appeared to be particularly weak due to the large amount of scatter among points, indicating that these trends are unreliable.

Table 2.5. Multiple linear regression models (a - r) of environmental response variables regressed against the habitat transformation variables (predictors), given the spatio-temporal covariables. Only significant relationships are presented here (α = 0.05, with the exception of agriculture, where α = 0.10). To maximise parsimony, covariable subsets were pre-selected for each model using step-wise regression of each response variable on the full list of possible covariables (see Appendix 1). For each predictor variable, results are listed in decreasing order of relationship strength based on P values. Only partial relationships between the response and predictor variables are reported here, not the full model results.

Natural - indigenous vegetation; Invaded - alien invasive vegetation; Agriculture - agricultural land; Urban - urban area. The areal cover of these variables is represented within 100 and 500 m radii of each wetland edge, measured on an ordinal scale. Time - Number of days since the first sampling event; β – standardized regression coefficient; SE – standard error of regression coefficient; Partial r^2 – coefficient of partial determination for each respective predictor variable; Res. df – residual degrees of freedom; r^2 (Covariables) = Full model r^2 - Partial r^2 (predictor).

	Predictor variables	Response variables	β	SE	Partial r^2 (Predictor)	t	Res. df	P	Covariables	r^2 (Covariables)
a)	Natural 100 m	pH	-0.354	0.081	0.187	-4.374	83	<0.001	Longitude, latitude, time, altitude, Western strandveld	0.329
b)	Natural 100 m	Phosphates	-0.214	0.083	0.073	-2.591	86	0.011	Longitude, latitude, Shale renosterveld	0.371
c)	Natural 100 m	Oxygen	-0.216	0.092	0.061	-2.357	86	0.021	Altitude, Ferricrete fynbos	0.256
d)	Natural 100 m	Turbidity	-0.163	0.070	0.060	-2.326	85	0.022	Longitude, latitude, Shale renosterveld	0.543
e)	Natural 500 m	pH	-0.261	0.087	0.097	-2.993	83	0.004	Longitude, latitude, time, altitude, Western strandveld	0.365
f)	Natural 500 m	Oxygen	-0.229	0.093	0.066	-2.467	86	0.016	Altitude, Ferricrete fynbos	0.255
g)	Natural 500 m	Phosphates	-0.195	0.084	0.059	-2.317	86	0.023	Longitude, latitude, Shale renosterveld	0.377
h)	Natural 500 m	Turbidity	-0.152	0.071	0.051	-2.138	85	0.035	Longitude, latitude, Shale renosterveld	0.548
i)	Invaded 100 m	pH	0.391	0.088	0.229	4.423	66	<0.001	Time, altitude, Western strandveld	0.295
j)	Invaded 100 m	Phosphates	0.251	0.098	0.090	2.570	67	0.012	Longitude, latitude	0.274
k)	Invaded 100 m	Oxygen	0.248	0.102	0.079	2.418	68	0.018	Altitude	0.209
l)	Invaded 500 m	Oxygen	0.318	0.102	0.123	3.108	69	0.003	Altitude	0.191
m)	Agriculture 100 m	Phosphates	0.371	0.161	0.210	2.307	20	0.032	Latitude, Sand fynbos	0.410
n)	Agriculture 500 m	Ammonium	-0.399	0.149	0.285	-2.676	18	0.015	Latitude	0.332
o)	Agriculture 500 m	% Open water	-0.316	0.156	0.194	-2.025	17	0.059	Time, Sand fynbos	0.476
p)	Urban 100 m	Turbidity	0.457	0.135	0.253	3.395	34	0.002	Latitude	0.158
q)	Urban 100 m	pH	0.341	0.131	0.167	2.610	34	0.013	Sand fynbos	0.252
r)	Urban 500 m	Simple vegetation	0.363	0.121	0.148	3.010	52	0.004	Western strandveld	0.097

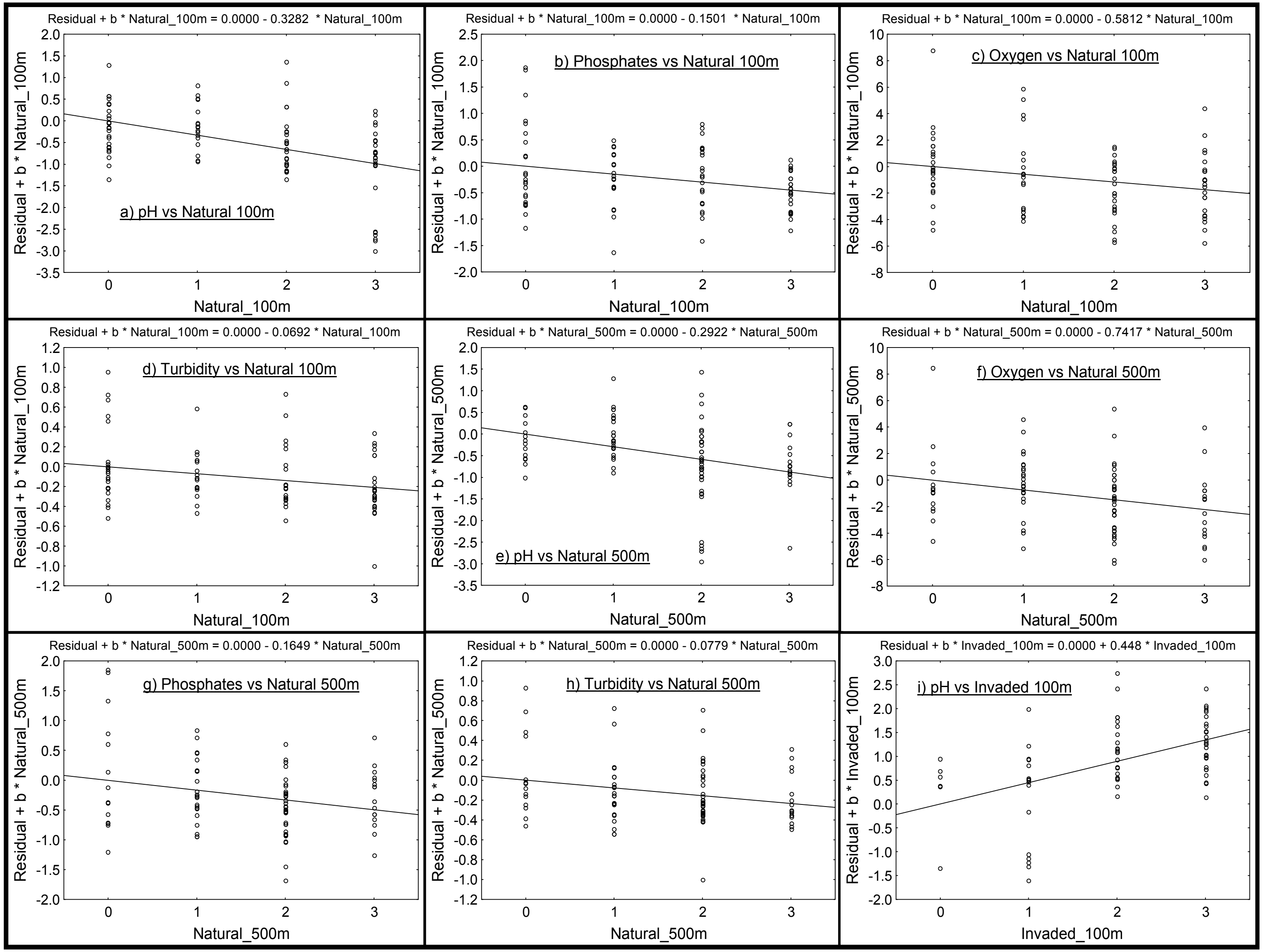
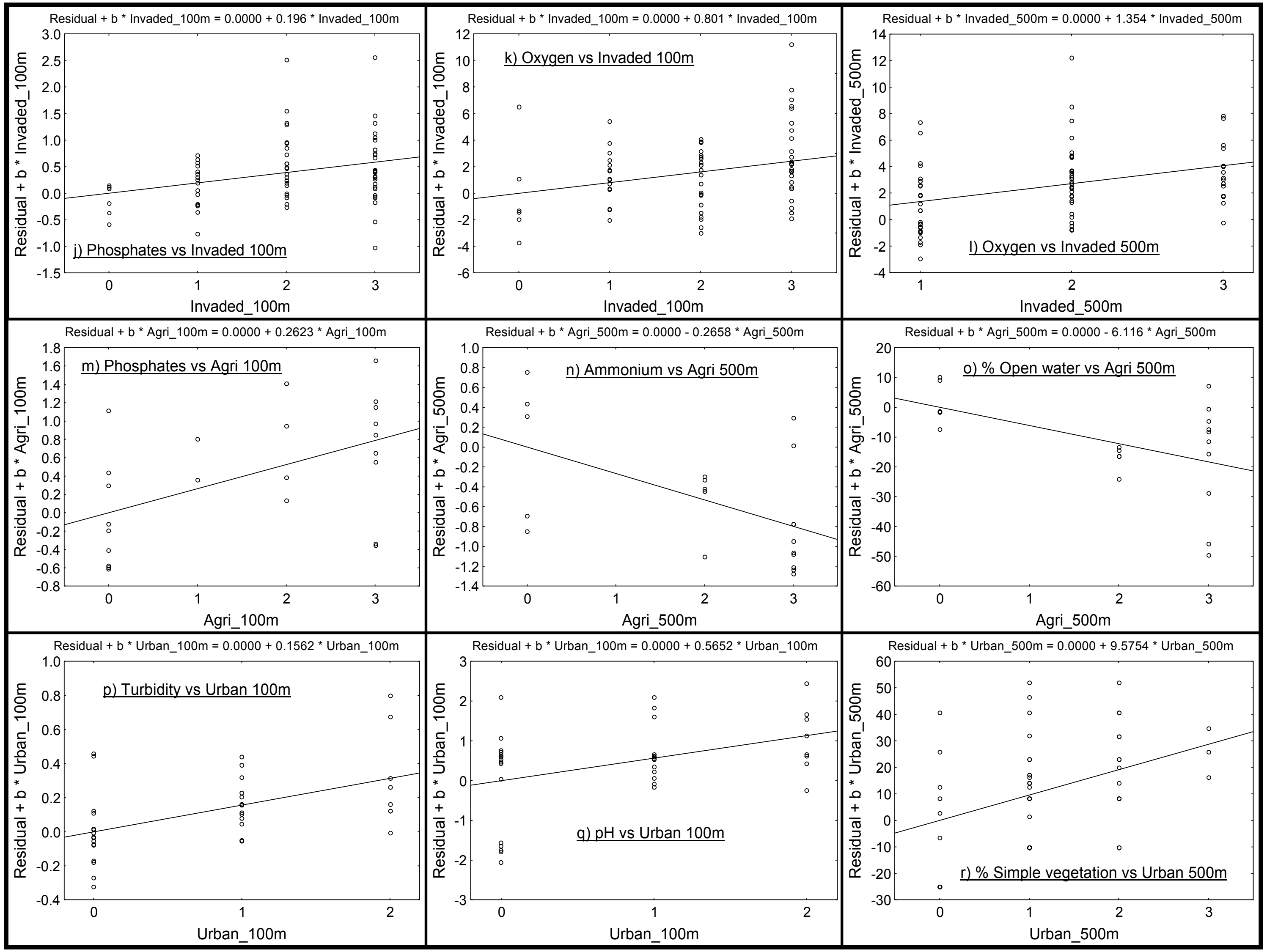


FIGURE 2.8. Partial residual plots displaying the relationships a – r presented in Table 2.5. Environmental response variables are depicted in relation to the habitat transformation variables (predictors, x axes), holding the spatio-temporal covariables constant. Natural - indigenous vegetation; Invaded - alien invasive vegetation; Agri - agricultural land; Urban – urban area. The areal cover of these variables is represented within 100 and 500m radii of each wetland edge, measured on an ordinal scale: 0 – none; 1 – sparse; 2 – moderate; 3 – extensive. For more detailed information regarding each model, refer to Table 2.5.

NOTE: The residuals on the vertical axis of each plot come from the regression of the response variable against all the predictors except the one of interest. The residuals for the horizontal axis of each plot come from the regression of the predictor variable of interest against all other predictors. Each residual scatterplot shows the relationship between a given univariate response variable and a predictor variable of interest, holding the other predictor variables constant. The regression equation for each relationship has been indicated, with each slope being equal to the non-standardized regression coefficient (b) in the full multiple regression model in which the parameter was included. ‘0.0000’ indicates that the intercept value is <0.0001.

Figure 2.8. (Continued)



2.4. DISCUSSION

2.4.1. Physico-chemical conditions

Relationships with surrounding indigenous vegetation cover

The multivariate regression relationships presented in this study indicate that human transformation of the landscape surrounding temporary isolated depression wetlands in the south-western Cape is associated with physico-chemical conditions in these wetlands. This statement refers to overall transformation of adjacent habitats as represented by the remaining indigenous vegetation cover (i.e. the predictor variables “Natural 100 m” and “Natural 500 m”). This pattern appears to be slightly stronger for habitat transformation taking place within 100 m of wetlands than for 500 m, although significant trends were seen at both spatial scales (Table 2.2). The contribution to the percentage variation in the physico-chemical conditions explained by variables representing the different types of habitat transformation was very low (ranging between 2.08 and 5.57%), despite being significant in some cases (Table 2.2). This explained variation was generally in the region of one order of magnitude lower than that explained by the spatio-temporal covariables in the multivariate models (ranging between 35.47 and 63.71%, Table 2.2). At the broad scale of this study, the primary influence on physico-chemical conditions in wetlands thus appeared to come from spatio-temporal factors, although a significant signal was still detected for certain habitat transformation factors over and above the spatio-temporal influence. This indicates firstly, that spatio-temporal variation in environmental conditions is high for these wetlands, and secondly, that habitat transformation has played a meaningful role (albeit relatively weak in comparison to that of spatio-temporal factors) in altering the physico-chemistry of these wetlands, as was hypothesized at the outset of this study.

My results are in line with those of a similar study by Declerck *et al.* (2006) on the water quality of 99 small permanent ponds (natural and artificial) affected by agriculture in Belgium. These authors recorded land use at multiple spatial scales up to 3.2 km around ponds and found that the maximum amount of variation in a set of water quality variables explained by crop land was 2.3% and by the amount of indigenous forest cover was 4%, both measured at a scale of 100 m around ponds and both were statistically significant results. Their study also corroborates my finding that habitat transformation influences on the physico-chemistry of small, isolated wetlands appear to be strongest within 100 m of wetlands, although only a slight difference was found between the 100 and 500 m scales in this study. Given that small isolated wetlands have been shown elsewhere to drain localised catchments, these wetlands would not be expected to be affected by broader catchment-scale processes as might be the case for rivers or lakes (Davies *et al.* 2008). This probably explains why the strongest relationships between wetland physico-chemical conditions and surrounding land cover are reported at the 100 m scale in this study and that of Declerck *et al.* (2006).

Negative linear relationships were reported between surrounding indigenous vegetation cover within 100 and 500 m of wetlands and the pH, phosphates, dissolved oxygen and turbidity levels in these wetlands (Table 2.5). Turbidity has been shown to increase with transformation of the surrounding landscape for other wetland ecosystems, particularly as a result of sedimentation from agricultural or urban runoff (Crosbie and Chow-Fraser 1999, Declerck *et al.* 2006). Replacement of natural vegetation often leads to de-stabilization of soils (Skagen *et al.* 2008) and thus various forms of habitat transformation could be responsible for increased sediment input to wetlands through increased surface water flows during rain events. The negative relationship between wetland pH and natural vegetation cover within 100 m is most likely an effect of removing fynbos, which is known to release acidic leachates into the soil (Gardiner 1988, Raubenheimer and Day 1991, Midgley and Schafer 1992). The resultant physico-chemical effect would be an increase in the pH of wetlands as surrounding fynbos is lost. However, this can only be hypothesized until causality is established. This alteration of pH with transformation of the landscape might be hypothesized to occur only in areas where soils are naturally acidic and the vegetation type is sclerophyllous fynbos, which contains high levels of acidic tannins. For example, the Western strandveld cluster occurs on naturally alkaline soils, due to the intrusion of calcareous sediments of marine origin (Rebelo *et al.* 2006), and the vegetation is not sclerophyllous, but dominated by succulents. Thus replacing this vegetation type with alien vegetation, one would not expect a consequent effect on soil or surface water pH. However, Sand fynbos is a vegetation type that occurs on well-leached, naturally acidic soils and the vegetation itself is sclerophyllous, containing high levels of acidic tannins as a defence against herbivory (Rebelo *et al.* 2006). Therefore, the loss of fynbos in this area can be hypothesized to raise soil and surface water pH. For example, closer inspection of plots “a” and “e” in Figure 2.8 reveals that five of the sites appeared to have a high leverage on the strength of the trend between indigenous vegetation cover and the pH of wetlands (although omitting these sites still yielded significant regression results, see section 2.3.2). These sites occurred inside the Kenilworth racetrack on the Cape Flats and were among the most pristine wetlands in the Sand fynbos cluster, because the land has been minimally disturbed inside the racetrack (McDowell 1989, Hitchcock 2006, Hopkins 2006, Turner 2006, Hitchcock *et al.* 2008). The low pH values are thus most likely a real reflection of the vast amount of undisturbed fynbos vegetation surrounding these sites. In summary, the relationship between the amount of natural vegetation and pH levels in wetlands was patchy and driven mostly by sites occurring within Sand fynbos. One cannot expect to observe this relationship in areas where the natural vegetation type does not contain acidic tannins.

The negative association between phosphate concentrations and natural vegetation cover within 100 and 500 m could be due to the effects of alien vegetation, agriculture or urban development. The following sections further explore the physico-chemical variables that appear to have been most affected by each type of habitat transformation. The negative relationships between surrounding indigenous vegetation cover and dissolved oxygen levels were surprising given that previous studies have found human disturbance of the landscape to be generally associated with increased levels of nutrients and decreased levels of oxygen in aquatic ecosystems (e.g. Carpenter *et al.* 1998, Leavitt *et al*. 2006, Pham *et al*. 2008). Further investigation would be required to establish any underlying causes in this regard.

Alien invasive vegetation

Considering the three types of habitat transformation separately, only invasive vegetation cover was significantly related to physico-chemical conditions at both 100 and 500 m spatial scales (Table 2.2) and thus appears to be an influential form of habitat transformation. Using various modelling approaches, Rouget *et al.* (2003) predicted that between 27.2 and 30% of remaining untransformed habitat in the Cape Floristic Region (into which the south-western Cape falls) is likely to be invaded by alien plants over the next 20 years (i.e. from the time of their study). My results suggest that this predicted spread of alien invasive plants into untransformed areas in the near future is likely to impact significantly on temporary wetland environments occurring in those areas without “polluting” or physically altering them. To my knowledge, these are the first data to provide evidence that terrestrial alien plants are indeed affecting water quality conditions (as proxied in this study by physico-chemical variables) of aquatic ecosystems in the region, despite numerous research efforts that have focussed only on the effects of alien plants on water quantity. Furthermore this has importance in the light of changes in biotic assemblages that could potentially be induced by these physico-chemical effects, given the potential importance of physico-chemistry in structuring aquatic assemblages such as invertebrates.

The positive association between dissolved oxygen concentrations in wetlands and surrounding invasive vegetation cover within 100 and 500 m was difficult to explain and no literature appears to report similar findings. Further investigation is required to explore possible mechanisms governing this trend, although it should be noted that these relationships were not convincing as reflected by the low partial r^2 values (0.079 and 0.123 at 100 and 500 m respectively). Phosphate concentrations (see summary statistics for this variable in Appendix 4) were related to invasive vegetation cover within 100 m, but not 500 m, suggesting a localised nutrient input from invasive vegetation into groundwater. Once again the relationship was weak as judged by the small extent of explained variation in phosphates due to the predictor variable "Invaded 100 m" (partial r^2 = 0.090). A possible mechanism governing this trend is the elevation of soil phosphorus in adjacent terrestrial soils due to infestation by alien shrubs (soil phosphorus was not however measured in this study), which may then leach into wetlands. This is postulated based on the findings of Witkowski and Mitchell (1987), who reported a significant increase in soil phosphorus in stands of *Acacia saligna* (also the dominant invader in the current study) compared to surrounding natural lowland fynbos vegetation and established that this was due to higher litterfall from acacias, which released leaves into the soil with a significantly higher phosphorus content than those of lowland fynbos vegetation. A controlled experiment is required to validate this ecological mechanism.

The relatively strong ($P = 0.001$, partial r^2 = 0.229) positive relationship between pH and alien vegetation cover within 100 m (Table 2.5) is most likely a consequence of the loss of natural vegetation which accompanies the transformation of habitats by invasive alien vegetation. As discussed earlier, I hypothesized that the loss of natural vegetation in the Sand fynbos area would cause an increase in soil and surface water pH through the loss of acidic tannins that characterise natural fynbos ecosystems in this area. The predominant disturbance type in this area was alien vegetation and thus it was positively associated with levels of pH, even though it is not expected that alien vegetation itself raises the soil pH, but rather that it is associated with higher levels of pH as a consequence of the loss of naturally acidic vegetation to the system.

Agriculture

Previous studies, mostly on permanent wetlands, have indicated that agriculture has significant impacts on the water chemistry of wetlands (e.g. Lougheed *et al*. 2001, Rhazi *et al*. 2001, Declerck *et al*. 2006, Skagen *et al*. 2008), whilst no significant effects were detected in this study. This may, to some extent, be an artefact of the relatively small sample size for the agricultural datasets (100 m scale: n = 24; 500 m scale: n = 21), which reduces the statistical power to detect an effect (Nakagawa 2004). The primary agricultural areas of the study region occur mostly on relatively fertile shale soils (Rebelo *et al.* 2006), where wheat agriculture has transformed the landscape so intensively that least impaired wetlands were difficult to find and it was necessary to search for small fragments of remaining natural vegetation that also happened to house temporary wetlands. In the Sand fynbos cluster, least impaired sites were not too difficult to find, and these were compared with sites occurring within pasture areas (the predominant form of agriculture in this area). However, the difficulty in this case was in finding enough sites within moderately and extensively transformed pasture areas. My data on agriculture is thus limited and although no effect on wetland physico-chemistry was found, this should be interpreted with caution until a larger set of data is available. The lack of un-impacted depression wetlands that could be found within the extensively transformed wheat farming areas highlights the plight of these wetlands in lowland agricultural areas.

Urban development

The association between urban cover within 100 m and physico-chemical conditions in the studied wetlands (Table 2.2) is in line with the few previous studies which have addressed the topic for temporary wetlands (Brooks *et al.* 2002, Faulkner 2004), however certain affected variables appear to be different (see below). The significant positive relationship between amount of urban area (within 100 m in this study) and wetland turbidity has been reported elsewhere (Azous and Horner 1997, Brooks *et al.* 2002) and could be attributed to sedimentation from increased surface runoff, amongst other factors. The positive relationship between pH and urban cover within 100 m is once again likely to be due to the rise in pH associated with the loss of fynbos vegetation as habitat is converted to urban surfaces. It was surprising that nutrient concentrations were not associated with surrounding urban cover, as previous literature has reported this for other temporary wetland systems (Brooks *et al.* 2002, Faulkner 2004) and it was expected that the major form of disturbance for urban-exposed wetlands would be in the form of increased nutrient levels. Furthermore, one would expect that the effects of urban development would extend beyond 100 m, given the intensity of this land use, and that a significant association would have been found between physico-chemical conditions and urban cover within 500 m.

Spatial patterns

The PCA ordinations indicated that the spatial scale of sampling was positively associated with the amount of variation in physico-chemical conditions in the temporary wetlands. Although wetland clusters did not clearly separate out on the basis of their physico-chemical constituents, the amount of variation within each cluster was linked to the spatial area covered (Fig. 2.6). The Sand fynbos cluster covered the broadest area of sampling and showed by far the most variation in physico-chemical conditions (as evidenced by scatter in the PCA plot among sites for this cluster), and the Western strandveld was the second largest cluster showing the second-highest levels of variation. With the exception of Sandstone fynbos, which showed considerable variation among few sites, the remaining small wetland clusters displayed correspondingly lower levels of variation than for the bigger clusters. The ordinations further indicated that the spatial scale with the clearest pattern of influence on the physico-chemical variables was at the level of broad latitudinal regions (Agulhus Plain, Cape Flats and West coast, Fig. 2.7). This appears to be consistent with the pattern described above (although at a broader scale) of increased variation with increased spatial scale and reinforces the pattern of a link between spatial extent of sampling and increasing variation of physico-chemical conditions in the region. This is perhaps not surprising given that one expects more variation in physico-chemistry as the area sampled broadens, due to an associated increased variation in natural environmental factors such as geology and local climate. However, very little information exists on these basic aspects of spatial variation of environmental conditions in temporary wetlands of the region (but see Silberbauer and King 1991b, De Roeck 2008) and thus it is important to document such patterns. One should bear in mind that there is a certain degree of confounding from temporal differences between clusters (they were sampled sequentially), which cannot be accounted for in the PCA ordinations, but which were partialled out of the multiple regression models.

2.4.2. Biotope characteristics

Multivariate relationships between biotope characteristics and the extent of habitat transformation around wetlands showed a lack of convincing patterns, with the possible exception of the trend with urban cover within 500 m of wetlands. The multivariate relationship with the variable “Urban 500 m” was probably driven largely by the positive relationship between this variable and % simple-structured vegetation cover within wetlands, as evidenced by the univariate regression results (Table 2.5). The partial residual plot (Fig. 2.8, plot “r”) for this trend showed however that there was considerable spread among the residual points for each level of urban cover and the pattern appears weak and unreliable. The only other significant univariate relationship between any of the biotope and habitat transformation variables was the negative relationship between % open water and agriculture within 500 m (Table 2.5). Once again, examination of the partial residual plot (Fig. 2.8, plot “o”) showed this to be an unreliable trend due to the large amount of spread in the residuals for sites surrounded by extensive agriculture (cover category 3).

I hypothesized at the outset of this study that the structural complexity of biotopes within wetlands would be negatively related to habitat transformation around wetlands. There were however no consistent patterns indicating an increase in the cover of simple-structured biotopes (% simple vegetation, % open water and % benthic un-vegetated habitat) or a decrease in the cover of complex-structured biotopes (% complex vegetation) with transformation of habitats around wetlands. Taken as a whole, the multivariate and univariate patterns presented in this study indicate a lack of convincing relationships between habitat transformation and biotope characteristics in wetlands. These results are in contrast to those of Declerck *et al*. (2006) who observed a negative relationship between both the amount of cropland and degree of trampling by cattle around wetlands, and the structural complexity of aquatic vegetation habitats for a set of 99 small permanent ponds (natural and artificial) differentially affected by agriculture across Belgium. They attributed this to the indirect effects of increased turbidity and nutrients due to agriculture on vegetation complexity through a decrease in light penetration, which allows phytoplankton to dominate and this in turn continues to increase turbidity. As neither phytoplankton nor chlorophyll *a* was measured in the current study, it is difficult to gauge whether the increased levels of turbidity and phosphates associated with habitat transformation were linked to a shift in the ratio between macrophyte and phytoplankton dominance. However, the role of phytoplankton in temporary wetlands is generally expected to be less pronounced than for permanent wetlands due to the inconsistency of the water regime and thus macrophytes generally dominate the primary production component of these systems (Marty 2004, Williams 2006, De Roeck 2008). Perhaps the shift in turbidity and phosphates associated with increasing levels of habitat transformation in this study were not pronounced enough to alter vegetation complexity of the temporary wetlands. Mean and median turbidity values were low (< 6 NTU) across all clusters, with the exception of Shale renosterveld (mean: 235.3 NTU; median: 105.7 NTU; Appendix 4), and thus one cannot exclude the possibility that turbidity levels in this study were below some threshold required for causing an impact on vegetation structure. Another explanation could be that the adaptations of macrophytes in these temporary wetlands to constantly fluctuating hydrological and physico-chemical environments (hydrology and physico-chemistry may have separate effects on vegetation structure) gives them a natural resilience to more moderate and longer-term fluctuations in physico-chemical conditions associated with surrounding habitat transformation.

Declerck *et al.* (2006) reported that relationships between land use and physico-chemical conditions in wetlands were clearer than those observed for vegetation structural complexity, as was also observed in the current study. Similarly, Rhazi *et al.* (2001) observed impacts of agriculture in the catchment on water and sediment quality of ten temporary wetlands in Morocco, but did not find a significant influence on vegetation structural complexity. A more in-depth study on 30 Moroccan temporary wetlands by Rhazi *et al.* (2006) established significant changes in temporary wetland vegetation composition as a result of agricultural activities around wetlands, but these authors did not address the structural complexity of wetland vegetation biotopes as did Declerck *et al.* (2006). My results, and those of previous studies, do not show a clear and consistent effect of habitat transformation on the structural complexity of biotopes within temporary wetlands. It is more likely that vegetation structural complexity will be affected by habitat conversion if this is associated with pronounced variation in physico-chemical conditions in wetlands, which was not the case in this study even though physico-chemical variation was found to be statistically significant. Measures of plant taxonomic composition may have been more appropriate for elucidating impacts of land use on wetland vegetation, but these were beyond the scope of this study. It is interesting to note the similarities between my study results and those reported above, given that my study has incorporated a range of habitat transformation types (agriculture, urban development, alien vegetation) and those reported above only deal with the impacts of agriculture. There is a complete lack of studies that have addressed the impacts of multiple types of land use (or any impact other than agriculture for that matter) on temporary wetland ecosystems and further such investigations would enable better validation of the statements made in the current study.

2.4.3. Hydro-morphometry

There exists a very limited literature on the influence of habitat transformation in the immediate landscape on permanent wetland hydro-morphometry (Azous and Horner 1997, Reinelt *et al.* 1998, Bruland *et al.* 2003), and none so for temporary wetlands. No clear hypotheses could be established in this regard and an exploratory approach was taken to assess whether there were any prevalent relationships between temporary wetland hydro-morphometry and surrounding habitat transformation in the region. This study did not attempt to address the topic in detail and only quantified two variables (total surface area and maximum depth); mostly because these variables are known to have important influences on wetland invertebrates (the topic of the next chapter) and thus habitat transformation effects on these variables would have potentially important ramifications for the analyses in chapter 3. A variety of mechanisms might explain changes in surface area or depth of wetlands as they become encroached by alien vegetation invasion (e.g. reduction of groundwater availability to wetlands), agricultural activities (e.g. reduced surface water availability to wetlands due to artificial drainage networks) or urbanisation (e.g. increasing depth or surface area of wetlands due to increased runoff from hardening of adjacent landscapes); however the data presented in this study does not suggest that any of these mechanisms were in effect because there were no significant multivariate or univariate relationships between hydro-morphometry and surrounding habitat transformation. More detailed hydro-morphometrical measures such as basin profiles and measurements of depth over time may have revealed relationships with habitat transformation, but this was not practical given the broad scale of sampling and the limited time available at each wetland. The major hydrological modification to temporary wetlands in the region has been the alteration of hydroperiod through the damming of temporary wetlands in order to make them more perennial (e.g. to provide drinking water for livestock in summer or for general irrigation, Silberbauer and King 1991a, De Roeck 2008). This study did not address this issue because only natural depressions with a seasonal inundation regime (< 2 m deep) were selected.

2.4.4. Conclusions

Small, isolated depression wetlands are highly heterogeneous systems, especially temporary wetlands which have pronounced temporal variation (Williams 2006). The environmental characteristics of these wetlands were thus expected to be influenced by various spatial and temporal factors acting at a landscape scale (e.g. geological context or local climate) and the sampling and analysis of wetlands in comparable clusters helped aid the detection of any influences from surrounding habitat conversion. The fact that significant physico-chemical signals (both multivariate and univariate) from habitat transformation were still detected over and above strong spatio-temporal influences suggests that further studies at smaller spatial scales (e.g. within wetlands clusters), and better accounting for temporal variation (through repeated sampling), would be worthwhile for elucidating more specific information on the nature of the impacts of habitat transformation on the environments of temporary wetlands (see chapter 4). However this study provides important first-level data for these wetlands in the region and offers insight into the broad-scale patterns between habitat conversion and its effects on temporary wetland environments. My data indicate that the physico-chemical environment of these temporary wetlands is significantly influenced by human transformation of natural habitat within adjacent landscapes (< 500 m). Relationships were generally stronger at the scale of 100 m around wetlands than for 500 m, which indicates that preservation of narrow buffer strips of indigenous vegetation around these wetlands may afford significant protection of water quality. Restoration of even small fragments of terrestrial vegetation supporting temporary wetlands is likely to yield significant improvements in water quality towards the original least impaired state. Variation in biotope characteristics was related to urban cover within 500 m of wetlands, but otherwise the multivariate and univariate trends were weak for biotope variables in relation to surrounding habitat transformation. Thus, no clear evidence was established in this study for a negative effect of transforming natural habitat around wetlands on the structural complexity of aquatic biotopes in these wetlands, as was hypothesized. It is expected that clearer patterns between habitat transformation and biotope characteristics will be revealed at smaller spatial scales of study, following the reasoning stated above for environmental conditions in general. There was no evidence of any association between habitat transformation and wetland hydro-morphometry for temporary wetlands in the region.

This chapter has addressed relationships between environmental conditions in temporary wetlands and surrounding levels of habitat transformation. The following chapter explores whether this transformation of landscapes around temporary wetlands of the region appears to have influenced the aquatic invertebrate assemblages inhabiting these wetlands and, if so, whether environmental changes in wetlands related to habitat transformation have played a role in mediating these associations.

CHAPTER 3

THE INFLUENCE OF HABITAT TRANSFORMATION ON AQUATIC INVERTEBRATE ASSEMBLAGES IN TEMPORARY WETLANDS

ABSTRACT

The effects of human land use and associated transformation of habitats on the ecology of wetlands is a topic that is gathering momentum in the worldwide scientific literature. Temporary wetlands, the dominant wetland type in drier parts of the planet, have seldom been studied in this regard. Aquatic invertebrates are a ubiquitous and characteristic fauna in these systems and have been proposed as useful indicators of human disturbance in other permanent wetland types. Whether they constitute effective indicators in temporary wetland environments is open to debate. This study tests the hypothesis that aquatic invertebrates of temporary wetlands are resilient to disturbances caused by adjacent habitat transformation and thus do not constitute effective indicators of human activities in the landscape. 90 temporary depression wetlands spread across the south-western Cape mediterranean-climate region of South Africa were sampled for aquatic macroinvertebrates and various environmental constituents. A subset of 41 of these sites was also sampled for microcrustaceans (Copepoda, Ostracoda and Cladocera). Distance-based redundancy analysis (dbRDA) was used to relate invertebrate assemblage composition to variation in natural spatio-temporal and environmental factors, as well as anthropogenic factors represented by levels of adjacent habitat transformation within 100 m and 500 m of wetlands. Individual families and metrics were also regressed against gradients of habitat transformation using univariate multiple linear regression (MLR) in order to assess their use as indicators of human disturbance for the future ecological assessment of these wetlands in the region. Relationships between invertebrate assemblage composition and gradients of habitat transformation were in several cases significant (or P values were very close to the significance level), but were very weak in comparison to the effects of natural variation among wetlands. It appears that natural variation effects mask anthropogenic effects at the broad scale of this study. This was reflected in the overall poor performance of macroinvertebrate and microcrustacean families and metrics as indicators of human disturbance, as proxied by habitat transformation around wetlands and an index of human disturbance for each wetland. The data collected in this study strongly indicates that temporary wetland invertebrates do not show potential for inclusion in a biotic index for the region. The hypothesis that temporary wetland invertebrates are resilient to human disturbances in adjacent landscapes was not fully supported by this data, because significant (albeit weak) relationships were detected between invertebrate assemblage composition and several of the habitat transformation variables. It is expected that at smaller spatial scales (with reduced natural variation) the influence of habitat transformation on invertebrates is likely to increase relative to that of natural variation, thus allowing better detection of anthropogenic effects.

3.1. INTRODUCTION

3.1.1. Background

Wetland environments are highly threatened by human activities, particularly in low-lying landscapes such as coastal plains where urban development and conversion of land to agriculture are most prevalent. Temporary wetlands constitute the most abundant wetland type in the drier parts of our planet, yet historically have been completely overlooked in terms of their conservation importance (Williams *et al.* 2001, Oertli *et al.* 2005, Williams 2006, Zacharias *et al.* 2007). The impact of human activities in the landscape on the structure and functioning of these ecosystems has thus scarcely been addressed in the scientific literature. As a means towards bridging this gap in knowledge, the present study investigates aquatic invertebrate assemblages in temporary wetland environments in relation to human transformation of adjacent habitats.

In the light of recent recognition of the ecological importance of temporary wetlands (Gibbs 1993, Semlitsch and Bodie 1998, Nicolet 2001, Williams *et al.* 2001, De Meester *et al.* 2005, Williams 2006, Gómez-Rodríguez *et al.* 2009), it is of interest to assess how human transformation of terrestrial landscapes, in which temporary wetlands are embedded, affects these aquatic ecosystems. The previous chapter of this thesis addressed the abiotic component of temporary wetland ecosystems in relation to human disturbance, whilst this chapter focuses on a characteristic biotic component, namely the aquatic invertebrates. A set of 90 temporary depression wetlands in the south-western Cape region of South Africa was sampled for their aquatic invertebrate fauna and environmental variables. Invertebrate assemblage variation is assessed in relation to gradients of habitat transformation due to human activities and also to natural variation in environmental and spatio-temporal factors. As in the previous chapter, overall levels of habitat transformation are proxied in the current study using the areal cover of remaining natural vegetation habitat around wetlands (see section 2.2.3).

3.1.2. Temporary wetland invertebrates

Aquatic invertebrates form an abundant and diverse component of the temporary wetland fauna and dominate secondary production in these systems (Williams 1997, Williams 2006).

They are the most ubiquitous and characteristic biotic group to inhabit temporary wetlands (Williams 1997, Williams 2006). The composition of aquatic invertebrate assemblages in temporary wetlands generally differs from those of permanent wetlands and the former are known to harbour unique species found in no other environments (Wiggins *et al.* 1980, Williams 1996, Brooks 2000, Eitam *et al.* 2004, Urban 2004, Tarr *et al.* 2005, Williams 2006, Waterkeyn *et al.* 2008, Porst and Irvine 2009b, a). The temporary wetland fauna is dominated by two major groups of invertebrates (Williams 2006). First are the macroinvertebrates, which are generally greater than 1mm in length (adults) and dominated by benthic insects, molluscs and water mites. Second are the microcrustaceans, which consist of three major groups: Copepoda (limnetic); Cladocera (mostly limnetic); and Ostracoda (mostly benthic). Studies in temporary wetlands addressing the “zooplankton” component are mostly referring to limnetic copepod and clodoceran microcrustaceans. The present study will deal with both the macroinvertebrate and microcrustacean fauna. The term “invertebrates” herein refers to both macroinvertebrates and microcrustaceans. To my knowledge, this study is the first to address both macroinvertebrate and microcrustacean components of the temporary wetland biota (or any wetland type for that matter) in relation to gradients of anthropogenic disturbance in the landscape.

All animal species favouring temporary wetlands require adaptations that enable them to not only deal with dry phases (e.g. diapause or migration stage) but also to cope with the temporal fluctuations in physico-chemical conditions associated with varying water levels (e.g. physiological tolerance or life history modification) (Wiggins *et al.* 1980, Williams 1996, Williams 2006). Ecological theory suggests that biotic communities adapted to life in temporary environments are adapted to high levels of natural disturbance and thus may be expected to be resilient to perturbations of their environment from human activities (Lahr 1997, Angeler and Moreno 2007). In this regard, aquatic invertebrates in temporary wetlands, adapted to constantly fluctuating and physiologically demanding environments, are expected to show some degree of resilience to disturbance of their environment (e.g. physico-chemical alteration) from human land-use activities.

3.1.3. Literature review

Aquatic invertebrates are successfully used as indicators for the biological assessment (bioassessment) of ecosystem integrity in rivers (for reviews see Rosenberg and Resh 1993, Barbour *et al*. 1999, Bonada *et al*. 2006, Ollis *et al*. 2006), lakes (e.g. Pinel-Alloul *et al*. 1996, Stemberger and Miller 1998, O'Connor *et al.* 2000, Lewis *et al*. 2001, Blocksom *et al*. 2002, White and Irvine 2003, Pinto-Coelho *et al*. 2005, Tall *et al*. 2008) and more recently in some permanent wetlands (e.g. Chessman *et al*. 2002, Helgen 2002, Lougheed and Chow-Fraser 2002, Uzarski *et al*. 2004, Davis *et al*. 2006, Solimini *et al*. 2008, Trigal *et al*. 2009). Despite their use as indicators of human impacts in other aquatic ecosystems, empirical studies concerning invertebrate responses to human disturbance in temporary wetlands are lacking. Euliss and Mushet (1999) evaluated the influence of intensive agriculture on the resting eggs, shells and cases of invertebrates in 38 temporary wetlands (19 occurring in natural grasslands and 19 in agricultural lands) sampled during the dry state in North Dakota, USA. They found significantly more taxa and greater numbers of cladoceran ephippia, planorbid and physid snail shells and ostracod shells in wetlands surrounded by grasslands than in those surrounded by agriculture.

Conversely, several studies have found little effect of agriculture on temporary wetland invertebrates. Mahoney *et al.* (1990) found no evidence for a difference in zooplankton species richness or assemblage composition among 23 temporary wetlands in South Carolina differentially impacted by agricultural land use. These authors did find however, that hydroperiod exerted significant influence on cladoceran species richness. Brose (2003) found that hydroperiod and distance between wetlands were important determinants of the diversity of semi-aquatic carabid beetles in 36 temporary wetlands across six different agricultural landscapes in north-eastern Germany, but this diversity was not related to cultivation intensity in surrounding agricultural land. Similarly, Bagella *et al.* (2010) found no relationship between grazing intensity and crustacean assemblages among six temporary wetlands in Sardinia, whereas hydroperiod and distance between wetlands were important in determining these assemblages. No studies could be found which have related whole invertebrate assemblages (either macroinvertebrates or microcrustaceans) in temporary wetlands to surrounding land-use intensity or which have addressed more than just agriculture as a land use type. Drawing from the limited literature mentioned above, only one study (i.e. Euliss and Mushet 1999) appears to have found a link between land use and temporary wetland invertebrate assemblages, thus suggesting a generally poor response of the biota to surrounding land-use practices.

In terms of ecotoxicology testing, Angeler and Moreno (2007) showed that zooplankton species richness declined with experimental additions of a fire retardant chemical pollutant (Fire Trol 934) in artificially constructed temporary wetlands. These authors suggested however that temporary wetland zooplankton appeared to have a high resilience in that the assemblages recovered quickly from perturbation. Similarly, Lahr *et al.* (2000) reported that the chemical pollution of temporary wetlands in Senegal with insecticides caused negative effects on richness and abundance of various macroinvertebrate and microcrustacean taxa, but that populations of most taxa recovered to a least impaired state fairly quickly (within 6 weeks). The studies of Angeler and Moreno (2007) and Lahr *et al.* (2000) involve rather extreme forms of direct chemical pollution of temporary wetlands and it is perhaps not surprising that the invertebrate assemblages were affected to some degree. However, human activities in the landscape often emit more moderate perturbations on aquatic resources and effects on biota are often indirect (e.g. bottom-up effects of agriculturally-induced eutrophication on invertebrates mediated through changes in aquatic macrophytes and algae). In this regard, the ecosystem effects of transforming landscapes in which temporary wetlands are embedded may often be difficult to predict.

As mentioned earlier, the theoretical literature leads one to expect a certain degree of resilience of temporary wetland invertebrate assemblages in the face of human disturbance of landscapes surrounding wetlands. Various studies on the ecology of temporary wetland invertebrates in relation to natural variations in environmental factors can help one to formulate predictions in the light of human disturbances. For example, Batzer *et al.* (2004) reported that macroinvertebrate assemblages were generally unresponsive to environmental factors that varied over a set of 66 relatively pristine temporary wetlands in northern Minnesota, USA. They argued that the lack of response could be attributed to temporary wetland macroinvertebrates being habitat generalists that can endure pronounced variations in their natural environment. Similar findings of a lack of responsiveness of aquatic invertebrates to natural variations in environmental factors among temporary wetlands have been reported by Wissinger *et al.* (1999), Battle and Golladay (2001), Spencer *et al.* (2002), Studinski and Grubbs (2007) and Ganguly and Smock (2010). The results of these studies suggest that if mild or moderate changes in environmental conditions of temporary wetlands are caused by human activities in the landscape then this would not be likely to induce a significant response from aquatic invertebrates. However, literature findings are equivocal and a number of studies have conversely found a distinct structuring effect of environmental variables on temporary wetland invertebrates (Mahoney *et al.* 1990, Eitam *et al.* 2004, Vanschoenwinkel *et al.* 2007, Waterkeyn *et al.* 2008, Bilton *et al.* 2009, Bagella *et al.* 2010). It would appear therefore that results vary among different geographic regions and a universal theory cannot simply be applied to all temporary wetlands.

One is required to draw on previous studies for the specific region being investigated in order to make more accurate predictions of invertebrate responses to environmental factors. Importantly in this regard, and as mentioned in the previous chapter (section 2.1.2), the PhD study of De Roeck (2008) investigated the ecology of 57 temporary depression wetlands in the same region as covered in the current study (i.e. south-western Cape). De Roeck sampled macroinvertebrates and related them to variations in physico-chemistry (e.g. pH, nutrients, conductivity), biotope characteristics (e.g. cover of open water, complex- and simple-structured biotopes) and hydro-morphometry (e.g. hydroperiod, surface area, depth) among wetlands. Her findings indicated a significant and important influence of all three sets of factors on invertebrate assemblages. Although the wetland type and region investigated by De Roeck (2008) were the same as investigated in the current study, her study design incorporated random selection of wetlands across the pre-defined study area without taking into account human impacts in surrounding landscapes. Despite acknowledging that certain of her study wetlands were situated within disturbed landscapes, these disturbances were neither quantified nor incorporated as factors in the analysis. Her results do suggest however that if gradients in environmental factors among temporary wetlands are sufficiently pronounced, one can expect a corresponding shift in invertebrate assemblage composition. These results are particularly meaningful for the current study in that they were drawn from the same wetland type and region, and also at a similar scale (covering the south-western Cape). The implications of her work for the hypotheses of this study are further discussed in the following section.

3.1.4. Study aims

This chapter addresses three broad questions:

1) Are invertebrate assemblages in temporary wetlands significantly associated with levels of habitat transformation adjacent to these wetlands?
2) What is the relative influence of natural and anthropogenic factors in determining the composition of invertebrate assemblages?
3) Do temporary wetland invertebrates show potential as indicators of anthropogenic disturbance in and around wetlands (for use in a biotic index)?

More specific questions addressed within each section include the following:

Invertebrate assemblage composition

1) Is the composition of invertebrate assemblages in temporary wetlands associated with the overall transformation of adjacent habitat (as proxied by the cover of remaining natural vegetation)?
2) Which types of transformation (if any) appear to exert the most effect on assemblage composition? The categories considered are alien invasive vegetation, agriculture and urban development.
3) What is the effect of taxonomic resolution (coarse family-level *versus* the best achievable resolution) on the detection of patterns between invertebrates and habitat transformation?
4) What is the relative influence of variables grouped in sets (spatio-temporal factors, hydro-morphometry, physico-chemistry, biotope characteristics and habitat transformation) in determining assemblage composition in these temporary wetlands?
5) What is the relative influence of natural environmental and spatio-temporal variation *versus* human-induced habitat transformation on the composition of invertebrate assemblages?
6) Which individual environmental variables (if any) appear to most influence assemblage composition?

Taxon richness and diversity

7) Is the transformation of habitats adjacent to temporary wetlands significantly associated with changes in invertebrate taxon richness and diversity in these wetlands?

Invertebrates as indicators of anthropogenic disturbance

8) Do individual families of aquatic invertebrates in these temporary wetlands present themselves as useful indicators of human disturbance?
9) Can metrics as summaries of assemblage composition be used to indicate the effects of human disturbance on temporary wetland ecosystems?

3.1.5. Hypotheses

Based on current literature findings, I argue that temporary wetland invertebrates are likely to possess a certain degree of resilience to perturbations of their natural environment and thus are unlikely to respond significantly to mild human-induced changes to environmental conditions in wetlands. Studies such as that of De Roeck (2008) do indicate, however, that the fauna will be affected if environmental changes are pronounced. Therefore temporary wetland invertebrates do not appear to be impervious to environmental variation. The previous chapter of this thesis investigated the same set of 90 temporary wetlands as covered in the current chapter, although the focus was on environmental conditions. Results indicated that human transformations of landscapes adjacent to temporary wetlands were associated with significant changes in physico-chemical conditions in the wetlands. These physico-chemical responses to habitat transformation were relatively weak however, with the variation in physico-chemical conditions explained by habitat transformation ranging between ~2 and ~5.5%. Biotope characteristics and hydro-morphometry, both important determinants of invertebrate assemblage composition in lentic environments, showed no clear association with gradients of habitat transformation. Given that only physico-chemical conditions appeared to be affected by adjacent habitat transformation, and that even this response was reasonably weak, it is expected that the invertebrate assemblages in these temporary wetlands will not display a significant response to gradients of habitat transformation around wetlands. This finding is expected to apply to analyses both on taxonomic composition (questions 1 and 2) and on richness/diversity (question 7).

In accordance with studies in other aquatic systems (Bailey *et al.* 2001, King and Richardson 2002, Trigal-Dominguez *et al.* 2010) one expects better detection of human influences using genus- or species-level data than using coarser family-level data (question 3). In terms of the relative influence of variables grouped in sets (question 4), one expects habitat transformation to feature least among the categories listed as possible determinants of assemblage composition. The exact hierarchy of importance of the remaining variables sets in question 4 is difficult to predict, but spatio-temporal factors, hydro-morphometry, physico-chemistry and biotope characteristics have all been reported as influential factors in previous studies on wetlands and are thus expected to have significant influence on assemblages in this study. Given the high degree of spatio-temporal heterogeneity of environmental conditions reported in the previous chapter, it is expected that the influence of natural spatio-temporal factors and environmental changes will far outweigh that of human influence on invertebrate assemblages (question 5). The findings of De Roeck (2008) suggest that hydroperiod and conductivity will be the predominant individual environmental variables influencing invertebrate assemblages in the region (question 6). Lastly, given previous comments on invertebrate resilience, it is not expected that individual families and metrics will produce patterns that suggest an invertebrate index of human impairment for these wetlands is a feasible option (questions 8 and 9).

3.2. METHODS

This chapter deals with the aquatic invertebrate component of the set of 90 wetlands described in chapter 2. The invertebrate sampling was concurrent to the sampling of environmental variables for these wetlands and thus the reader is referred to chapter 2, sections 2.2.1 – 2.2.4 for information on site selection, study design and the sampling of environmental variables.

3.2.1. Invertebrate sampling

Wetlands were sampled for aquatic invertebrates using a square-framed, long-handled sweep net with a 235 mm mouth and 80 µm mesh. Various studies have suggested that sweep-net sampling of shallow wetlands is the most effective sampling method where the goal is comparing invertebrate assemblages among wetlands (e.g. Cheal *et al.* 1993, Turner and Trexler 1997, Gernes and Helgen 2002, Bowd *et al.* 2006).

Four biotope types (complex-structured vegetation, simple-structured vegetation, open water and benthic un-vegetated habitat: see chapter 2 section 2.2.4 for description of these) were sampled for aquatic invertebrates in each wetland as these formed the major available biotopes for this fauna. To standardise sampling effort, three separate invertebrate samples were taken within all wetlands. These three samples came from a maximum of three different biotopes as all four of the possible biotopes were never found to occur within the same wetland. Where only two biotopes were present within a wetland, two samples were collected in the more widespread biotope and one sample in the remaining biotope. For wetlands with one homogenous habitat (e.g. wetlands entirely covered by submerged vegetation), three standardized samples were collected from three different areas to maximise spatial representation.

Samples were standardized and made as quantifiable as possible through a strict sampling method. The goal of the sweep net sampling was to collect a representative sample of the aquatic invertebrate fauna of each wetland. The sampling method follows that of Rundle *et al.* (2002) who established that five replicate 1 m sweeps from each biotope using a standard sweep net (area 200 x 250 mm) consistently sampled 60 – 80% of the total invertebrate species pool from that biotope for a set of 16 temporary isolated depression wetlands in Cornwall, south-west Britain. I modified the protocol of Rundle *et al.* (2002) by sampling each biotope within three different areas of each wetland so as to maximise spatial representation of each biotope. The method for each 1 m sweep follows that prescribed by various authors for sampling temporary and permanent wetland invertebrates (Gernes and Helgen 2002, Rundle *et al.* 2002, Bowd *et al.* 2006), whereby each sweep constituted dragging the net down from the water surface at a 45^o angle until nearly touching the bottom and then completing the sweep arc by returning the net back to the surface at a 45^o angle, covering a distance of one metre with each full sweep. For cases where habitats were shallow (< 30 cm) this method had to be modified to a straight one metre sweep keeping the net immediately above the bottom substrate. Each biotope sample comprised 3 × 1 m sweeps for three different areas of the wetland, so that one sample was a pooled combination of 9 × 1 m sweeps from three different areas where the habitat was found in the wetland. This method thus provided three replicate sweeps at each location in a wetland as well as three replicates across different areas in the wetland where the habitat was found. Ideally a pilot study to assess species accumulation with different numbers of sweeps would have been performed to decide on the optimal number of sweeps per biotope, but this was not achievable given time constraints. The nine sweeps per biotope is likely to represent the majority of the invertebrate assemblage for that biotope, given the recommendation of Rundle *et al.* (2002) of five sweeps per biotope. As the procedure was in turn repeated three times per wetland (to obtain three biotope samples), this produced a total effort of 27 × 1 m sweeps evenly divided over the spatial area of each wetland and representing the major biotopes that were present. The three samples from each wetland were preserved and stored separately.

3.2.2. Invertebrate sample processing

All samples were fixed on site in buffered 10% formalin and replaced with a 70% ethanol solution after 24-48 hours for long-term preservation (Davies and Day 1998). In the laboratory, plant material was removed from the samples and all macro-invertebrates visible to the naked eye were picked out. The microcrustaceans (defined as copepods, ostracods and cladocerans) were extracted from the remaining sample and enumerated using a sub-sampling procedure, because extremely high densities of these taxa occurred in some samples. Each sample was first shaken and then emptied onto an 80 µm filter divided into 64 square cells (each 2.25 cm^2). The contents were examined one cell at a time (using a random numbers generator) in a Bogorov tray placed under a dissecting microscope until 200 individuals had been counted, the square containing the 200th individual being completely picked. Abundances were standardised to whole-sample estimates (i.e. 64 cells) by extrapolation. The use of 200 individuals as a stopping point for sub-sampling was adapted from the recommendations of various studies in rivers (Barbour and Gerritsen 1996, Somers *et al.* 1998, Barbour *et al.* 1999), and more recently from a study in wetlands (King and Richardson 2002). Furthermore, several sub-sampling trials were performed using 300 and 400 individuals. These trials indicated that counts > 200 organisms were too labour intensive given the time constraints in this study and in almost all cases the full complement of microcrustacean taxa was encountered within a 200-organism count. Upon completion of each sub-sample, the entire sample was scanned for 5 minutes for large rare (LR) taxa (defined as < 10 individuals), as adapted from the recommendations of Vinson and Hawkins (1996) and King and Richardson (2002) for the macroinvertebrate bioassessment of rivers and wetlands respectively.

Due to challenges faced in identifying and enumerating microcrustacean taxa (especially problematic were the Ostracoda), a subset of 41 wetlands was chosen for micro-crustacean analysis from areas with the strongest gradients of habitat transformation among wetlands (more information in section 3.2.4). The finest practicable level of taxonomic resolution for the various macro and micro taxa depended on information available for each taxon, but comprised mostly genus- and species-level identifications. Macroinvertebrates were identified to family (sub-family for Chironomidae), genus or species; for the majority of taxa it was possible to obtain genus- or species-level identifications (Appendix 5). Microcrustaceans were identified to genus and species (Appendix 6). The major reference source for keying out invertebrate taxa was the series of “Guides to the Freshwater Invertebrates of Southern Africa” (Day *et al.* 1999, Day *et al.* 2001a, Day *et al.* 2001b, Day and de Moor 2002b, a, Day *et al.* 2003, de Moor *et al.* 2003b, a, Stals and de Moor 2007). Problematic taxa which required expertise (e.g. Ostracoda) were identified by a specialist taxonomist for the given taxon.

3.2.3. Index of human disturbance

Although the focus of this thesis is the ecological impacts of habitat transformation on temporary wetlands, invertebrate metrics and families were also tested against scores derived from an index of human disturbance. These scores, herein referred to as “human disturbance scores” or “HDS” (following the terminology of Gernes and Helgen 2002), were the output of a simple index used to gauge the amount of disturbance on each wetland from human activities in the surrounding landscape and also within the wetland itself. Impacts to wetlands may be integrated across various sources and it is often the case that a wetland is not affected by a single human stressor, but by a combination of interacting stressors that cannot be directly measured in the wetland. These stressors may come in different forms and can be assigned to general classes of disturbance such as hydrological, physical, habitat and water chemistry (Teels and Adamus 2002). The development and use of an index that scores multiple wetland stressors is useful for classifying wetlands along a gradient of human disturbance. Invertebrate metrics and potential indicator taxa can be regressed against this disturbance gradient when assessing the feasibility of a biotic index (Danielson 2002, Teels and Adamus 2002).

No single standard protocol currently exists for the rapid assessment of human impacts on wetlands, although several comprehensive indices have been established (e.g. Ohio Rapid Assessment Method for Wetlands: Mack 2001; the South African WET-Health index: Macfarlane *et al*. 2008). The WET-Health index, which scores impacts to wetlands based on measures of hydrology, geomorphology and vegetation, was still being developed and a full version of the index was not available for use when this study was being designed (although a draft version was available for preliminary use in this study). Gernes and Helgen (2002) present a rapid assessment index for scoring human disturbance on wetlands in Minnesota (USA), which they used to establish a gradient of human impairment among wetlands. Although their index was found to be useful in Minnesota, the types of landscape disturbance it scored are specific to the types of human activities in Minnesota and would not be particularly meaningful in the south-western Cape. Furthermore, the index is over-simplified and does not appropriately score human impacts across different distances from each wetland (e.g. within wetland, within 100m, within 500m). The HDS index used in this chapter was developed specifically for the work presented in this thesis. It was designed to score impacts in and around isolated depression wetlands in the south-western Cape and was modified from the indices of Mack (2001), Gernes and Helgen (2002) and the draft version of Macfarlane *et al.* (2008). The index is not intended to be definitive, but is a first attempt to rank the degree of impact on temporary wetlands caused by human activities in and around these wetlands.

The expected influence of various human activities on each wetland's water quality, hydrology and physical structure was scored semi-quantitatively (using ordinal scoring) as set out in Appendices 7 and 8. It must be emphasized that the "expected" disturbances of human activities on each of these three categories (water quality, hydrology and physical structure) were subjective and were drawn from the author's judgment after reference to literature on the impacts of different land uses on aquatic environments. A workshop at the University of Cape Town was also held prior to sampling (April 2007) to collate local expertise on the topic of human land-use impacts on wetland water quality, hydrology and physical structure. An additional category for plant community indicators was also included following the rationale of Mack (2001) and Macfarlane *et al.* (2008), both of which include an assessment of vegetation as an indication of human impacts on wetlands. Scores for each impact type and for the plant community indicators were summed and contributed to an overall "human disturbance score" ("HDS") for each wetland. To ease interpretation, HDS values were produced as percentage scores through division of actual scores by the total possible score per site (a score of 70) and then multiplying by 100. The "Extent" column in Appendix 7 was used as guide to aid the determination of impacts and was not itself analyzed quantitatively. Three distance bands were used to score local human impacts at each wetland (distance categories: within wetland; within 100 m radius of wetland edge; within 500 m radius of wetland edge). Within each distance band, the expected impacts of human activities were scored (ordinal rank scoring from 0 = "Least" to 5 = "Highly extensive", see Appendix 7 for details) in terms of the expected impacts of each human activity on the water quality, hydrology and physical structure of wetlands. For each column scored for human impacts (and in turn within each of the distance bands), the maximum score of impact across all human activities was used in the next step, which was to sum the maximum scores of impact across all impact categories (namely water quality, hydrology and physical structure) and distance bands. This score was added to the sum of the plant community indicator scores to produce the final HDS. This was divided by the maximum possible score (70) to obtain the HDS (%) score for each wetland. Appendix 7 presents the template score sheet and gives explanations for scoring criteria. Appendix 8A provides an example score sheet for calculating % HDS at SAN13 (a relatively impacted site) and Appendix 8B is an example score sheet for SAN23 (a minimally impacted site). Appendix 9 presents the human disturbance score calculated for each wetland in this study.

3.2.4. Data analysis

Data preparation and broad analysis approach

The relative abundances of invertebrates were standardised to density (no.m^{-2}) by dividing relative abundances per sample by the area swept. The surface area swept per sample was calculated using the dimensions of the net and the length swept (i.e. 0.235 m x 9 m per sample). Data from the three separate macroinvertebrate samples from each wetland were merged to produce an average density per wetland for each taxon. The abundances for each sample (no.m^{-2}) were multiplied by the proportional cover of the biotope from which the sample was collected. The resulting proportional abundances from each habitat were summed to produce an overall average density of that taxon in the wetland, having thus adjusted for proportional cover of the various habitats. Where only two habitats were present in a wetland, a similar procedure was followed to estimate average density as explained above, except that the two samples from the more abundant habitat were averaged before multiplying by the proportional cover of that habitat in the wetland. Where only one habitat was present in a wetland, the average density per wetland was simply an average density across the three samples from the wetland. In all cases, the final densities averaged per wetland (no.m^{-2}) were used for further statistical analyses and individual wetlands were thus treated as replicates.

Due to time constraints, only one biotope sample could be processed from each wetland for microcrustaceans. The chosen habitat in this regard was submerged vegetation as previous studies have generally reported a higher abundance and diversity of aquatic invertebrates from more structurally complex biotopes in lentic environments (Voigts 1976, Beckett *et al.* 1992, Cardinale *et al.* 1998, Cattaneo *et al.* 1998). In a few cases where submerged vegetation was not present in a wetland, the emergent vegetation sample was processed as this was the second most structurally complex biotope found in these wetlands. Final estimated densities for microcrustacean taxa used in further analyses were thus an average for the sweeps taken from one habitat and were not averages per wetland (unlike the macroinvertebrate data). As mentioned earlier, microcrustaceans were analysed in the laboratory for only a subset of the sites. The subset of 41 sites was chosen from the entire dataset of 90 sites based on examination of the habitat transformation scores. The sites were chosen so as to maximise replication across the full gradient of habitat transformation, whilst minimising the number of sites selected and the spatial extent covered. The chosen microcrustacean sites occurred within two wetland clusters, namely Sand fynbos and Western strandveld. Macroinvertebrate and microcrustacean data were analysed separately because, firstly, the extremely high densities of microcrustaceans would interfere with the detection of patterns for macroinvertebrates, secondly, microcrustacean data was available only for a subset of the sites sampled for macroinvertebrates, and thirdly, it was of interest to assess the separate response of these groups to human disturbance in the landscape.

Relationships between aquatic invertebrate assemblages, habitat transformation, environmental variables and spatio-temporal covariables were addressed using multiple linear regression models. Following the reasoning outlined in chapter 2 (section 2.2.5), a regression approach was chosen over an ANOVA (categorical) approach in this study for relating invertebrate assemblages to the ordinal habitat transformation variables. Detrended Correspondance Analysis (DCA) indicated that gradient lengths in the macroinvertebrate and microcrustacean data were best suited to linear rather than unimodal analyses (gradients lengths were all < 3, Lepš and Šmilauer 2003), as was the case for the environmental data analysed in chapter 2. The full list of predictor variables (and covariables) incorporated into the analyses of this study is reported in Table 2.1 (chapter 2). The only modification is for the environmental response variables in Table 2.1, which are treated as predictor variables in the current study. Four broad types of invertebrate response data were analysed for both the macroinvertebrate and microcrustacean datasets: (1) assemblage composition (multivariate); (2) taxon richness and diversity (univariate); (3) family-level tests to establish taxa indicative of human disturbance (univariate); and (4) testing for metrics indicative of human disturbance (univariate). The following sections address analyses related to each type of response data.

Invertebrate assemblage composition

a) Associations with habitat transformation variables

Invertebrate assemblage composition was first converted to a multivariate similarity matrix using the Bray-Curtis coefficient (Bray and Curtis 1957). Macroinvertebrate abundances were ln(x+1) transformed for all analyses in this study to down-weigh the influence of the most abundant taxa. All microcrustacean abundances were 4th root transformed, which is a more severe transformation than ln(x+1), but was appropriate given the extremely high densities reached for certain microcrustacean taxa. Invertebrate assemblage composition, represented by the Bray-Curtis similarity matrices, was step-wise regressed against the full set of covariables in a manner similar to the analyses of environmental conditions in chapter 2. For information on the list of covariables incorporated into analyses see section 2.2.5 in chapter 2 and also Appendix 1. Significant covariables retained in the step-wise models were included in the next step, which was to regress the invertebrate similarity matrices against the habitat transformation variables. This step was performed in order to assess the amount of variation in invertebrate assemblage composition that could be explained by each of the habitat transformation variables, over and above the covariables, and whether these relationships were statistically significant. The influence of the covariables was first partialled out of these models in order to account for their confounding effects on assemblage composition before calculating the variation attributable to habitat transformation.

Regressions of invertebrate composition on habitat transformation were performed at two levels of taxonomic resolution. First was the finest achievable resolution (mostly genus- and species-level, herein referred to as “fine-scale” resolution) and the second was data grouped at family level. Interest lay in whether one can better detect relationships with habitat transformation at fine or coarse levels of taxonomic resolution, the latter presenting a more practical level for use in bioassessment indices. Distance-based redundancy analysis (dbRDA, Legendre and Anderson 1999, McArdle and Anderson 2001), introduced in chapter 2, was used to perform multivariate linear regressions on invertebrate assemblage composition. In order to analyse macroinvertebrate assemblages in relation to different types of habitat transformation around wetlands, the overall dataset (n = 90 sites) was analysed in subsets composed of sites affected by only one type of habitat transformation (e.g. agriculture). Section 2.2.5 in chapter 2 provides the necessary information on how data were divided into subsets and is not repeated here. The microcrustacean dataset (n = 41 sites) was analysed only against levels of habitat transformation in general (as proxied by the areal cover of natural vegetation within 100 and 500 m of wetlands) and not against the different types of transformation as the dataset was not large enough to accommodate these analyses. The microcrustacean data were therefore not divided into subsets.

b) Relationships with predictor variables grouped in sets

Invertebrate assemblage composition was also regressed (using dbRDA) against predictor variables grouped in sets. To achieve this, variables were coded as “indicators” (terminology of the DISTLM routine in PERMANOVA+ software) according to the following categories into which they could be classified: hydro-morphometry (maximum depth, total surface area); physico-chemistry (pH, conductivity, average temperature, turbidity, dissolved oxygen, phosphates, nitrates + nitrites, ammonium); biotope (% complex-structured vegetation, % simple-structured vegetation, % open water, % benthic un-vegetated); habitat transformation (cover of natural vegetation within 100 and 500 m); and spatio-temporal covariables (longitude, latitude, time, altitude, wetland cluster). The invertebrate similarity matrices (Bray-Curtis) were regressed against these sets of variables in order to gauge the relative contribution to variation of invertebrate assemblages that could be attributed to each set of conditions. The statistical significance of each set was also assessed. These tests were performed on the full macroinvertebrate and microcrustacean datasets, using data at the fine-scale level of taxonomic resolution. The reduced subsets of covariables (selected from the step-wise regression of each invertebrate similarity matrix on the full covariable set, as outlined earlier) were first partialled out of all models, the obvious exception being when the full covariable set was itself tested for significance.

c) Variation partitioning

A variation partitioning procedure was applied to the macroinvertebrate and microcrustacean compositional datasets to partition variability among three broad sets of explanatory variables, namely habitat transformation, environmental conditions and spatio-temporal covariables. The technique estimates the unique contribution of each set of explanatory variables that is independent of the other sets. It also estimates components of variation that are shared between variable sets (interactive effects). The method of partitioning the variation among sets of predictor variables in ecological datasets was first demonstrated by Borcard *et al.* (1992) for two sets of explanatory variables (spatial and environmental). The method was later modified by Anderson and Gribble (1998) for use with three sets of variables (spatial, environmental and temporal). I have used the same approach as described by these authors, but the terms incorporated have different names (i.e. habitat transformation, environmental conditions and spatio-temporal covariables). For a detailed account of the procedure and discussion thereof, the reader is referred to Anderson and Gribble (1998). A summary follows.

The method for this data results in eight different components of variation: (1) Pure environmental (E); (2) pure spatio-temporal (S); (3) purely due to transformation of adjacent habitat (T); (4) pure spatio-temporal component of environmental (SE), this is the overlap in the variation explained by spatio-temporal and environmental variables; (5) pure transformation component of environmental (TE), similar to (4), this is the overlap in the variation explained by transformation of habitat and environmental variables; (6) pure combined spatio-temporal and transformation component (ST), which is the fraction of the variation in the invertebrate data that is not related to the environmental variables, but which can be attributed to the pure combination of spatio-temporal and habitat transformation patterns; (7) combined spatio-temporal and transformation component of environmental (STE), the fraction of the variation that can be explained by the combined action of spatio-temporal, transformation of habitat and environmental variables; (8) Unexplained (U), the remaining variation in the invertebrate data that cannot be explained by the environmental, spatio-temporal or transformation variables that have been recorded in this study. Figure 3.1 illustrates this partitioning of variation among three sets of variables.

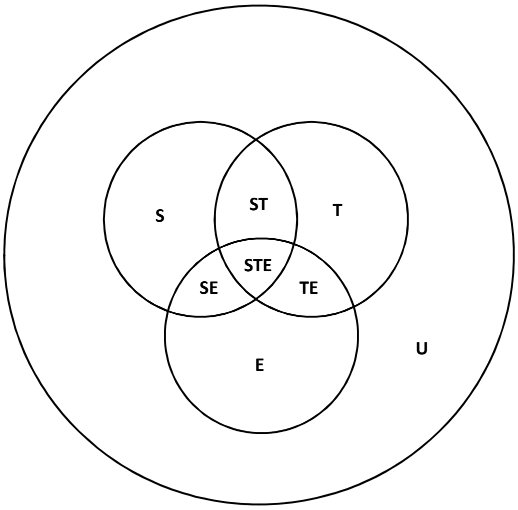


Figure 3.1. A Venn diagram showing the partitioning of variation according to three sets of explanatory variables, spatio-temporal (S), transformation of habitat from human activities (T) and environmental (E). The largest circle is the set corresponding to the total variation in the dependent (invertebrate) data. Each area of overlap of the three smaller circles is representative of the intersection of the three sets, S, T and/or E in terms of their explained variation. For example, the intersection of circles S and T, but where the circle E does not intersect, represents the variation explained by spatio-temporal factors and transformation of habitat (ST). The area of the largest circle (total variation) that the three smaller circles do not cover represents the unexplained variation (U). Adapted from Anderson and Gribble (1998).

Anderson and Gribble (1998) performed their multivariate analyses using Canonical Correspondance Analysis (CCA) on chi-squared distances. I use the same set of analysis steps as per their procedure, but perform the multivariate analyses using dbRDA on Bray-Curtis similarity with an adjusted R^2 criterion. The 12 steps in the analysis (modified for use in this study) are outlined in Table 3.1.

Table 3.1. Steps in the process performed using dbRDA in PERMANOVA+ (adapted from Anderson and Gribble 1998). The species matrix refers to the invertebrate similarity matrix.

Step	Description
[1]	dbRDA of species matrix, constrained by environmental matrix
[2]	dbRDA of species matrix, constrained by spatio-temporal matrix
[3]	dbRDA of species matrix, constrained by habitat transformation matrix
[4]	dbRDA of species matrix, constrained by environmental matrix, spatio-temporal variables treated as covariables
[5]	dbRDA of species matrix, constrained by environmental matrix, habitat transformation variables treated as covariables
[6]	dbRDA of species matrix, constrained by environmental matrix, spatio-temporal + habitat transformation variables treated as covariables
[7]	dbRDA of species matrix, constrained by spatio-temporal matrix, environmental variables treated as covariables
[8]	dbRDA of species matrix, constrained by spatio-temporal matrix, habitat transformation variables treated as covariables
[9]	dbRDA of species matrix, constrained by spatio-temporal matrix, environmental + habitat transformation variables treated as covariables
[10]	dbRDA of species matrix, constrained by habitat transformation matrix, environmental variables treated as covariables
[11]	dbRDA of species matrix, constrained by habitat transformation matrix, spatio-temporal variables treated as covariables
[12]	dbRDA of species matrix, constrained by habitat transformation matrix, environmental + spatio-temporal variables treated as covariables

Once the 12 dbRDA models have been run, the final eight components of variation (mentioned earlier) can be calculated according to Table 3.2. These components of variation allow one to determine the unique and shared effects of the major variable sets incorporated into this study. The partitioning procedure was performed once on each of the full sets of sites for the macroinvertebrate (n = 90) and microcrustacean (n = 41) datasets. The full sets of spatio-temporal and environmental variables were included in the models. Habitat transformation was represented by the two variables “Natural 100 m” and “Natural 500 m”, which are measures of overall transformation of the landscape within 100 and 500 m of wetlands respectively. Variable sets were coded using the “indicators” function of DISTLM in PERMANOVA+ and the cumulative effects of the individual variables in each set were thus incorporated into the dbRDA models for each variation partitioning procedure.

Table 3.2. Summary of calculations for the partitioning of variation among the three sets of explanatory variables: spatio-temporal; transformation of habitat adjacent to wetlands; and environmental (adapted from Anderson and Gribble 1998). The numbers in square brackets refer to the numbered steps in the analysis described in Table 3.1.

Component	**Calculation**
Spatio-temporal = S	[9]
Transformation = T	[12]
Environmental = E	[6]
SE	[1] - [4] - STE
TE	[1] - [5] - STE
ST	[2] - [8] - STE
STE	[9] + ([2] - [7]) + ([2] - [8]) - [2]
Total explained, Ω	[1] + [7] + [12]
Unexplained	100% - Ω

d) Relative importance of individual environmental variables

Step-wise multiple regression models (adjusted R^2 selection criterion) were used to explore which environmental variables were most correlated with invertebrate assemblage composition (the habitat transformation variables were also included here as "environmental" variables). Final models selected by the step-wise procedure were cross-validated with results from an all-subsets multivariate regression routine. This was done to assess whether variables selected by step-wise regressions were also included in the most parsimonious models when all possible combinations of variables (all-subsets) were considered. This follows the recommendation of Quinn and Keough (2002) to compare all possible subsets of variables rather than just use step-wise selection to arrive at a "best" subset model, due to certain logical and statistical flaws associated with step-wise regressions (James and McCulloch 1990, Neter *et al.* 1996, Mac Nally 2000). The all-subsets regression procedure incorporated an AICc selection criterion, a modification of the traditional "Akaike Information Criterion" (AIC) (Akaike 1973). AICc has been shown by several authors (Sakamoto *et al.* 1986, Hurvich and Tsai 1989, Burnham and Anderson 2002) to perform better than traditional AIC in datasets where the ratio of the sample size to the number of predictor variables is small or moderate, as is often the case in ecological studies. Models that have an AICc score within 2 units of the overall lowest score can be considered as potential "best" models (Burnham and Anderson 2002, 2004). The effects of covariables were first partialled out of the step-wise and all-subsets models because interest lay in understanding which of the environmental variables were most influential, given existing spatio-temporal variation.

The step-wise and all-subsets regression models were performed using both the macroinvertebrate and microcrustacean datasets (models were applied separately to each dataset), incorporating the full data arrays (n = 90 and 41 sites respectively) and at the fine-scale of taxonomic resolution. The step-wise and all-subsets models were performed using non-parametric dbRDA.

Taxon richness and diversity

To test for relationships between taxon richness/diversity and the transformation of habitat adjacent to wetlands, univariate multiple linear regression models were applied. For the macroinvertebrate and microcrustacean datasets (full datasets), five measures of taxon richness/diversity were independently regressed against the two variables representing overall levels of conversion of surrounding habitat (cover of natural vegetation within 100 and 500 m). As per previous analyses, each response variable was first regressed against the full set of possible covariables using step-wise regression (adjusted R^2 selection criterion) and the significant covariables from this procedure were then partialled out of subsequent tests for linear relationships between each response variable and habitat transformation. The terms “taxon” richness and “taxon” diversity are used here, because the level of taxonomic resolution in this study was not always to species. Five commonly used measures of richness or diversity were incorporated into these analyses:

First was taxon richness (S), represented simply by the total number of taxa;

Second was Margalef’s index (d), a richness index, given by the equation:

$d = (S - 1) / \log N$;

Third was the Shannon diversity index (H’), given by the equation:

$H' = - \sum_i p_i \log (p_i)$, where p_i is the proportion of the total count arising from the ith taxon;

Fourth was Pielou’s index of evenness (J’), given by the equation:

$J' = H' / \log S$;

Fifth was the Simpson diversity index ($1 - \lambda$), which once again expresses evenness and is given by the equation:

$1 - \lambda = 1 – (\sum p_i^2)$.

Family-level tests for indicator taxa

To assess potential feasibility of using aquatic invertebrates as indicators of human disturbance in the landscape, regressions of invertebrate families on the habitat transformation variables and the human disturbance scores were explored using univariate multiple linear regressions. Rare families, defined as being present in < 5% of samples, were omitted from these analyses as the number of occurrences was too low for regression analysis. Macroinvertebrate family abundances were ln(x+1) transformed prior to analysis, whilst the extremely high abundances for microcrustaceans made a 4^{th} root transformation appropriate.

Testing potential metrics

A list of macroinvertebrate metrics developed for previous wetland bioassessment programs was collated from available literature (Hicks and Nedeau 2000, Gernes and Helgen 2002) and applied to the invertebrate data collected in this study. Relationships between the calculated metrics, levels of habitat transformation and the human disturbance scores were once again explored using multiple regressions. Various metrics were developed specifically for this study. No microcrustacean metrics for wetland bioassessment could be sourced from the literature and thus a fairly crude set of metrics was developed and tested in this study. For the full list of macroinvertebrate and microcrustacean metrics tested refer to Appendix 10. It should be noted that the terms “tolerant” and “intolerant” used for certain macroinvertebrate metrics in this study are derived from Hicks and Nedeau (2000) for New England (USA) and are not based on levels of known tolerance for taxa in the south-western Cape study region. As was the approach with previous analyses, each response variable (in this case each invertebrate family or metric) was first regressed against the full set of possible covariables using step-wise regression (adjusted R^2 criterion). The significant covariables from this procedure were then partialled out of subsequent tests for linear relationships between these response variables and habitat transformation. Macroinvertebrate abundance metrics (non-percentage metrics) were ln(x+1) transformed prior to analysis, whilst microcrustacean abundance metrics were 4^{th} root transformed.

Visualizing patterns

Relationships between individual predictor variables (“Natural 100 m”, “Natural 500 m”, “HDS”) and univariate response variables (richness/diversity measures, families and metrics), given spatio-temporal covariables, were visualized using partial residual plots (see chapter 2, section 2.2.5 for more information on these plots). Non-metric multidimensional scaling (MDS) ordinations were used to visualize the multivariate patterns in invertebrate assemblage composition (Bray-Curtis similarity). Samples were coded according to levels of habitat transformation, wetland cluster or geographic (latitudinal) region in order to assess whether these factors formed distinct groupings on the two-dimensional plots. All multidimensional plots, herein referred to as MDS plots, are non-metric *sensu* Kruskal (1964).

Transforming environmental data; significance levels; and software used

For all analyses in this study which incorporated environmental variables, these variables were $\log_{10}$ transformed where appropriate to improve normality and for the same purpose the percentage variables were arcsine square root transformed. The "HDS" percentage variable was not arcsine square root transformed as it was normally distributed. The significance level (α) for all regression tests in this study was 0.05. An exception to this was for tests related to agriculture, as the smaller sample sizes of the two agricultural datasets (100 m: $n = 24$; 500 m: $n = 21$) indicated that the possible lack of power to detect effects could be balanced by interpreting P values < 0.10 as offering some evidence against the null hypothesis. DCA ordinations were performed using CANOCO for Windows v4.5 (Ter Braak and Šmilauer 2002). All dbRDA models (including the step-wise and all-subsets procedures) were implemented using the DISTLM routine of the PERMANOVA+ software package (Anderson *et al.* 2008). P values for dbRDA models were tested by 9999 permutations of residuals under the reduced model. Univariate multiple regressions (including step-wise models and partial residual plots) were performed using STATISTICA v10 software (Statsoft Inc. 2010, Tulsa, Oklahoma, USA). MDS ordinations were performed using PRIMER v6 software (Clarke and Gorley 2006).

3.3. RESULTS

3.3.1. Biodiversity characteristics

Describing biodiversity patterns of aquatic invertebrate fauna was not an aim of this study and the reader is referred to Mlambo *et al.* (2011) for more detailed description of aquatic macroinvertebrate biodiversity patterns in temporary wetlands of the south-western Cape. A broad sketch of some biodiversity aspects of the wetlands sampled in this study is provided for general reference, but is not discussed further in section 3.4.

A total of 100 macroinvertebrate taxa, belonging to 47 families, was collected from the 90 wetlands sampled for macroinvertebrates (Appendix 5). Samples processed for microcrustaceans (41 wetlands) yielded 43 taxa belonging to 10 families (Appendix 6). The ubiquitous families are reported in Table 3.3. The families Dytiscidae, Chironomidae and Baetidae were all found in more than 70% of the wetlands sampled. The majority of macroinvertebrate taxa identified to the fine-scale resolution (mostly genus and species) showed relatively localised distributions, with 65 of the 100 taxa occurring in < 5% of wetlands sampled. Representatives of all three major microcrustacean groups (Copepoda, Ostracoda and Cladocera) were very widespread among the sampled wetlands, with the families Cyclopidae, Cyprididae and Daphniidae all being present in more than 85% of sites. The microcrustacean taxa identified beyond family-level were generally more widespread than the macroinvertebrates, with only 14 of the 43 taxa being present in < 5% of wetlands. This may partly be an artefact of the smaller sample size and spatial area covered by the microcrustacean samples however.

Table 3.3. The ten most widespread families of macroinvertebrates and microcrustaceans among the wetlands sampled in this study. The occurrence (number of wetlands in which the family was present) and percentage occurrence (as a percentage of all wetlands sampled) are listed in descending order.

Macroinvertebrates (n = 90 wetlands)	Occurrence (no. of wetlands)	% Occurrence	Microcrustaceans (n = 41 wetlands)	Occurrence (no. of wetlands)	% Occurrence
Dytiscidae	87	96.7	Cyclopidae	38	92.7
Chironomidae	68	75.6	Cyprididae	38	92.7
Baetidae	66	73.3	Daphniidae	35	85.4
Culicidae	57	63.3	Diaptomidae	29	70.7
Corixidae	52	57.8	Cypridopsidae	21	51.2
Notonectidae	51	56.7	Macrothricidae	14	34.1
Hydrophilidae	38	42.2	Moinidae	11	26.8
Pomatiopsidae	35	38.9	Chydoridae	7	17.1
Physidae	26	28.9	Ameiridae	1	2.4
Pleidae	26	28.9	Limnocytheridae	1	2.4

Note: Only ten families were recorded for microcrustaceans and thus all ten of these are listed here in descending order of occurrence.

The mean number of taxa per wetland was 12.4 (± 5.2) for macroinvertebrates and 8.3 (± 2.7) for microcrustaceans. This mean richness (number of taxa) per wetland was significantly greater for macroinvertebrates than microcrustaceans ($t = 4.74$, $p < 0.001$). Microcrustacean samples were characterised by very high densities of organisms. The median macroinvertebrate density across all wetlands was just 28 ind.m^{-2} (25th percentile = 15, 75th percentile = 51), whilst for microcrustaceans the median density was extremely high at 8503 ind.m^{-2} (25th percentile = 3703, 75th percentile = 22979). A Mann-Whitney U Test confirmed that this difference was highly significant ($Z = -9.2$, $p < 0.001$). Nonparametric summary statistics are reported here due to the non-normal, skewed nature of the density data for both groups. It appears therefore that temporary wetlands in the region possess a richer macroinvertebrate than microcrustacean fauna, but the density of microcrustaceans is considerably higher. The design of this study (non-random site selection) cannot establish this result with a high degree of confidence and further studies would be useful for confirming this finding.

3.3.2. Invertebrate assemblage composition

Associations with habitat transformation variables

Overall levels of habitat transformation as reflected by the variables “Natural 100 m” and “Natural 500 m” were not significantly related to macroinvertebrate assemblage composition at either scale of taxonomic resolution. The P values were however very close to the 0.05 significance level (“Natural 100 m”: $P = 0.076$; “Natural 500 m”: $P = 0.056$; Table 3.4), thus providing some suggestion of a relationship despite being non-significant. In terms of relationships with the different agents of habitat transformation, only agricultural cover within 100 m was significantly associated with macroinvertebrate composition (Table 3.4). The percentage variation in macroinvertebrate assemblage composition explained by overall levels of habitat transformation, as well as by each of the types of habitat transformation, was low (ranging between 1.31 and 6.26%). This explained variation was especially low when compared to that explained by the subsets of spatio-temporal covariables (ranging between 17.10 and 46.24%). These patterns of explained variation were similar across both levels of taxonomic resolution (Table 3.4 vs. Table 3.5). P values indicated slightly less detection of a relationship with the “Natural 100 m” and “Natural 500 m” predictors using family-level data ($P = 0.109$ and 0.083 respectively), but the percentages of explained variation were very similar for most predictor variables (Table 3.5).

Microcrustacean assemblage composition displayed a highly significant (P < 0.001) relationship with overall transformation of habitat within 100 m of wetlands, but did not appear to be influenced by transformation at the broader scale of 500 m (P = 0.415, Table 3.6). Interestingly, at the coarser family level microcrustaceans were significantly related to habitat transformation at both spatial scales, although the relationship with "Natural 100 m" was not highly significant as for the genus/species-level data. As was observed for the macroinvertebrate data, the amount of variation in microcrustacean assemblage composition explained by gradients of habitat transformation (ranging between 1.77 and 5.91%) was low relative to that explained by the subsets of spatio-temporal covariables (ranging between 24.12 and 35.77%).

Table 3.4. Non-parametric multivariate regression tests (dbRDA) for relationships between habitat transformation gradients and aquatic macroinvertebrate assemblage composition at the fine-scale of taxonomic resolution (mostly genus- and species-level). Natural - indigenous vegetation; Invaded - alien invasive vegetation; Agriculture - agricultural land; Urban - urban area. The areal cover of these variables is represented within 100 and 500 m of each wetland edge. To maximise parsimony, covariable subsets were pre-selected for each model using step-wise regression of each response matrix on the full list of possible covariables (see Appendix 1). % Var. - the percentage of variation in each Bray-Curtis similarity matrix that is explained by the respective predictor variable or covariable set in each model; Time – number of days since the first sampling event; Res. df – residual degrees of freedom for each model; SF – Sand fynbos; WS – Western strandveld; SR – Shale renosterveld; FF – Ferricrete fynbos. Significant P values are presented in boldface (α = 0.05, with the exception of agriculture, where α = 0.10).

Predictor variable	Res. df	F	P	% Var	Covariables	% Var (covariables)
Natural 100 m	81	1.503	0.076	1.31	Longitude; latitude; time; altitude; SF; WS; SR	27.85
Natural 500 m	81	1.591	0.056	1.39	Longitude; latitude; time; altitude; SF; WS; SR	27.85
Invaded 100 m	63	1.334	0.158	1.54	Longitude; latitude; time; altitude; SF; WS	25.77
Invaded 500 m	64	1.358	0.139	1.55	Longitude; latitude; time; altitude; SF; WS	25.56
Agriculture 100 m	18	2.198	**0.006**	6.18	Longitude; latitude; time; SR	43.25
Agriculture 500 m	16	1.263	0.227	4.31	Latitude; time; FF	41.16
Urban 100 m	30	1.152	0.300	2.65	Longitude; latitude; time; altitude; WS	28.27
Urban 500 m	49	0.955	0.495	1.53	Latitude; time; altitude; SF	19.98

Table 3.5. Non-parametric multivariate regression tests (dbRDA) for relationships between habitat transformation gradients and aquatic macroinvertebrate assemblage composition at the coarse-scale taxonomic resolution (family-level). Natural - indigenous vegetation; Invaded - alien invasive vegetation; Agriculture - agricultural land; Urban - urban area. The areal cover of these variables is represented within 100 and 500 m of each wetland edge. To maximise parsimony, covariable subsets were pre-selected for each model using step-wise regression of each response matrix on the full list of possible covariables (see Appendix 1). % Var. - the percentage of variation in each Bray-Curtis similarity matrix that is explained by the respective predictor variable or covariable set in each model; Time – number of days since the first sampling event; Res. df – residual degrees of freedom for each model; SF – Sand fynbos; WS – Western strandveld; SR – Shale renosterveld; FF – Ferricrete fynbos. Significant P values are presented in boldface (α = 0.05, with the exception of agriculture, where α = 0.10).

Predictor variable	Res. df	F	P	% Var	Covariables	% Var (covariables)
Natural 100 m	82	1.558	0.109	1.35	Longitude; latitude; time; altitude; SF; WS	27.57
Natural 500 m	82	1.655	0.083	1.43	Longitude; latitude; time; altitude; SF;WS	27.57
Invaded 100 m	62	1.226	0.263	1.39	Longitude; latitude; time; altitude; SF; WS; Urban100m	28.35
Invaded 500 m	64	1.567	0.112	1.77	Longitude; latitude; time; altitude; SF; WS	26.09
Agriculture 100 m	19	2.424	**0.019**	6.26	Latitude; time; SR	44.69
Agriculture 500 m	16	1.532	0.151	4.70	Latitude; time; FF	46.24
Urban 100 m	31	1.407	0.174	3.19	Longitude; latitude; altitude; SF	26.50
Urban 500 m	50	0.972	0.461	1.58	Latitude; time; altitude	17.10

Table 3.6. Non-parametric multivariate regression tests (dbRDA) for relationships between habitat transformation gradients and microcrustacean assemblage composition in wetlands. Results are presented for fine-scale (genus- and species-level) and coarse-scale (family-level) taxonomic resolution. The predictor variables represent overall levels of habitat transformation, as proxied by the areal cover of indigenous vegetation around wetlands. The variables "Natural 100 m" and "Natural 500 m" are the cover of indigenous vegetation within 100 and 500 m of each wetland edge respectively. To maximise parsimony, covariable subsets were pre-selected for each model using step-wise regression of each response matrix on the full list of possible covariables (see Appendix 1). % Var. - the percentage of variation in each Bray-Curtis similarity matrix that is explained by the respective predictor variable or covariable set in each model; Time – number of days since the first sampling event; Res. df – residual degrees of freedom for each model. Significant P values are presented in boldface (α = 0.05).

Predictor variable	Res. df	F	P	% Var	Covariables	% Var (covariables)
Genus/species-level						
Natural 100 m	36	3.648	**<0.001**	5.91	Longitude; time; Sand fynbos	35.77
Natural 500 m	36	1.023	0.415	1.77	Longitude; time; Sand fynbos	35.77
Family-level						
Natural 100 m	37	2.954	**0.019**	5.61	Longitude; time	24.12
Natural 500 m	37	2.942	**0.020**	5.59	Longitude; time	24.12

Relationships with predictor variables grouped in sets

Regressions of macroinvertebrate composition against predictor variables grouped in sets revealed that all variable sets explained a significant proportion of variation in the macroinvertebrate composition data (Table 3.7). The spatio-temporal variables explained more variation in macroinvertebrate assemblage composition than any of the other variable sets (28.63%). Following this were the physico-chemical variables, which explained roughly half that amount of variation (13.35%). Variables describing biotope characteristics in turn explained roughly half the amount of variation as for physico-chemical conditions (7.16%). The hydro-morphometry and habitat transformation variable sets were associated with comparatively small amounts of variation in macroinvertebrate assemblage composition (3.15 and 2.73% respectively), despite both being statistically significant relationships. Interestingly, when the variables “Natural 100 m” and “Natural 500 m” were grouped as a set in this manner, their combined effect was statistically significant, but when assessed individually the variables were not significant (Table 3.7 c.f. Table 3.5).

The amounts of variation in microcrustacean assemblages explained by the predictor sets were generally higher than for macroinvertebrate assemblages (Table 3.7), although this is likely to be a sample size effect to some degree. With fewer samples and spatial area covered in the microcrustacean than in the macroinvertebrate dataset, there is not likely to be as much variation requiring explanation as for the macroinvertebrate dataset and thus the models tend to show a better statistical fit. As for the macroinvertebrate dataset, spatio-temporal factors were associated with a considerable amount of the microcrustacean assemblage variation (41.03%, Table 3.7). Once again this was followed by physico-chemistry (23.99%) and biotope characteristics (9.52%). Habitat transformation appears to have played an important role in determining microcrustacean assemblage composition among wetlands, with 8.53% of the variation (highly significant contribution, $p < 0.001$) being explained by the combined effects of the two habitat transformation variables (i.e. “Natural 100 m” and “Natural 500 m”) taken as a set.

Table 3.7. Non-parametric multivariate regression tests (dbRDA) of association for sets of predictor variables and invertebrate assemblage composition (macroinvertebrates and microcrustaceans) in wetlands. The first model for each of the invertebrate groups is a test of association with the full set of spatio-temporal variables, which were then partialled out of all subsequent models as covariables. % Var - the percentage of variation in each Bray-Curtis similarity matrix that is explained by the respective set of predictor variables in each model; Res. df – residual degrees of freedom for each model. Significant P values are presented in boldface (α = 0.05).

Predictor variable set	Res. df	F	P	% Var
Macroinvertebrates				
Spatio-temporal covariables	81	4.062	**<0.001**	28.63
Hydro-morphometry	79	1.826	**0.004**	3.15
Physico-chemistry	73	2.100	**<0.001**	13.35
Biotope characteristics	77	1.694	**<0.001**	7.16
Habitat transformation	79	1.571	**0.021**	2.73
Microcrustaceans				
Spatio-temporal covariables	35	4.870	**<0.001**	41.03
Hydro-morphometry	33	1.098	0.346	3.68
Physico-chemistry	27	2.315	**<0.001**	23.99
Biotope characteristics	31	1.155	0.234	9.52
Habitat transformation	33	2.7377	**<0.001**	8.39

Note: The variables included in each set were the following: spatio-temporal covariables (full set) – longitude, latitude, time, altitude, and vegetation group (wetland cluster); hydro-morphometry - total surface area and maximum depth; physico-chemistry – pH, conductivity, average temperature, turbidity, dissolved oxygen, phosphates, nitrates + nitrites and ammonium; biotope characteristics - % complex vegetation, % simple vegetation, % open water, % benthic un-vegetated; habitat transformation - areal cover of natural vegetation within 100 and 500 m of wetlands.

Variation partitioning: macroinvertebrates

Environmental factors appeared to have the largest unique effect on macroinvertebrate composition (19.51%, P < 0.001, Fig. 3.2c), followed by spatio-temporal factors (12.44%, P < 0.001, Fig. 3.2a). The shared effect of these two sets of variables (spatio-temporal x environmental) explained 14.92% (Fig. 3.2d) of the total macroinvertebrate variation, which was the second highest contribution after the unique effect of environmental conditions. The unique contribution of habitat transformation to macroinvertebrate assemblage variation was comparatively low, explaining just 1.70% of the variation (P = 0.300, Fig. 3.2b). The shared effects of habitat transformation with environmental (Fig. 3.2e) and spatio-temporal factors (Fig. 3.2f) were also low (1.03 and 1.01% respectively). The variation partitioning approach used here could only provide significance values (P) for unique contributions (segments “a”, “b” and “c” in Fig. 3.2) because the other interactive effects (all remaining segments in Fig. 3.2) were calculated by adding or subtracting components and those resulting fractions could not be tested for statistical significance. From the above it is apparent that whilst the unique effects of spatio-temporal and environmental factors were highly significant (P < 0.001), habitat transformation alone did not account for a significant proportion of the variation in macroinvertebrate assemblage composition (P = 0.300). The cumulative influence of habitat transformation on macroinvertebrate assemblage composition, controlling for spatio-temporal factors as covariables, is the sum of segments “b” and “e” in Fig. 3.2 (2.73%). This cumulative influence of habitat transformation equates to the contribution tested in Table 3.7 and was significant (P = 0.021). Therefore whilst the effect of habitat transformation alone on macroinvertebrates was not significant, the cumulative effect (i.e. including associated environmental effects) was significant, albeit a comparatively small contribution in relation to the variation explained by changes in natural environmental and spatio-temporal factors.

Variation partitioning: microcrustaceans

The trends arising from the variation partitioning procedure for microcrustaceans were similar to those observed for macroinvertebrates, although the percentages explained were generally higher for the former (Fig. 3.3). The unique contribution from environmental factors was highest at 31.40% (P < 0.001, Fig. 3.3c), followed by the interactive effects of spatio-temporal x environmental at 25.77% (Fig. 3.3d) and spatio-temporal factors alone at 8.32% (P = 0.027, Fig. 3.3a). The remaining fractions (excluding the unexplained component) each contributed ~4% or less and included the unique effects of habitat transformation (4.24%, P = 0.021, Fig. 3.3b) and the effects of environmental factors associated with habitat transformation (4.15%, Fig. 3.3e). As for macroinvertebrates, the contribution from natural variations (i.e. independent of habitat transformation) in environmental and spatio-temporal factors (Fig. 3.3a, c and d) far outweighed the unique contribution from habitat transformation (Fig. 3.3b) and that from environmental changes associated with habitat transformation (Fig. 3.3e). The cumulative influence of habitat transformation on microcrustacean assemblages is once again the sum of segments “b” and “e” (8.39%, Fig. 3.3), which was reported as a highly significant contribution in Table 3.7 (P < 0.001). The transformation of habitats adjacent to wetlands appears to exert a small, but meaningful influence on microcrustacean assemblage composition.

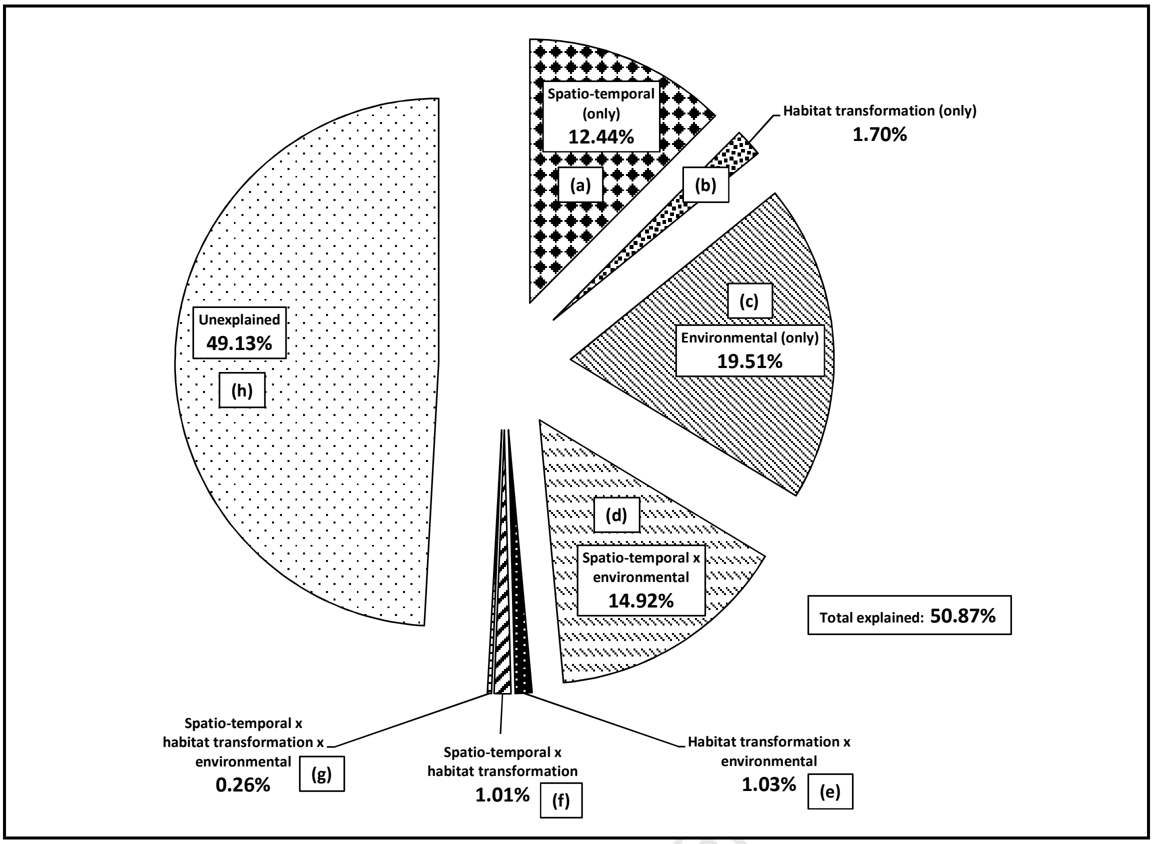


Figure 3.2. Results of the variation partitioning procedure for multivariate regressions of macroinvertebrate assemblage composition on spatio-temporal, environmental and habitat transformation variables. The contributions to explained variation in assemblage composition are represented by segments of the pie chart (a – h).

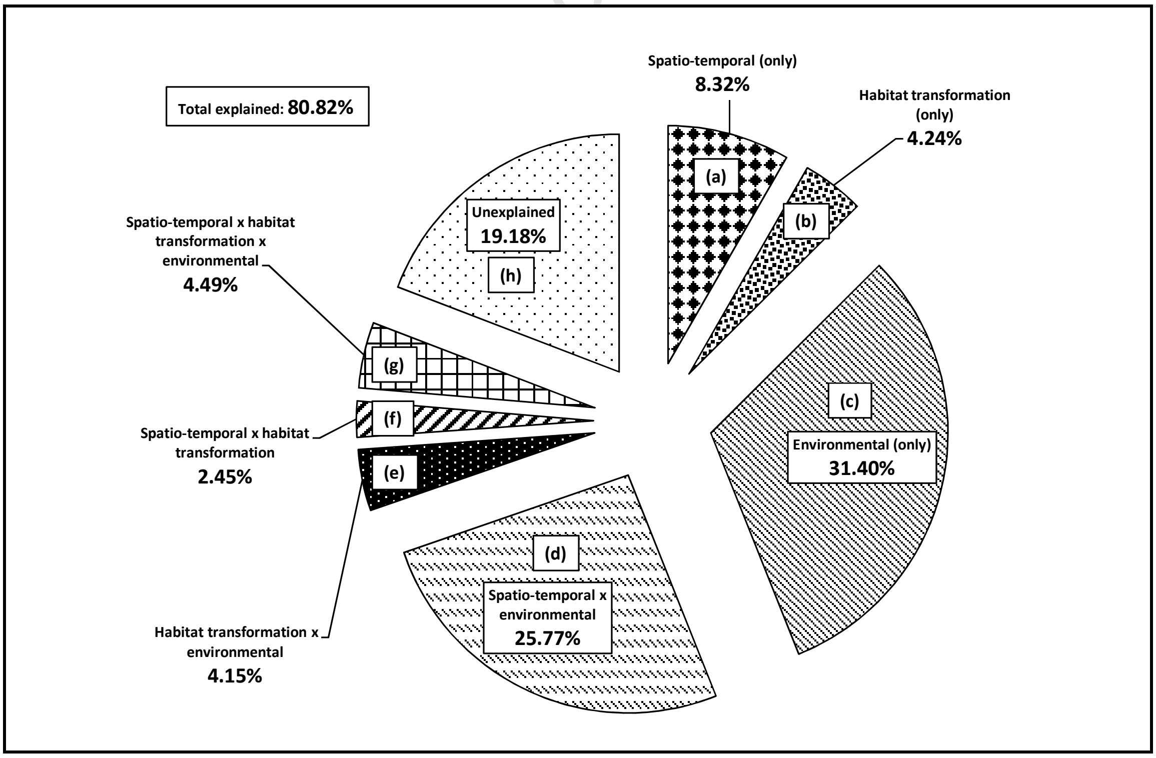


Figure 3.3. Results of the variation partitioning procedure for multivariate regressions of microcrustacean assemblage composition on spatio-temporal, environmental and habitat transformation variables. The contributions to explained variation in assemblage composition are represented by segments of the pie chart (a – h).

Relative importance of individual environmental variables

Step-wise dbRDA regressions indicate small but significant contributions from five environmental variables to the explained variation of macroinvertebrate composition (Table 3.8). Regressions using all possible combinations of predictor variables (Table 3.9) appear to validate the importance of the variables in Table 3.8, particularly so for pH, conductivity and nitrates + nitrites, which occur in nine of the ten most parsimonious models (pH and nitrates + nitrites were in all ten) and taken together form the model with lowest overall AICc rating (706.72). The top ten models in Table 3.9 are all within two AICc units of the lowest overall score and thus can all be considered as competing "best" models towards explaining macroinvertebrate assemblage composition. The habitat transformation variables did not feature strongly among the competing models in Table 3.9, but the variable "Natural 500 m" was included in the tenth model. The inclusion of the variables "% open water", "% complex vegetation", "% simple vegetation", "ammonium" and "Natural 500m" in the models of Table 3.9 indicates that the subset of variables chosen by the step-wise procedure (Table 3.8) was not necessarily the best set of environmental variables explaining the composition of macroinvertebrate assemblages.

Four environmental variables were reported as significant in explaining variation in microcrustacean assemblage composition according to dbRDA step-wise regression (Table 3.10). The contributions to explained variation appear to be dominated by pH (8.73%) and dissolved oxygen (5.40%). The importance of these two variables is once again validated by their inclusion in nine of the ten most parsimonious models (pH featured in all ten) reported by the all-subsets regression routine in Table 3.11. These two variables taken together formed the model with the lowest overall AICc score (310.10). All ten models were within two AICc units of the lowest score and thus should be considered as potentially important models. The inclusion of ammonium, % complex vegetation, total surface area, average temperature, maximum depth and % simple vegetation in some of the models in Table 3.11 indicates their potential importance as explanatory variables and suggests that the five significant variables reported by step-wise regression in Table 3.10 do not necessarily constitute the set of environmental variables best explaining the composition of microcrustacean assemblages. Neither of the habitat transformation variables was reported among the most important explanatory variables for microcrustaceans.

Table 3.8. Significant variables (α = 0.05) from the step-wise dbRDA regression (adjusted R^2 criterion) of macroinvertebrate assemblage composition on environmental variables. This is a partial model and thus conditioned upon the set of covariables, the effects of which were factored out of all subsequent tests. % Var. - the percentage of variation in each Bray-Curtis similarity matrix that is explained by the respective predictor variable in each test; Cum. % var. – the cumulative percentage variation across all tests; Res. df – residual degrees of freedom associated with each test.

Variable	F	P	% Var.	Cum. % var.	Res. df
Covariables	4.523	<0.001	27.85	27.85	82
Nitrates + nitrites	3.630	<0.001	3.09	30.95	81
pH	3.708	<0.001	3.06	34.01	80
Conductivity	2.893	<0.001	2.33	36.34	79
Maximum depth	1.859	0.015	1.48	37.82	78
Dissolved oxygen	1.888	0.013	1.48	39.31	77

Note: The subset of covariables used here was chosen beforehand using step-wise regression of macroinvertebrate assemblage composition against all covariables (see section 3.2.4, Appendix 1). The resulting seven covariables are: longitude; latitude; time; altitude; Sand fynbos; Western strandveld; Shale renosterveld.

Table 3.9. The ten models with highest parsimony for the environmental variables associated with macroinvertebrate assemblage composition. Models were selected from all possible combinations of predictor variables (all-subsets) using dbRDA multiple regression and are listed in descending order according to the AICc criterion for parsimony. The total percentage variation (% Var.) in macroinvertebrate composition (Bray-Curtis similarity matrix) explained by each model and the number of variables (No. vars) are included. These are partial models and thus the effects of covariables are included in each model.

AICc	% Var.	No. vars	Selections
706.72	36.34	10	pH; conductivity; nitrates + nitrites; + covariables
707.27	37.82	11	pH; conductivity; nitrates + nitrites; maximum depth; + covariables
707.36	34.01	9	pH; nitrates + nitrites; + covariables
707.39	37.74	11	pH; conductivity; nitrates + nitrites; % open water; + covariables
707.39	37.74	11	pH; conductivity; nitrates + nitrites; dissolved oxygen; + covariables
707.43	37.71	11	pH; conductivity; nitrates + nitrites; % complex vegetation; + covariables
707.56	37.62	11	pH; conductivity; nitrates + nitrites; % simple vegetation; + covariables
707.64	37.57	11	pH; conductivity; nitrates + nitrites; ammonium; + covariables
707.83	39.31	12	pH; conductivity; nitrates + nitrites; maximum depth; dissolved oxygen; + covariables
707.86	37.41	11	pH; conductivity; nitrates + nitrites; Natural 500 m; + covariables

Note: The subset of covariables used here was chosen beforehand using step-wise regression of macroinvertebrate assemblage composition against all covariables (see section 3.2.4, Appendix 1). The resulting seven covariables are: longitude; latitude; time; altitude; Sand fynbos; Western strandveld; Shale renosterveld.

Table 3.10 Significant variables (α = 0.05) from the step-wise dbRDA regression (adjusted R^2 criterion) of microcrustacean assemblage composition on environmental variables. This is a partial model and thus conditioned upon the set of covariables, the effects of which were factored out of all subsequent tests. % Var. - the percentage of variation in each Bray-Curtis similarity matrix that is explained by the respective predictor variable in each test; Cum. % var. – the cumulative percentage variation across all tests; Res. df – residual degrees of freedom associated with each test.

Variable	F	P	% Var.	Cum. % var.	Res. df
Covariables	6.868	<0.001	35.77	35.77	37
pH	5.665	<0.001	8.73	44.50	36
Dissolved oxygen	3.771	<0.001	5.40	50.00	35
Phosphates	1.922	0.036	2.68	52.58	34
Complex vegetation	1.887	0.039	2.57	55.15	33

Note: The subset of covariables used here was chosen beforehand using step-wise regression of macroinvertebrate assemblage composition against all covariables (see section 3.2.4, Appendix 1). The resulting three covariables are: longitude; time; Sand fynbos.

Table 3.11 The ten models with highest parsimony for the environmental variables associated with microcrustacean assemblage composition. Models were selected from all possible combinations of predictor variables (all-subsets) using dbRDA multiple regression and are listed in descending order according to the AICc criterion for parsimony. The total percentage variation (% Var.) in microcrustacean composition (Bray-Curtis similarity matrix) explained by each model and the number of variables (No. vars) are included. These are partial models and thus the effects of the three covariables are included in each model.

AICc	% Var.	No. vars	Selections
310.1	49.90	5	pH; dissolved oxygen; + covariables
310.76	52.58	6	pH; dissolved oxygen; phosphates; + covariables
310.81	52.53	6	pH; dissolved oxygen; ammonium; + covariables
310.86	52.47	6	pH; dissolved oxygen; % complex vegetation; + covariables
310.89	52.44	6	pH; dissolved oxygen; total surface area; + covariables
310.97	52.35	6	pH; dissolved oxygen; average temperature; + covariables
311.04	52.26	6	pH; dissolved oxygen; maximum depth; + covariables
311.35	51.90	6	pH; dissolved oxygen; nitrates + nitrites; + covariables
311.39	51.85	6	pH; dissolved oxygen; % simple vegetation; + covariables
311.53	44.50	4	pH; + covariables

Note: The subset of covariables used here was chosen beforehand using step-wise regression of macroinvertebrate assemblage composition against all covariables (see section 3.2.4, Appendix 1). The resulting three covariables are: longitude; time; Sand fynbos.

Multidimensional Scaling (MDS) plots

MDS plots depict the similarity of sites according to the multivariate composition of their invertebrate fauna. Plots "a" and "b" in Figure 3.4 do not indicate any distinct structuring of macroinvertebrate composition according to the cover of natural vegetation within 100 m and 500 m of wetlands. As these two variables proxy overall levels of habitat conversion around wetlands, it would not appear that this conversion has any consistent effect on macroinvertebrates inhabiting temporary wetlands. One weakness of the MDS approach, however, is that it cannot partial out spatio-temporal structure in the dataset. The influence of spatio-temporal factors (covariables) can thus interfere with patterns in the MDS plots and may mask the effect of habitat conversion. Results from the multiple regression models are more useful in this regard. The stress values in Fig. 3.4 are reasonably high at 0.25 and these plots should therefore be treated with some caution (Clarke and Warwick 2001), although 3-dimensional versions of these plots (with a lower stress value of 0.19) showed similar results, indicating that the 2-dimensional patterns were reasonably reliable.

Plot "c" (Fig. 3.4) shows some distinction among clusters of wetlands classified according to natural vegetation group, however this distinction is not clear and there is a lot of overlap among the Sand fynbos and Western strandveld clusters. Shale Renosterveld, Sandstone fynbos and Ferricrete fynbos were reasonably distinct from the Sand fynbos and Western strandveld sites. Variation does appear to be spatially linked in that the clusters with the most sites (Sand fynbos n = 44, followed by Western strandveld n = 28) showed the most spread in the plot, whilst the other three smaller clusters (n = 6 in each) were more tightly grouped. The classification of sites by latitudinal region (Fig. 3.4, plot "d") produced a clearer pattern of separation, although there was still considerable overlap towards the middle of the plot. The Cape Flats shows the most variation among sites, which is possibly an artefact of the greater number of samples collected from this region. Interestingly the Cape Flats shows slightly more spread among sites, despite the West Coast being sampled over a much larger spatial area. This offers some evidence that the number of samples collected may be more important than the spatial extent of sampling in determining the amount of dispersion or spread in the macroinvertebrate assemblages among sites. Plots "a" and "b" in Figure 3.5 show similar trends in the microcrustacean data as for the corresponding macroinvertebrate plots in Figure 3.4. Although there was some separation among individual levels of habitat conversion (e.g. between levels 2 and 3 in plot "b"), there was no overall trend in the separation of groups. Once again the multiple regression routines reported earlier are more useful than MDS for disentangling the effects of habitat conversion from those caused by intrinsic spatio-temporal structuring, which may interfere with the MDS patterns. Sites in Figure 3.5c (classified by natural vegetation group) appear to group more clearly than those in plots “a” and “b”, although there is a large amount of spread among the Sand fynbos sites. Further clearer distinction of sites was obtained when classifying sites by the latitudinal region in which they occurred, as evidenced by the clear split between the two groups in Figure 3.5d. The comments on stress values for Figure 3.4 are mirrored here for Figure 3.5.

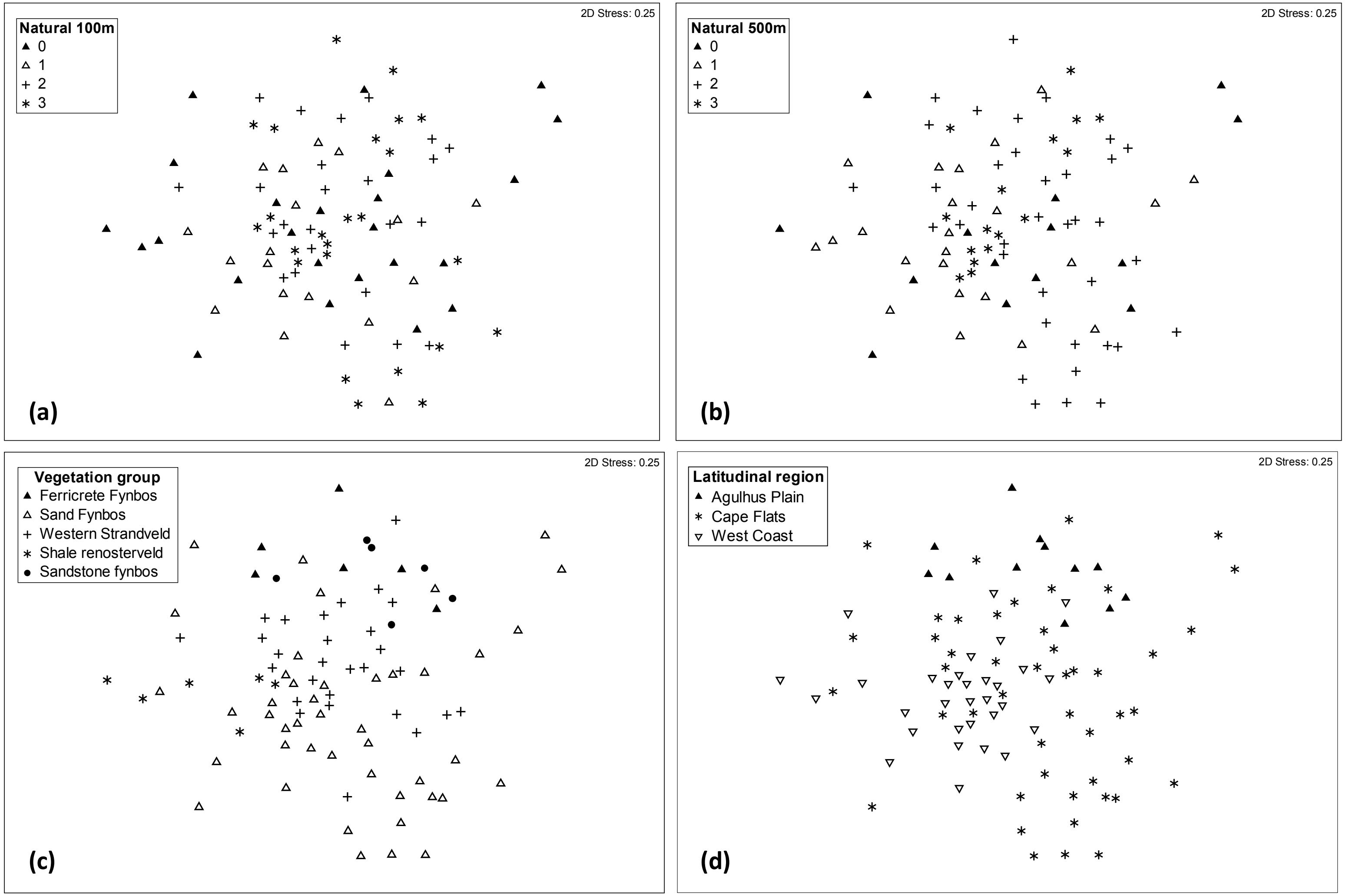


Figure 3.4. Multidimensional scaling (MDS) plots of macroinvertebrate assemblage composition, represented by the Bray-Curtis similarity among sites (n = 90). The factors "Natural 100 m" (a), "Natural 500 m" (b), vegetation group (wetland cluster) (c) and latitudinal region (d) have been coded on the respective plots. "Natural 100 m" and "Natural 500 m": sites are coded according to the areal cover of natural (indigenous) vegetation within 100 and 500 m of each wetland edge: 0 - absent; 1 – sparse cover (<33%); 2 – moderate cover (33-66%); 3 – extensive cover (> 66%).

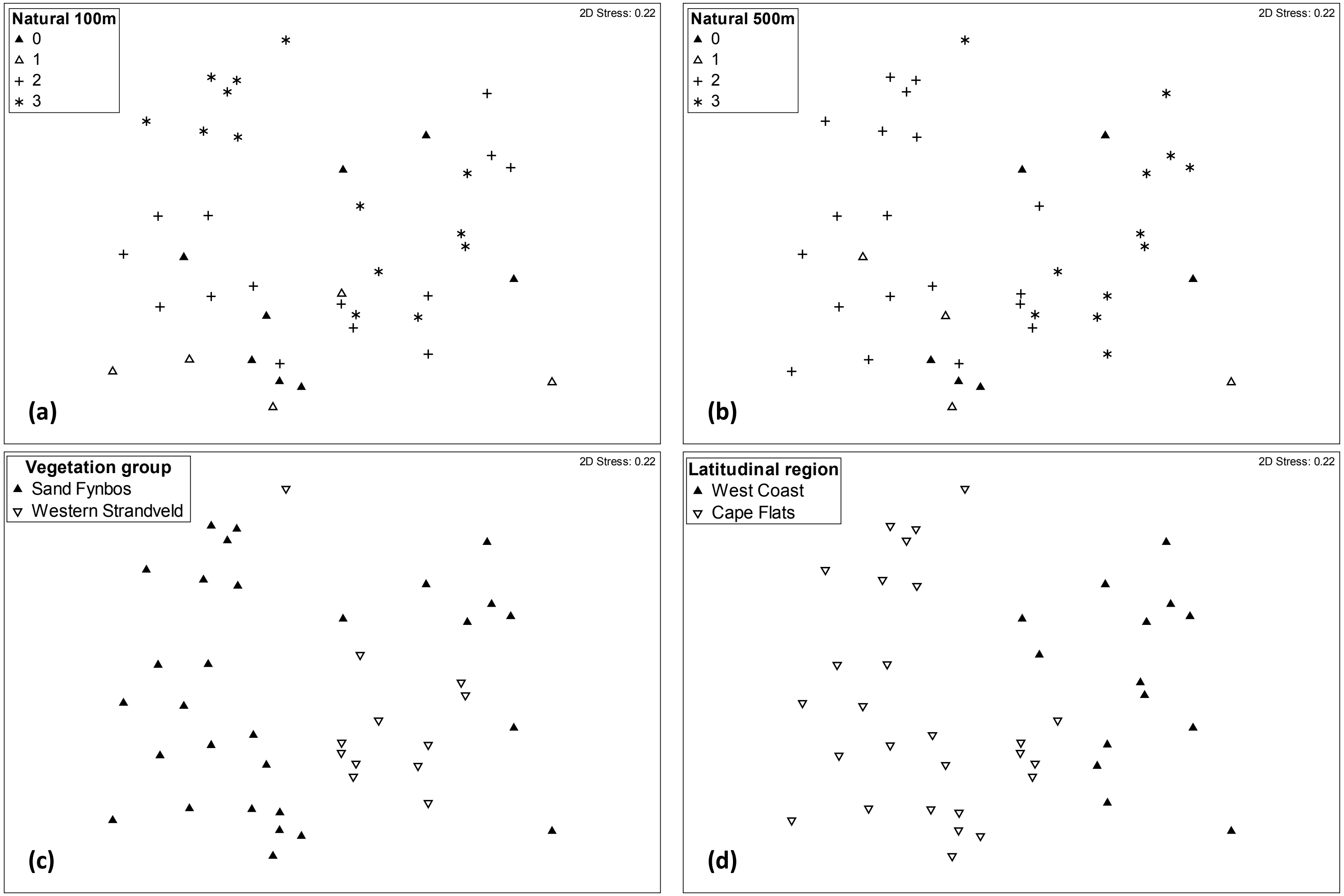


Figure 3.5. Multidimensional scaling (MDS) plots of microcrustacean assemblage composition, represented by the Bray-Curtis similarity among sites (n = 41). The factors “Natural 100 m” (a), “Natural 500 m” (b), vegetation group (wetland cluster) (c) and latitudinal region (d) have been coded on the respective plots. “Natural 100 m” and “Natural 500 m”: sites are coded according to the areal cover of natural (indigenous) vegetation within 100 and 500 m of each wetland edge: 0 - absent; 1 – sparse cover (< 33%); 2 – moderate cover (33-66%); 3 – extensive cover (> 66%).

3.3.3. Taxon richness and diversity

No significant relationships were found between any of the macroinvertebrate richness/diversity measures and the two predictor variables representing habitat transformation ("Natural 100 m" and "Natural 500 m"). Three univariate measures of microcrustacean richness/diversity showed significant response to habitat transformation within 100 m of wetlands ("Natural 100 m"). These measures were the number of taxa (S), Margalef's richness index (d) and the Shannon diversity index (H', Table 3.12). Interestingly, these were all negative relationships, indicating that increasing transformation around wetlands tended to be associated with higher microcrustacean richness/diversity in terms of these three measures. No significant relationships were found between the predictor variable "Natural 500 m" and the richness/diversity measures applied to the microcrustacean dataset. Figure 3.6 offers a visual depiction of relationships between the cover of natural vegetation within 100 m and each of the three significant response variables, holding the other covariables in each model constant. The residual points on the plots occur in vertical bands because of the ordinal nature of the predictor variable "Natural 100 m". Wetlands scored as level 2 for the variable "Natural 100 m" (i.e. moderate cover of natural vegetation – 33-66%, see chapter 2, section 2.3) tend to go against the negative linear trend in these plots, displaying reasonably high richness/diversity values. Otherwise the negative trend in all three plots is quite apparent for the other score levels and there are no pronounced outliers. Models reporting non-significant relationships between richness/diversity response variables and the habitat transformation predictor variables ("Natural 100 m" and "Natural 500 m") are not presented here.

3.3.4. Family-level tests for indicator taxa

The macroinvertebrate and microcrustacean families that were significantly related to the predictor variables representing gradients of overall habitat transformation around wetlands ("Natural 100 m" and "Natural 500 m") and the human disturbance scores ("HDS") are presented in Table 3.13. Non-significant relationships are not presented here. Only seven macroinvertebrate families (Table 3.13a - h) and one microcrustacean family (Daphniidae, Table 3.13i) presented significant relationships with the habitat transformation variables or human disturbance scores. The partial r^2 values associated with each of these relationships were very low (maximum was 0.113), indicating weak relationships. The performance of individual families as indicators of human disturbance was further gauged by visual assessment of the partial residual plots presented in Figure 3.7. A good indicator taxon would present a clear linear trend, with low scatter among points, preferably with no outliers and have few or no points with high leverage. None of the plots in Figure 3.7 actually meets these requirements, although the relationship between the Daphniidae and the variable “Natural 100m” is better than the others and is plagued by only one outlier (bottom left of plot “i"). The eight macroinvertebrate plots depict relationships that are either dominated by outliers or contain high amounts of scatter, thus generally presenting weak trends. The outliers in the plots for Gerridae and Paramelitidae are likely to be responsible for the overall significant P values being attached to these relationships in Table 3.13. It should be noted that the multiple regressions which did not include any covariables (i.e. step-wise selection retained no significant covariables in the equation) are essentially equivalent to least squares regressions with only one predictor variable. The corresponding partial residual plots will show the same pattern as a simple linear regression plot with one predictor.

Table 3.12. Multiple linear regression models (a - c) for the three microcrustacean richness/diversity measures that were significantly related to the habitat transformation variable (“Natural 100 m”), given the spatio-temporal covariables. Only significant relationships are presented here ($\alpha = 0.05$). To maximise parsimony, covariable subsets were pre-selected for each model using step-wise regression of each response variable on the full list of possible covariables (see Appendix 1). Only partial relationships between the response and predictor variables are reported here, not the full model results.

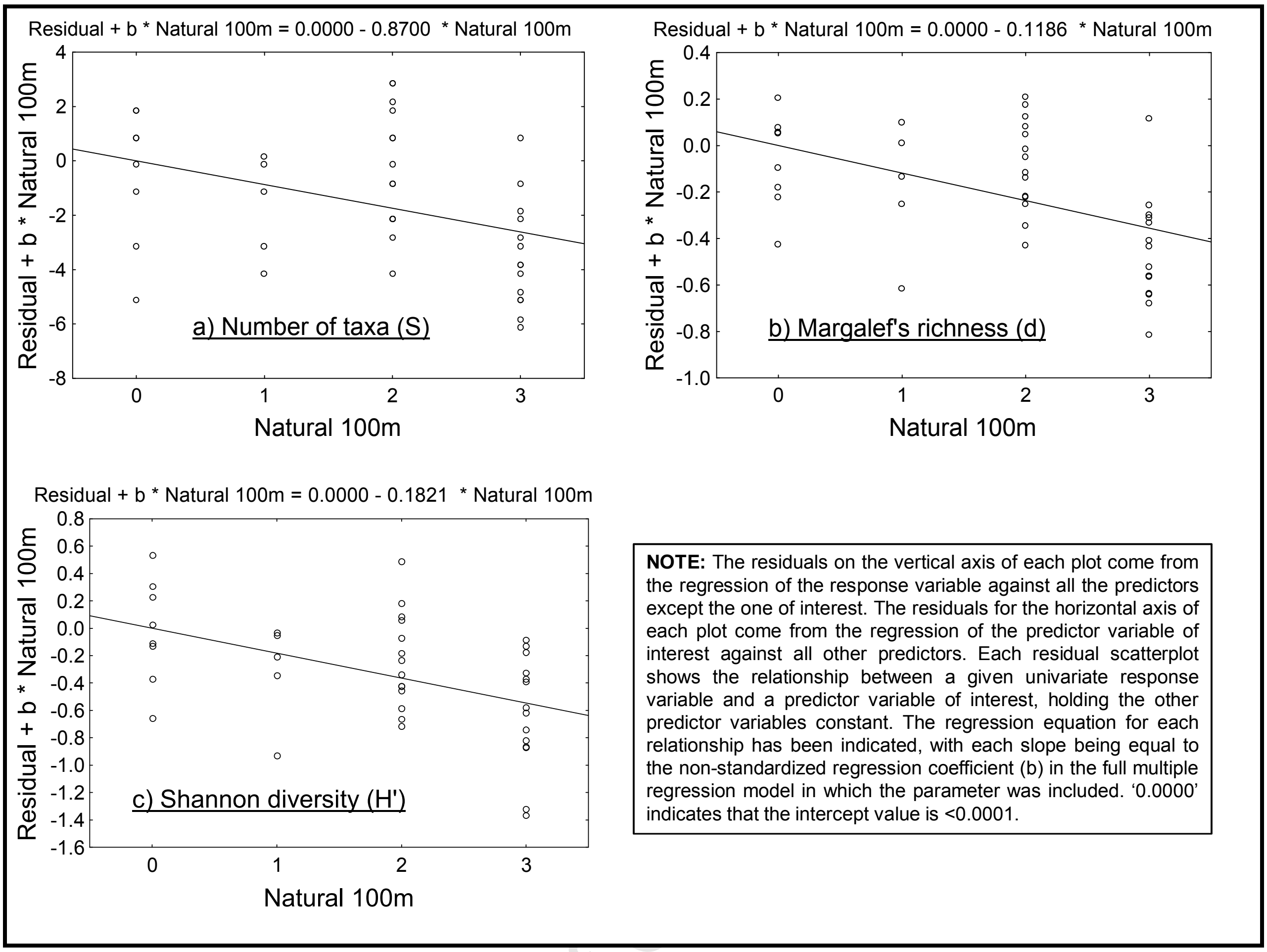
“Natural 100 m” - areal cover of indigenous vegetation within 100 m of each wetland edge, measured on an ordinal scale; Time - Number of days since the first sampling event; β – standardized regression coefficient; SE – standard error of regression coefficient; Partial r^2 – coefficient of partial determination for each respective predictor variable; Res. df – residual degrees of freedom; r^2 (Covariables) = Full model r^2 - Partial r^2 (predictor).

	Predictor variables	Response variables	β	SE	Partial r^2 (predictor)	t	Res. df	P	Covariables	r^2 (Covariables)
a)	Natural 100m	Number of taxa (S)	-0.365	0.156	0.126	-2.340	38	0.025	Sand fynbos	0.092
b)	Natural 100m	Margalef's richness index (d)	-0.430	0.143	0.196	-3.004	37	0.005	Time, Sand fynbos	0.189
c)	Natural 100m	Shannon diversity index (H')	-0.408	0.164	0.147	-2.488	36	0.018	Latitude, time, Sand fynbos	0.279

Table 3.13. Multiple linear regression models of macroinvertebrate (a - h) and microcrustacean (i) family abundances regressed on the habitat transformation variables (“Natural 100 m” and “Natural 500 m”) and human disturbance scores (“HDS”), given the spatio-temporal covariables. Only significant relationships are presented here ($\alpha = 0.05$). To maximise parsimony, covariable subsets were pre-selected for each model using step-wise regression of each response variable on the full list of possible covariables (see Appendix 1). Only partial relationships between the response and predictor variables are reported here, not the full model results. Family abundances were ln(x+1) transformed for macroinvertebrates and 4th root transformed for microcrustaceans.

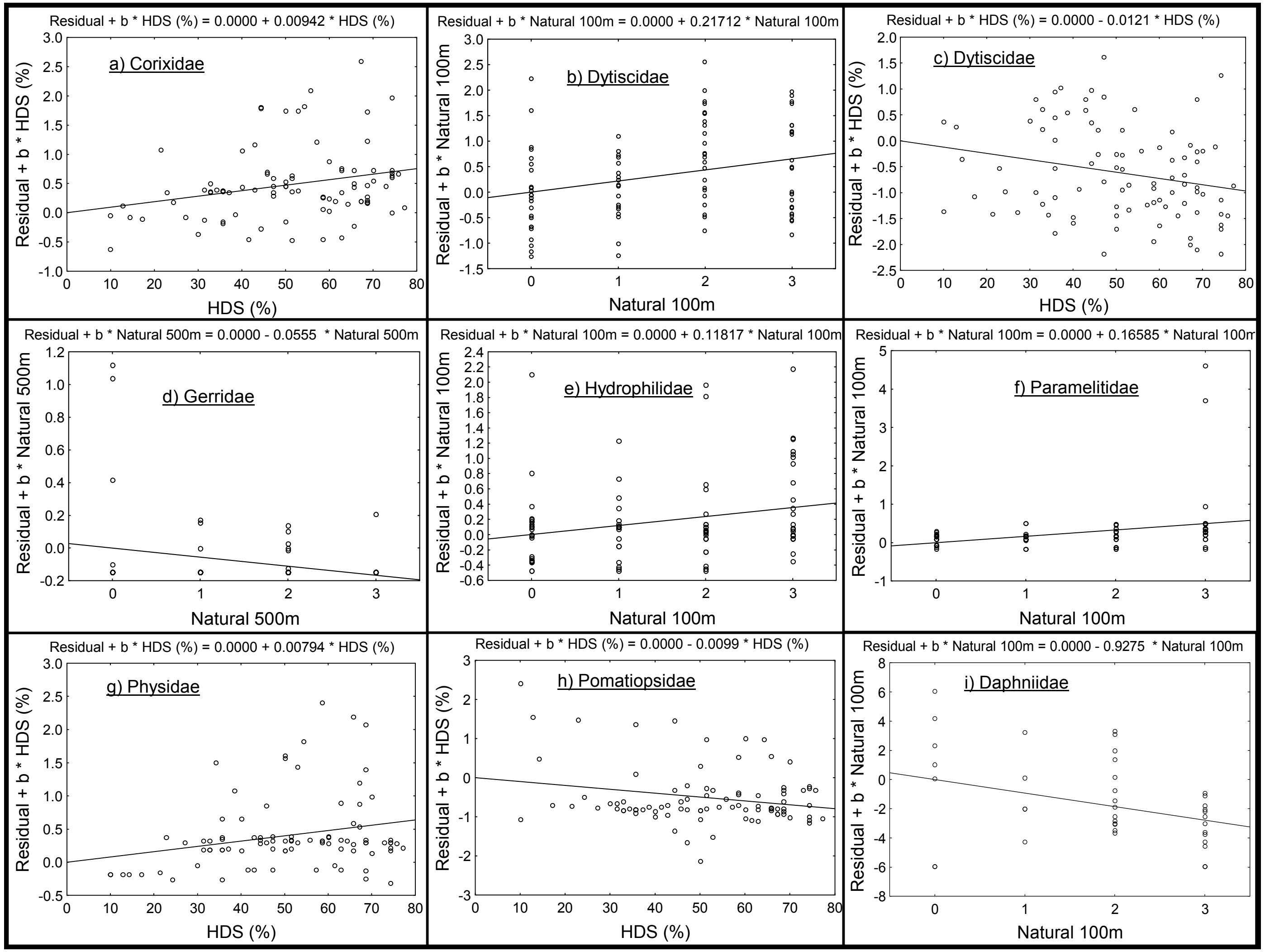
“HDS” – human disturbance scores from the rapid assessment index; “Natural 100 m” and “Natural 500 m” - areal cover of indigenous vegetation within 100 and 500 m radii of each wetland edge, measured on an ordinal scale; Time - Number of days since the first sampling event; β – standardized regression coefficient; SE – standard error of regression coefficient; Partial r^2 – coefficient of partial determination for each respective predictor variable; Res. df – residual degrees of freedom; r^2 (Covariables) = Full model r^2 - Partial r^2 (predictor).

	Predictor variables	Response variables	β	SE	Partial r^2 (predictor)	t	Res. df	P	Covariables	r^2 (Covariables)
	Macroinvertebrates									
a)	HDS	Corixidae	0.216	0.093	0.060	2.315	84	0.023	Longitude, time, altitude, Ferricrete fynbos	0.307
b)	Natural 100m	Dytiscidae	0.256	0.099	0.073	2.596	85	0.011	Longitude, latitude, time	0.164
c)	HDS	Dytiscidae	0.320	0.124	0.073	2.593	85	0.011	Longitude, latitude, time	0.144
d)	Natural 500m	Gerridae	-0.264	0.103	0.070	-2.573	88	0.012	No covariables	NA
e)	Natural 100m	Hydrophilidae	0.222	0.101	0.053	2.197	86	0.031	Latitude, time	0.124
f)	Natural 100m	Paramelitdae	0.296	0.103	0.087	2.863	86	0.005	Time, Western strandveld	0.050
g)	HDS	Physidae	0.227	0.098	0.059	2.314	86	0.023	Time, Western strandveld	0.140
h)	HDS	Pomatiopsidae	-0.153	0.075	0.047	-2.044	85	0.044	Altitude, Ferricrete fynbos, Sandstone fynbos	0.525
	Microcrustaceans									
i)	Natural 100m	Daphniidae	-0.336	0.151	0.113	-2.229	39	0.032	No covariables	NA



NOTE: The residuals on the vertical axis of each plot come from the regression of the response variable against all the predictors except the one of interest. The residuals for the horizontal axis of each plot come from the regression of the predictor variable of interest against all other predictors. Each residual scatterplot shows the relationship between a given univariate response variable and a predictor variable of interest, holding the other predictor variables constant. The regression equation for each relationship has been indicated, with each slope being equal to the non-standardized regression coefficient (b) in the full multiple regression model in which the parameter was included. ‘0.0000’ indicates that the intercept value is <0.0001.

Figure 3.6. Partial residual plots displaying relationships between the three univariate measures of microcrustacean taxon richness/diversity (a – c) and the habitat transformation variables, holding the covariables constant. These three measures were significantly related to the habitat transformation variable “Natural 100 m” in the full linear regression models (see Table 3.12). No significant relationships with the variable “Natural 500m” were reported. Measures of richness/diversity which were not significantly related to habitat transformation gradients are not reported here. “Natural 100 m” refers to the areal cover of indigenous vegetation within a 100 m radius of each wetland edge, measured on an ordinal scale: 0 – none; 1 – sparse; 2 – moderate; 3 – extensive.



NOTE: The residuals on the vertical axis of each plot come from the regression of the response variable against all the predictors except the one of interest. The residuals for the horizontal axis of each plot come from the regression of the predictor variable of interest against all other predictors. Each residual scatterplot shows the relationship between a given univariate response variable and a predictor variable of interest, holding the other predictor variables constant. The regression equation for each relationship has been indicated, with each slope being equal to the non-standardized regression coefficient (b) in the full multiple regression model in which the parameter was included. ‘0.0000’ indicates that the intercept value is <0.0001.

Figure 3.7. Partial residual plots displaying relationships of macroinvertebrate (a – h) and microcrustacean (i) families regressed on the habitat transformation variables and human disturbance scores, holding the covariables constant. These nine families presented significant relationships in Table 3.13. Non-significant relationships are not reported here. “HDS” – human disturbance scores from the rapid assessment index; “Natural 100 m” and “Natural 500 m” - areal cover of indigenous vegetation within 100 m and 500 m radii of each wetland edge, measured on an ordinal scale: 0 – none; 1 – sparse; 2 – moderate; 3 – extensive. Family abundances were ln(x+1) transformed for macroinvertebrates and 4th root transformed for microcrustaceans.

3.3.5. Testing potential metrics

Macroinvertebrate and microcrustacean metrics that were significantly related to the habitat transformation predictor variables ("Natural 100 m" and "Natural 500 m") and the human disturbance scores ("HDS") are presented in Table 3.14, Figure 3.8 (macroinvertebrates) and Figure 3.9 (microcrustaceans). A total of eight macroinvertebrate and five microcrustacean metrics showed significant association with at least one of the predictor variables. Partial r^2 values indicate that relationships were weak throughout and the maximum percentage of explained variation due to habitat transformation was 16.3% (i.e. partial $r^2 = 0.163$, Table 3.14w). Inspection of the partial residual plots reveals that the FBI metric derived from the Family Biotic Index for wetlands in Minnesota, USA (Appendix 10), can immediately be ruled out because the significant result appears to be caused by one pronounced outlier (Fig. 3.8o - p). The plots in Figures 3.8 and 3.9 are characterised by a generally high level of scatter, which is expected to account for the low r^2 values commented on earlier. As the predictor variables representing habitat transformation are ordinal, the points on the corresponding plots occur in vertical bands and can be interpreted similarly to box plots because the amount of spread for each bar is indicative of the amount of variation at each level of natural vegetation cover. The spread of the residual points in this regard is high throughout the plots, indicating that one would not be able to reliably infer levels of adjacent habitat disturbance using these metrics.

Table 3.14. Multiple linear regression models of macroinvertebrate (a - p) and microcrustacean (q – w) metrics against the habitat transformation variables (“Natural 100 m” and “Natural 500 m”) and human disturbance scores (“HDS”), given the spatio-temporal covariables. Only significant relationships are presented here ($\alpha = 0.05$). To maximise parsimony, covariable subsets were pre-selected for each model using step-wise regression of each response variable on the full list of possible covariables (see Appendix 1). Only partial relationships between the response and predictor variables are reported here, not the full model results. Abundance metrics were ln(x+1) transformed for macroinvertebrates and 4th root transformed for microcrustaceans.

“HDS” – human disturbance scores from the rapid assessment index; “Natural 100 m” and “Natural 500 m” - areal cover of indigenous vegetation within 100 m and 500 m radii of each wetland edge, measured on an ordinal scale; Time - Number of days since the first sampling event; SF – Sand fynbos; WS – Western strandveld; FF – Ferricrete fynbos; β – standardized regression coefficient; SE – standard error of regression coefficient; Partial r^2 – coefficient of partial determination for each respective predictor variable; Res. df – residual degrees of freedom; r^2 (Covariables) = Full model r^2 - Partial r^2 (predictor).

	Predictor variables	Response variables	β	SE	Partial r^2 (predictor)	t	Res. df	P	Covariables	r^2 (Covariables)
	Macroinvertebrates									
a)	Natural 100m	Total number of individuals	0.298	0.098	0.100	3.047	84	0.003	Longitude, time, SF, WS	0.151
b)	Natural 500m	Total number of individuals	0.262	0.102	0.073	2.580	84	0.012	Longitude, time, SF, WS	0.155
c)	HDS	Total number of individuals	-0.257	0.098	0.076	-2.637	84	0.010	Longitude, time, SF, WS	0.155
d)	Natural 100m	Total number of “tolerant" coleopteran individuals	0.250	0.102	0.067	2.449	83	0.016	Longitude, latitude, time, altitude, SF	0.184
e)	Natural 500m	Total number of “tolerant” coleopteran individuals	0.277	0.104	0.079	2.673	83	0.009	Longitude, latitude, time, altitude, SF	0.182
f)	HDS	Total number of “tolerant” coleopteran individuals	-0.256	0.102	0.071	-2.510	83	0.014	Longitude, latitude, time, altitude, SF	0.184
g)	Natural 100m	Total number of coleopteran individuals	0.289	0.103	0.086	2.794	83	0.006	Longitude, latitude, altitude, SF, WS	0.147
h)	Natural 500m	Total number of coleopteran individuals	0.313	0.103	0.100	3.041	83	0.003	Longitude, latitude, altitude, SF, WS	0.145
i)	HDS	Total number of coleopteran individuals	-0.301	0.101	0.096	-2.973	83	0.004	Longitude, latitude, altitude, SF, WS	0.145
j)	Natural 500m	% Coleopteran individuals (of total sample count)	0.211	0.102	0.047	2.057	85	0.043	Longitude, latitude, altitude	0.121
k)	Natural 100m	Total number of individuals in dominant taxon	0.284	0.100	0.086	2.849	86	0.005	Longitude, time	0.078
l)	HDS	Total number of individuals in dominant taxon	-0.216	0.102	0.050	-2.124	86	0.037	Longitude, time	0.081
m)	HDS	Corixidae (as % of Coleoptera and Hemiptera)	0.206	0.095	0.052	2.156	84	0.034	Latitude, time, altitude, FF	0.265
n)	HDS	% Omnivores	0.213	0.090	0.061	2.379	87	0.020	Latitude	0.260
o)	Natural 100m	Average score per taxon (FBI)	-0.233	0.104	0.055	-2.252	88	0.027	No covariables	NA
p)	Natural 500m	Average score per taxon (FBI)	-0.306	0.101	0.094	-3.015	88	0.003	No covariables	NA
	Microcrustaceans									
q)	Natural 100m	Total number of taxa	-0.365	0.156	0.126	-2.340	38	0.025	SF	0.092
r)	HDS	Total number of taxa	0.406	0.162	0.142	2.503	38	0.017	SF	0.090
s)	HDS	Total number of families	0.347	0.164	0.111	2.115	36	0.041	Latitude, time, SF	0.307
t)	HDS	Total number of copepod individuals	-0.369	0.174	0.106	-2.123	38	0.040	SF	0.011
u)	Natural 100m	Total number of ostracod individuals	-0.346	0.134	0.150	-2.586	38	0.014	Latitude	0.178
v)	Natural 100m	% Copepod taxa (of all taxa)	0.368	0.149	0.136	2.475	39	0.018	No covariables	NA
w)	HDS	% Copepod taxa (of all taxa)	-0.403	0.147	0.163	-2.753	39	0.009	No covariables	NA

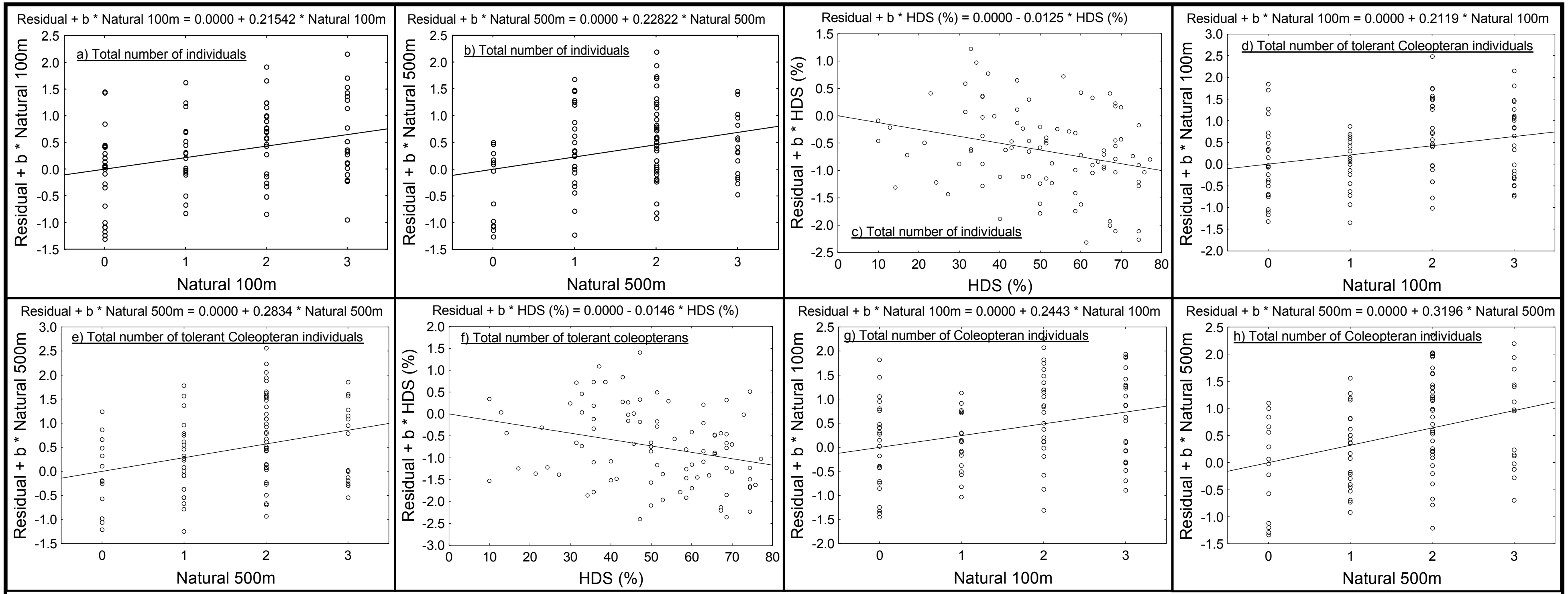


Figure 3.8. Partial residual plots displaying macroinvertebrate metrics (a – p in Table 3.14) regressed on the habitat transformation variables and human disturbance scores, holding the covariables constant. These 16 metrics presented significant relationships in Table 3.14. Non-significant relationships are not reported here. “HDS” – human disturbance scores from the rapid assessment index; “Natural 100 m” and “Natural 500 m” - areal cover of indigenous vegetation within 100 m and 500 m radii of each wetland edge, measured on an ordinal scale: 0 – none; 1 – sparse; 2 – moderate; 3 – extensive. Abundance metrics were ln(x+1) transformed.

NOTE: The residuals on the vertical axis of each plot come from the regression of the response variable against all the predictors except the one of interest. The residuals for the horizontal axis of each plot come from the regression of the predictor variable of interest against all other predictors. Each residual scatterplot shows the relationship between a given univariate response variable and a predictor variable of interest, holding the other predictor variables constant. The regression equation for each relationship has been indicated, with each slope being equal to the non-standardized regression coefficient (b) in the full multiple regression model in which the parameter was included. ‘0.0000’ indicates that the intercept value is <0.0001.

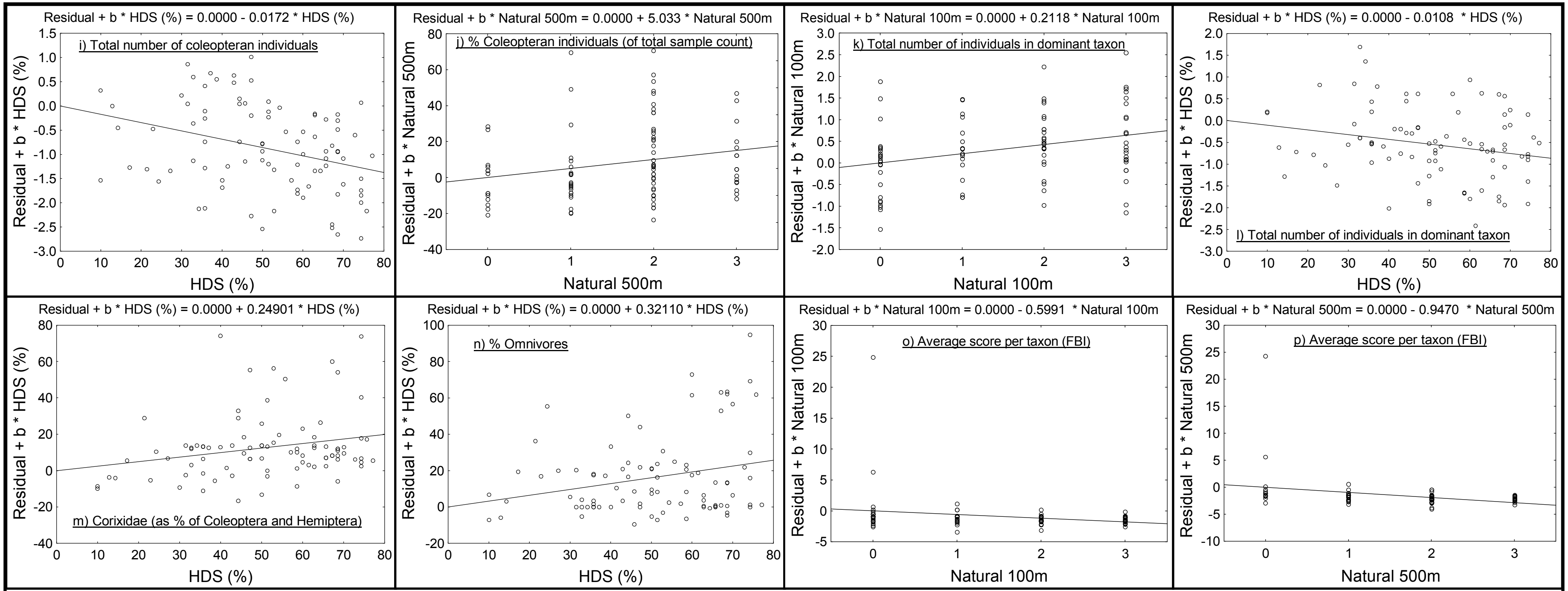
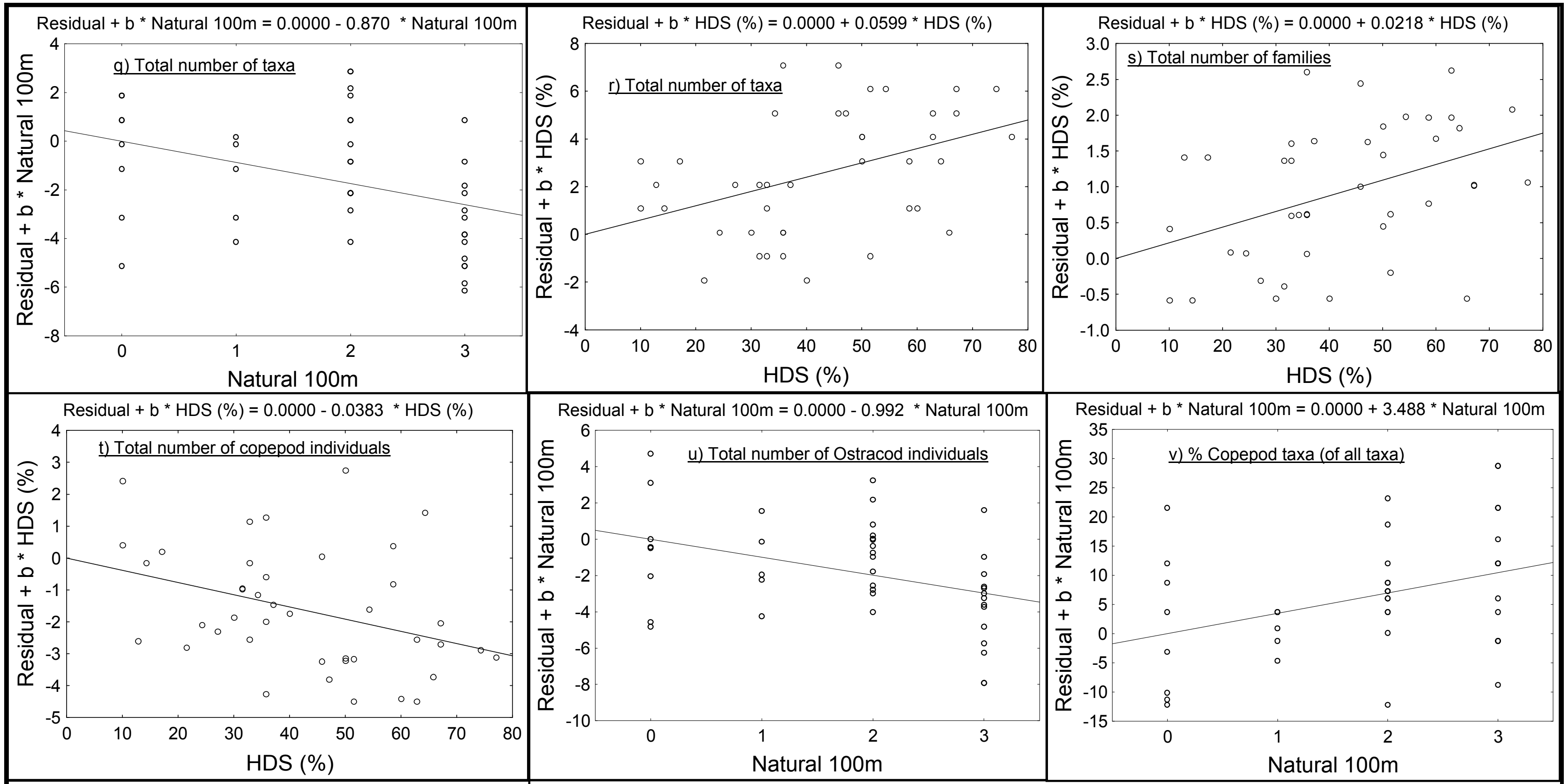
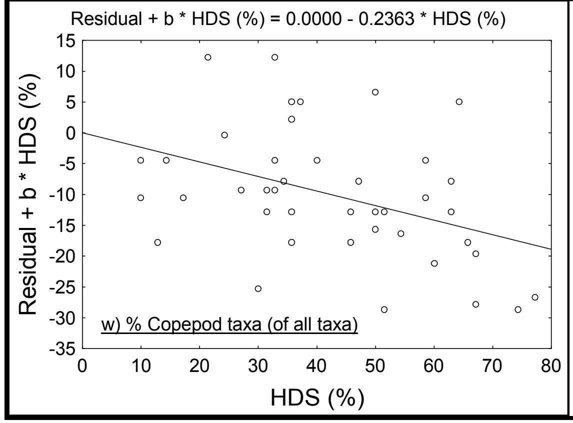


Figure 3.8 (continued)





NOTE: The residuals on the vertical axis of each plot come from the regression of the response variable against all the predictors except the one of interest. The residuals for the horizontal axis of each plot come from the regression of the predictor variable of interest against all other predictors. Each residual scatterplot shows the relationship between a given univariate response variable and a predictor variable of interest, holding the other predictor variables constant. The regression equation for each relationship has been indicated, with each slope being equal to the non-standardized regression coefficient (b) in the full multiple regression model in which the parameter was included. '0.0000' indicates that the intercept value is <0.0001.

Figure 3.9. Partial residual plots displaying microcrustacean metrics (q – w in Table 3.14) regressed on the habitat transformation variables and human disturbance scores, holding the covariables constant. These seven metrics presented significant relationships in Table 3.14. Non-significant relationships are not reported here. "HDS" – human disturbance scores from the rapid assessment index; "Natural 100 m" - areal cover of indigenous vegetation within a 100 m radius of each wetland edge, measured on an ordinal scale: 0 – none; 1 – sparse; 2 – moderate; 3 – extensive. Abundance metrics were 4th root transformed.

3.4. DISCUSSION

3.4.1. Invertebrate assemblage composition

Associations with habitat transformation variables

As expected, associations between the individual habitat transformation variables and the composition of macroinvertebrate assemblages were weak. Overall levels of habitat transformation, proxied by the variables “Natural 100 m” and “Natural 500 m”, were not significantly related to macroinvertebrate assemblage composition in these temporary wetlands (“Natural 100 m”: P = 0.076; “Natural 500 m”: P = 0.056). Yet the P values are very close to the significance level of 0.05 and thus one can conclude that there is some evidence of a relationship, though it is was not statistically significant in this study. The philosophy taken here (as prescribed by Verhagen *et al.* 2004) is that marginal P values should be interpreted in light of the percentage variation explained by the respective predictor variables. Variation in macroinvertebrate assemblage composition explained by the habitat transformation variables (both overall transformation and different types) was low throughout, especially relative to that explained by the spatio-temporal covariables. Regardless of whether these marginal P values are significant or not at the rather arbitrary α level of 0.05, the habitat transformation variables consistently explained only small proportions of variation, which in an ecological sense implies that they had a relatively minor effect.

The highly significant relationship between the composition of macroinvertebrate assemblages and agricultural cover within 100 m was surprising, given that agriculture was the only type of habitat transformation that did not show any significant association with wetland environmental conditions in chapter 2 (Table 2.2). It is likely that the effect of agriculture detected in this study is a result of the heavy direct physical disturbance of the area immediately adjacent to, and sometimes within, temporary wetlands of the region. Most agricultural wetlands in this study were surrounded by wheat fields, which are ploughed during the dry season and therefore the soil is heavily disturbed and natural vegetation often completely removed. Thus, despite no clear evidence of a physico-chemical effect of agriculture (chapter 2), direct physical disturbance of the wetland basin and immediately adjacent areas could well be expected to alter resting egg banks and dormant stages in the soil. Euliss and Mushet (1999) found significantly more taxa and greater numbers of planorbid and physid snail shells in temporary wetlands surrounded by grasslands than in those surrounded by agriculture. Brose (2003) did not find any clear links between agricultural land use and temporary wetland beetle assemblages, however. These were the only studies that could be found in the literature relating to agricultural effects on temporary wetland invertebrate assemblages and even this very sparse literature presented ambivalent results. The small sample sizes of the two agricultural datasets in this study ("Agriculture 100 m": n = 24; "Agriculture 500 m": n = 21) limits the ability to make conclusive statements, but from this limited data it appears that wheat cultivation in the south-western Cape has a significant impact on the assemblage composition of temporary wetland macroinvertebrates. Further research is recommended to establish whether this is indeed a consistent trend.

It appears that slightly lower detection of habitat transformation gradients was achieved using the macroinvertebrate family-level data when compared to the finer-resolution data. This statement is based on the slightly higher P values reported for the family-level data than for the data recorded at the best achievable taxonomic resolution (Table 3.4 vs. Table 3.5). However the percentages of explained variation for each predictor variable were very similar among the two datasets, indicating that not much detection power was gained with the increased resolution. King and Richardson (2002) found that taxonomic resolution did make a difference in detecting a gradient of eutrophication in the Florida Everglades using macroinvertebrates. They reported superior ability to detect impairment using generic data, but maintained that this was due only to the high number of genera and species within the family Chironomidae. Identification of chironomids beyond subfamily was not however feasible in the current study. The results of King and Richardson (2002) indicate that genus- or species-level identification of chironomids may have enabled better detection of the habitat transformation gradients in this study and also may have broadened the gap in detection power between the family-level and fine-scale datasets.

Despite limited research on the effect of macroinvertebrate taxonomic resolution for wetland bioassessment, debate over the use of family- *versus* genus- and species-level assessment is well documented for rivers (Hawkins *et al.* 2000, Bailey *et al.* 2001, Waite *et al.* 2004). As summarised by Bailey *et al.* (2001), it would appear that genus- and species-level resolution in rivers is only of significant advantage for regions of high species richness; otherwise family-level analyses tend to be sufficient to detect impacts. Especially important is the practicality of identifying samples beyond family level. Most bioassessment programs, notably the South African Scoring System in this country (SASS5, Dickens and Graham 2002), rely on non-specialists (volunteers or government employees) who identify taxa in the field. While genus- and species-level resolution may be useful for detailed academic studies relating human disturbance to invertebrate assemblages, this level of resolution is not an option for a practical wetland bioassessment index using invertebrates. This assertion is based on the extreme difficulty that was encountered during this study in obtaining such a level of taxonomic resolution, due mostly to the lack of local expertise for taxa such as the Coleoptera, Diptera and Acarina. Furthermore, not much improvement in detecting habitat transformation gradients has been observed in this study when going beyond family-level for macroinvertebrates.

Tests of association between the composition of microcrustacean assemblages and the habitat transformation variables revealed similar levels of explained variation as for the macroinvertebrate data. Significance levels showed some differences, however, and the relationship between microcrustacean assemblages and natural vegetation cover within 100 m of wetlands was highly significant ($P < 0.001$), whilst being clearly unrelated to natural vegetation within 500 m ($P = 0.415$). Thus, it appears that transformation of habitat within the immediate vicinity of wetlands (< 100 m) had a significant impact on microcrustacean assemblages, but apparently had very little impact when considered over a broader 500 m radius. This suggests that conservation of natural vegetation habitat within 100 m of temporary wetlands in the region may have significant benefits in terms of sustaining natural microcrustacean assemblages, although given the low percentages explained by habitat transformation it would appear that only a small fraction of variation is at stake. Caution is warranted here in making broad statements about the conservation implications of the microcrustacean results given that only two wetland clusters were analysed and only a small fraction of the region was assessed. These findings do suggest however that microcrustacean assemblages are not completely resilient to human activities in the adjacent landscape, despite my hypothesis that they would not be affected. Microcrustaceans are passive dispersers and are generally more sedentary than macroinvertebrates, many of which are able to actively disperse by flight. Batzer *et al.* (2004) observed that distribution patterns of aquatic insects (active dispersers) in temporary wetlands of Minnesota were better explained by environmental factors than were those of sedentary macroinvertebrates such as leeches. They argued that the logical interpretation was that sedentary invertebrates are more likely to develop resilience to environmental heterogeneity because they cannot escape it, whereas motile dispersers can select wetlands with suitable environmental conditions. Hence one would expect a more homogenous distribution of assemblages among wetlands for sedentary invertebrates such as microcrustaceans and more differentiated assemblages for motile invertebrates. This is, however, based on the assumption that sedentary forms will develop resilience to environmental changes. The observations in this study suggest, on the contrary, that relatively sedentary microcrustaceans have not developed full resilience to human disturbances, as inferred by their significant association with levels of habitat transformation among differentially impacted wetlands. A similar finding was reported by Euliss and Mushet (1999) for 38 temporary wetlands differentially affected by cropland agriculture in the Prairie Pothole Region of North Dakota, USA. Two studies elsewhere (Mahoney *et al*. 1990: South Carolina, USA; and Bagella *et al*. 2010: Sardinia, western Mediterranean) did not however report any significant association between microcrustacean assemblage composition and agricultural land use. Thus, results appear to vary considerably among regions and it does not necessarily appear that the significant association between microcrustaceans and habitat transformation, as established in this study, can be extrapolated to other areas.

The smaller sample size of the microcrustacean dataset (relative to that for macroinvertebrates) imposes lower statistical power (ability to detect significant differences) for tests conducted using this dataset. The highly significant relationship reported above (with “Natural 100 m”) is therefore convincing given this lowered statistical power. Given the smaller dataset, one would expect larger amounts of variation to be explained by the habitat transformation variables in that there is less overall variation within a smaller dataset that covers considerably less geographical area than for the macroinvertebrate sampling. The fact that percentages of explained variation were similarly low among the microcrustacean and macroinvertebrate datasets indicates that neither assemblage showed particularly strong relationships with gradients of transformation in adjacent landscapes, albeit that some highly significant results were detected.

Family-level classification of microcrustaceans yielded significant associations with both “Natural 100 m” and “Natural 500 m” variables. The amount of explained variation was also higher for “Natural 500 m” using family-level data than using genus/species-level data. This is an interesting result given the enormous level of difficulty that was encountered in identifying microcrustacean taxa to genus and species. These results indicate that family-level data for microcrustaceans were slightly more useful than genus/species-level for detection of habitat transformation gradients and that the large amount of effort to obtain genera or species lists may not be justified. That said, the use of microcrustaceans in a "hands on" bioassessment index is hindered by their small size and finicky identification, even to family-level (e.g. family-level identification of ostracods may require dissection of the inner soft parts). Their use in bioassessment will often require identification in the laboratory rather than in the field, unless effective metrics are developed which only require coarse identification, for example to order-level (see comments in section 3.4.4).

On the whole, variation in microcrustacean assemblages explained by anthropogenic habitat transformation was low for both "Natural 100 m" and "Natural 500 m" variables at both levels of taxonomic resolution, despite being statistically significant in some cases. This explained variation (ranging between 1.77 and 5.91%) was considerably lower than that explained by the subsets of spatio-temporal covariables, which ranged between 24.12 and 35.77%, thus indicating that microcrustacean assemblages were far more affected by natural spatio-temporal factors than by anthropogenic factors at the scale of this study. A similar trend was observed for the macroinvertebrates in this study.

Relationships with predictor variables grouped in sets

The major sets of predictor variables (spatio-temporal, physico-chemical, hydro-morphometrical, biotope characteristics and habitat transformation) were all significantly related to macroinvertebrate assemblage composition, whilst all except hydro-morphometry and biotope characteristics significantly explained microcrustacean assemblages. Excluding habitat transformation, most of these variable sets have been reported elsewhere as significant correlates of invertebrate composition in temporary wetlands (e.g. Vanschoenwinkel *et al*. 2007, De Roeck 2008, Waterkeyn *et al*. 2008, Bilton *et al.* 2009; for a review see Williams 2006). The hierarchical order of importance among these variable sets is not universal across studies and thus the order reflected in this study may be different in another region or wetland type. For instance, spatio-temporal factors were important at the fairly large scale of this study, but the relative importance of these factors can be expected to decrease at smaller spatial scales. The minor role of hydro-morphometry (maximum depth and total surface area variables) as a determinant of invertebrate assemblage composition in this study is surprising as it is probably the most universally important predictor for temporary wetland assemblages among other studies (e.g. Wiggins *et al*. 1980, Brooks 2000, Eitam *et al*. 2004, De Roeck 2008, and for a review see Williams 2006). De Roeck (2008) reported that hydro-morphometry and physico-chemistry explained similar proportions of variation in macroinvertebrate assemblage composition for a set of 57 temporary wetlands sampled across a comparable area in the south-western Cape. This was not supported by data in the current study, where physico-chemistry played a much stronger role than hydro-morphometry in determining macroinvertebrate composition (explaining roughly four times that explained by hydro-morphometry). The specific reasons for this difference are unknown, but broadly speaking my results show that the importance of hydro-morphometry as a determinant of invertebrate assemblage composition in temporary wetlands is not necessarily universal. In this region other environmental factors appeared to be more important than hydro-morphometry in structuring invertebrate assemblages. The role of hydro-morphometrical variables in this study is further discussed in section 3.4.1.

Another interesting feature of Table 3.7 is the prominent role of physico-chemistry as a determinant of macroinvertebrate and microcrustacean composition, occurring second in the hierarchy after spatio-temporal factors in both cases. Although the percentages of explained variation were not particularly large (13.35% for macroinvertebrates and 23.99% for microcrustaceans), they were highly significant ($P < 0.001$) and signal a considerable influence of physico-chemical conditions on temporary wetland invertebrates. The role of physico-chemical conditions in shaping temporary wetland invertebrate assemblages has been questioned by Batzer *et al.* (2004), who found few significant relationships between physico-chemical variables and invertebrate taxa among 66 seasonal woodland ponds in Minnesota, USA. They argued that there is little quantitative evidence to support the hypothesis that temporary wetland chemistry has an important influence on the invertebrate biota. My results offer evidence on the contrary, indicating a clear physico-chemical influence on invertebrates (both macro and micro). It is acknowledged that the influence is not particularly strong for the macroinvertebrate data in the sense that explained variation is not high and hence predictive power of these models is low. However the influence is certainly important relative to other measured variables and is highly significant. My findings are supported by those of Bilton *et al.* (2009), who reached a similar conclusion for a set of 76 temporary wetlands in two regions of southern England. The range of variation in physico-chemistry among the predominantly pristine wetlands studied by Batzer *et al.* (2004) in Minnesota is likely to have been considerably less than observed in the current study, given the heterogeneous habitats presented in the south-western Cape and the range of human impacts incorporated into my sampling design. Bilton *et al*. (2009) reported high levels of variation among their study wetlands (often exceeding two orders of magnitude for individual variables), which is more in line with the situation observed in the south-western Cape. The weak relationships between macroinvertebrates and physico-chemistry are therefore potentially an artefact of the more homogeneous landscape presented in Minnesota relative to that of the current study and Bilton *et al.* (2009).

The set of habitat transformation variables (“Natural 100 m” and “Natural 500 m”) explained relatively small proportions of the variation in both macroinvertebrate and microcrustacean data in relation to spatio-temporal and physico-chemical factors. For the macroinvertebrate dataset, habitat transformation was the least important factor, as hypothesized, but was still statistically significant. This indicates that the cumulative influence of the two variables as a group, which is essentially the full effect of habitat transformation over 500 m, had a small but statistically meaningful impact on macroinvertebrates. Habitat transformation appeared to play a slightly more important role in determining microcrustacean assemblage variation (explaining 8.53%) and its contribution was highly significant. Although explaining more variation than hydro-morphometry, habitat transformation still explained roughly three times less variation than the physico-chemical variables, thus fitting broadly in line with my hypothesis that habitat transformation effects would be low in comparison to other groups of variables. It is difficult to say whether the macroinvertebrate or microcrustacean fauna was comparatively more influenced by habitat transformation, due to differences in sample size (hence statistical power) and spatial area covered among the two datasets. A consistent pattern among datasets is that the habitat transformation variables (grouped as a set) were significantly associated with invertebrate assemblages in wetlands, but these effects appear to be far outweighed by spatio-temporal and physico-chemical effects.

It is important to note that the results discussed in this section do not address the unique contribution of predictors in that the statistical variation explained by each predictor set cannot be attributed purely to that set. Instead, some of the variation is due to interactive effects among the variable sets. For example, a certain proportion of the variation explained by the spatio-temporal variables is likely to be due to the effects of physico-chemical variables that change over space and/or time, rather than being purely spatio-temporal effects. Only results from the variation partitioning procedure, discussed in the next section, can address the unique and overlapping effects of variables. The procedure was therefore restricted to three sets of variables in the current study. Although one can theoretically partition among more than three matrices (see Økland 2003), computation becomes very complex and was not attempted in this study.

Variation partitioning

The partitioning procedure of Anderson and Gribble (1998), as modified for use in this study, proved to be a very useful tool for distinguishing the unique and shared contributions of spatio-temporal, environmental and habitat transformation factors in explaining variation in both macroinvertebrate and microcrustacean assemblages. The variables measured in this study explained roughly half (50.87%) of the total variation in macroinvertebrate assemblage composition among wetlands (Fig. 3.2). The remaining 49.13% of unexplained variation can be attributed either to factors which were not measured in this study or to stochastic factors (nondeterministic fluctuations) that cannot be measured (Borcard *et al.* 1992). Due to the scale of the study, it was not logistically feasible to record detailed environmental information at each wetland. Therefore it is not surprising that a considerable amount of the macroinvertebrate variation could not be explained by the set of variables measured in this study. This amount of unexplained variation is comparable to that found in other ecological studies that have incorporated variation partitioning procedures (see Borcard *et al*. 1992 for case studies). Most of the total variation in microcrustacean assemblage composition among wetlands was explained by the explanatory variables measured in this study (80.82%, Fig. 3.3). The larger proportion of explained variation than observed for the macroinvertebrate dataset is expected to be partly due the smaller number of samples (slightly less than half the number of macroinvertebrate samples) and smaller spatial scale covered by the microcrustacean dataset.

Two broad findings of interest were revealed by the variation partitioning procedure. The first was that natural variation in environmental factors had a considerable influence on aquatic invertebrate assemblages in these temporary wetlands. This can be said for pure environmental effects (independent of space, time or habitat transformation) and those which were associated with changes in space and/or time (i.e. spatio-temporal x environmental). Although environmental conditions were the major determinant of assemblage composition, for macroinvertebrates this explained variation was fairly low (~35.4%, sum of segments “c” and “d” in Fig. 3.2). This indicates that, given a prescribed set of environmental conditions at a wetland, the ability to predict the macroinvertebrate composition for that wetland would be poor (due to considerably uncertainty). As mentioned above, the remaining unexplained variation can be partly attributed to factors that were not measured in this study and partly to stochastic fluctuation in macroinvertebrate assemblages which cannot be measured. Although the relative influence of these two factors cannot be determined for this study, it is likely that a certain amount of the unexplained variation can be attributed to resilience to environmental variation on the part of the macroinvertebrate fauna. This does not detract from the fact that natural environmental heterogeneity across the study region had an important and highly significant influence on macroinvertebrate assemblages. Percentage variation in the microcrustacean dataset explained by natural environmental factors was higher (~57.1%, sum of segments "c" and "d" in Fig. 3.3) and indicates better predictive ability of microcrustacean fauna using environmental conditions and also less resilience to environmental variation.

The hypothesis forwarded by Batzer *et al.* (2004) that temporary wetland macroinvertebrates are unresponsive to natural variations in environmental factors, due to their being resilient habitat generalists, is not supported by the data in this study. That said, the reasonably high amount of unexplained variation does indicate a degree of resilience of macroinvertebrates to natural environmental variation. It is likely that the explained component of macroinvertebrate variation in this study was due to pronounced gradients that existed within the environmental data, whilst taxa probably displayed more resilience to weak or moderate environmental gradients. The large amount of explained variation in microcrustacean assemblages due to environmental factors indicates a clear structuring of these assemblages along environmental gradients and does not seem to offer evidence that these taxa are particularly resilient to the effects of natural environmental variation. This is in line with the findings discussed in section 3.4.1, which indicated that microcrustaceans were not completely resilient to human-induced alteration of adjacent habitats. Their sedentary lifestyle requires that microcrustaceans surviving in temporary wetlands must be able to adapt to changes in environmental conditions, whether these changes occur naturally or are human-induced. Evidence from this study suggests that they are structured quite markedly by natural environmental gradients and may not be as resilient to environmental change as suggested in the literature.

A second broad pattern of interest arising from the variation partitioning process is the minor role that human habitat transformation seems to play in determining invertebrate assemblage composition in relation to the influence of natural factors (spatio-temporal and environmental). Two similar investigations into the relative influence of natural *versus* anthropogenic factors in structuring invertebrate assemblages have been conducted elsewhere on permanent wetlands (Tangen *et al.* 2003, Trigal *et al.* 2007), with contrasting results. Thus, drawing on the limited number of studies for wetlands, the ratio of natural to human influence would appear to vary regionally and probably depends on important factors such as the natural heterogeneity of soils and vegetation across the region, as well as temporal heterogeneity of conditions experienced by invertebrates for the given wetland type. The results of Tangen *et al.* (2003) and Trigal *et al.* (2007) are further discussed in section 3.4.4, including their implications for bioassessment of wetlands using multimetric indices.

In the current study, the percentage of explained variation contributed from habitat transformation is represented by small segments of the pie charts (Figs 3.2 and 3.3). The cumulative amount of explained variation due to habitat transformation (controlling for spatio-temporal factors as covariables) was relatively low for both the macroinvertebrate and microcrustacean data (2.73% and 8.39% respectively: segments “b” + “e” in both figures), but these contributions were statistically significant and thus provide some evidence of a meaningful effect on assemblage composition. The significant (albeit relatively weak) signal from the effects of habitat transformation on invertebrates, over and above a large amount of background “noise” from environmental and spatio-temporal variation, suggests that a reduction in the amount of natural variation (data noise) would more clearly elucidate effects of transformation on invertebrates. This is likely to be indicative of a scale effect in that at the broad spatial scale covered in this study effects of habitat transformation are overridden by effects of natural variation, whereas at smaller spatial scales there is expected to be less natural variation interfering with habitat transformation patterns. Ultimately this leads one to hypothesize that invertebrate assemblages in temporary wetlands of the region are considerably more influenced by natural than human-induced variation at broad spatial scales (when natural variation is high). At smaller spatial scales (with reduced natural variation) one expects the relative influence of habitat transformation to increase, possibly matching or exceeding that of natural variation. This hypothesis will be further addressed in the next chapter.

Relative importance of individual environmental variables

When considering the contribution of each environmental variable to explained variation in the invertebrate datasets, it was clear that the “Natural 100 m” and “Natural 500 m” variables played a relatively unimportant role in comparison to other environmental factors. These two habitat transformation variables did not feature in either of the step-wise models and appeared only once in the all-subsets models (“Natural 500 m” was included in one of the macroinvertebrate models, Table 3.9). Instead, other environmental factors were more important. For instance, pH was selected in both step-wise models and was the only variable included in all of the most parsimonious AICc-selected models explaining macroinvertebrate and microcrustacean assemblage composition. pH has been widely reported as a key factor influencing invertebrate assemblage composition in a variety of aquatic ecosystems (e.g. rivers: Feldman and Connor 1992; lakes: Jackson and Harvey 1993; wetlands: Harrison 1962, Gardiner 1988, Woodcock *et al.* 2005, Becerra Jurado *et al.* 2009). Because pH is intimately linked to soil properties, a diversity or patchiness of soil types across a study area is often coupled with a wide variation in pH (Rebelo *et al.* 2006). This is especially true for the broad area covered in this study, which is known for its high turnover of soil types over relatively small spatial scales (Rebelo *et al.* 2006) and hence one observes a corresponding variety of aquatic chemistries (Silberbauer and King 1991b, and see chapter 2 of this thesis). Compounding this effect, the diversity of natural fynbos vegetation in the region differentially affects soil pH depending on whether or not plant species release acidic polyphenols (Gardiner 1988, Raubenheimer and Day 1991; see chapter 2, section 2.4.1 for more detailed discussion). This natural diversity of environmental factors, as described for pH, appears to be coupled with the composition of invertebrate assemblages and seems to largely override effects of habitat transformation on invertebrates.

Conductivity appeared to also be an important predictor of macroinvertebrate composition in these wetlands, as hypothesized (section 3.1.5). This variable was included in all but one of the ten most parsimonious models for macroinvertebrates, but did not feature in any of the microcrustacean models. It is likely that both macroinvertebrates and microcrustaceans are affected by conductivity fluctuations if they are sufficiently pronounced, because ionic changes in water are known to cause fundamental physiological effects on most aquatic organisms (Williams 1998, Williams 2006). The discrepancy in the importance of conductivity among the macroinvertebrate and microcrustacean datasets is likely to be an artefact of differences in the amount of fluctuation for this variable in the full macroinvertebrate dataset *versus* the subset of sites analysed for microcrustaceans. The larger sample size of the macroinvertebrate dataset is more reliable in this regard, and indicates an important influence of conductivity, despite the low amount of explained variation (2.33%) that was attributed to this variable in the step-wise regression output (Table 3.8).

It should be noted that environmental effects on invertebrates in this study were an accumulation of small contributions from numerous individual variables, as evidenced by the low and relatively similar amounts of variation explained by predictors in the two step-wise models (Tables 3.8 and 3.10). None of the individual variables appeared to exert a particularly dominant effect on invertebrates and instead effects were more gradual. One implication is that invertebrate samples collected from any given temporary wetland in the region may allow one to broadly classify the wetland in terms of its total environmental conditions (although even this will be fairly unreliable), but the ability of such models to predict levels of individual environmental variables will be too low to make any confident predictions.

The role of maximum depth and total surface area as predictors of invertebrate composition in these temporary wetlands once again (see comments in section 3.4.1) appears to be relatively unimportant when considering that they were expected to be the major determinants of invertebrate assemblage composition in these temporary wetlands. Maximum depth featured in two of the ten best AICc models for macroinvertebrates and total surface area and maximum depth each featured once among the ten best AICc models for microcrustaceans. Maximum depth was included as a significant variable in the step-wise model for macroinvertebrates, but explained only a small proportion of the variation (1.48%). Neither maximum depth nor surface area was included in the step-wise model for microcrustaceans, suggesting that these variables played no significant role in structuring microcrustacean assemblages. These results reiterate the dominance of other environmental variables (mostly physico-chemical) relative to the hydro-morphometrical variables as predictors of invertebrate assemblage composition in this study. Assuming maximum depth as a reasonable proxy for hydroperiod (*sensu* De Roeck 2008, but see comments by Brooks and Hayashi 2002), this study corroborates the findings of other authors who have also found a lack of evidence for hydroperiod as an environmental control of biotic assemblages in temporary wetlands (Wissinger *et al.* 1999, Batzer *et al.* 2004). The literature is inconclusive however and various studies report hydroperiod to be the key determinant of temporary wetland invertebrate assemblages in other regions (Wiggins *et al.* 1980, Mahoney *et al.* 1990, Brose 2003, Eitam *et al.* 2004, Bagella *et al.* 2010). Results presented in this study were surprising given that De Roeck (2008) found hydro-morphometry to be a key determinant of invertebrate assemblage structure in temporary wetlands of the same region. This topic is not a focus in the current study, but given the contrasting results between the current study and those of De Roeck (2008) it would appear that further work is required to clarify the role of depth and surface area as constraining factors for invertebrate fauna in temporary wetlands of the region. As this study focuses on habitat transformation effects and not hydro-morphometry, the latter was described crudely and further studies measuring actual time periods of inundation at each wetland would better account for the variable “hydroperiod” *per se*.

Multidimensional Scaling (MDS) plots

The MDS approach to visualising invertebrate assemblage composition among the wetlands of this study was useful for elucidating broad-scale patterns such as the distinction of clusters of wetlands based on the geographic region in which they occurred. This was the only pattern that could be clearly visualized in the multivariate data, and even for this there was considerable overlap of assemblages among the three regions for the macroinvertebrate data (see Fig. 3.4d). However, the inability to incorporate covariables into these plots means one cannot visualise smaller-scale effects of habitat transformation having accounted for covariable effects. The use of partial ordination models (in this case dbRDA) was found to be far more useful for detecting effects of anthropogenic disturbance in this study and was able to elucidate fairly subtle associations between habitat transformation and aquatic invertebrates. Irz *et al.* (2008) point out that most bioassessment studies which aim to establish relationships between the biota and measures of human disturbance fail to quantitatively incorporate effects of natural variation into linear models before determining effects of anthropogenic disturbance. The importance of taking into account natural variation before assessing anthropogenic effects is demonstrated by the detection of habitat transformation patterns (though subtle) in this study using partial ordination (dbRDA), whilst straightforward MDS did not reveal any habitat transformation patterns.

3.4.2. Taxon richness and diversity

Macroinvertebrate richness and diversity showed no significant associations with overall transformation of habitat within 100 and 500 m of wetlands. Thus, although transformation had some influence on macroinvertebrate assemblage composition, no effects on richness or diversity were detected in this study. Virtually no other empirical studies have addressed patterns of macroinvertebrate species richness/diversity in relation to terrestrial habitat transformation around temporary wetlands. In the only comparable study, Brose (2003) found no influence of agricultural practices on richness or diversity of semi-aquatic carabid beetle assemblages associated with temporary wetlands in north-eastern Germany. My results and those of Brose (2003) present evidence for a lack of effect of habitat transformation on temporary wetland macroinvertebrate richness or diversity. This is in line with my general hypothesis that the fauna is well adapted to these naturally variable systems and neither composition nor richness/diversity measures were expected to be strongly affected by changes in wetlands that were induced by human activities in the nearby landscape. Conversion of landscapes around wetlands was not strongly linked to changes in environmental conditions in wetlands (see chapter 2) and thus probably does not present a major disturbance to macroinvertebrates, particularly given the naturally high levels of environmental fluctuation intrinsic to temporary wetland environments (Williams 2006).

Certain measures of microcrustacean richness (number of taxa and Margalef's index) and diversity (Shannon index) were negatively associated with the natural vegetation cover within 100 m of wetlands, but showed no relationships at the 500 m scale. Stated inversely, microcrustacean richness and diversity showed positive association with increasing transformation of adjacent habitats. This refutes my expectation of a resilient fauna and is surprising given that other studies in temporary wetlands have found either no effect (Mahoney *et al.* 1990, Bagella *et al.* 2010) or a negative effect (Euliss and Mushet 1999, Lahr *et al.* 2000, Angeler and Moreno 2007) of anthropogenic disturbance in the landscape on microcrustacean richness and/or diversity. The specific causal mechanisms for this positive relationship are difficult to elucidate given the correlative nature of this study. The trend could be governed by the most generally accepted model in disturbance theory, that of the "intermediate-disturbance hypothesis" (Connell 1978, see review by Kondoh 2001), which may explain the increase in number of taxa and diversity with a moderate amount of disturbance imposed on temporary wetlands by habitat transformation effects. However the fact that no other studies in temporary wetlands have reported such a trend suggests that further independent data needs to be collected to establish the consistency of this trend in the region. As with previous results related to assemblage composition, the microcrustaceans have once again displayed a clearer response to habitat transformation gradients than have the macroinvertebrates, further suggesting their lower resilience to habitat transformation activities.

3.4.3. Family-level tests for indicator taxa

Individual macroinvertebrate and microcrustacean families showed poor linear relationships with gradients of habitat transformation and scores from the human disturbance index. Although some of the relationships were significant, closer inspection of these patterns in the partial residual plots revealed that the P values were misleading and were either influenced by strong outliers or the amount of scatter between points was too high to uphold any confidence in the relationship. The only family that showed decent potential as an indicator taxon was the Daphniidae (Cladocera), which was negatively related to natural vegetation cover within 100 m. This taxon therefore tends to be positively associated with increasing transformation of the adjacent landscape. No other families besides this presented themselves as reliable indicator taxa. These results are in line with the relatively weak responses observed for macroinvertebrate and microcrustacean assemblage composition in relation to habitat transformation gradients (as discussed earlier). The data collected in this study indicate quite clearly that the use of individual invertebrate families in a biotic index of human disturbance for temporary wetlands of the region would not be feasible and would produce poor results. The comments on metrics in the following section are in most cases equally applicable to the poor patterns observed here for invertebrate families in relations to human disturbance gradients.

3.4.4. Testing potential metrics

The array of macroinvertebrate metrics tested in this study performed poorly and did not show potential for inclusion in a biotic index for assessment of human disturbance. Those metrics significantly related to habitat transformation or the human disturbance scores showed unreliable patterns, which either contained a large amount of scatter in the residual plots or were dominated by a few outliers. This resulted in the metrics having low confidence in terms of their ability to infer levels of human disturbance. The poor response of metrics to anthropogenic factors in this study reflects the relatively minor role of habitat transformation around wetlands in relation to the stronger effects of natural environmental and spatio-temporal factors as determinants of macroinvertebrate assemblage composition. This pattern has been demonstrated in earlier sections regarding assemblage composition. As pointed out by Irz *et al.* (2008), multimetric approaches to index development in lentic environments seldom quantitatively incorporate natural variability into the statistical analysis stage, other than the usual controls for the specific type of system sampled and the region in which it is sampled. This may often lead to erroneous decisions regarding which metrics are useful as indicators of anthropogenic impacts. For example, the Irz *et al.* (2008) study investigated the feasibility of a fish-based multimetric index to assess lake condition using a set of 112 lakes in the north-eastern USA. They showed that failing to incorporate natural factors (as covariables) into multimetric index construction led to the selection of two metrics (percentage of intolerant species and percentage of omnivorous species) that did not display a response to stressors when the environment was controlled for. Furthermore they identified a metric (percentage of diadromous species) which responded to the impact of agricultural land use only once natural variability was controlled for. They recommended that for broad surveys of regions with high heterogeneity, each metric should not only be regressed against each human disturbance variable (simple linear regression), but should instead be regressed against each human disturbance variable having partialled out the effects of relevant influential covariables (natural factors) using multiple linear regression. This approach has been adopted for testing metrics in the current study and is to my knowledge the first study to do so for wetlands.

No studies to date have investigated the use of aquatic macroinvertebrates for biotic indices in temporary wetlands, but there is a growing literature of such studies that have successfully developed macroinvertebrate indices in permanent wetlands (e.g. Burton *et al*. 1999, Hicks and Nedeau 2000, Kashian and Burton 2000, Chessman *et al*. 2002, Gernes and Helgen 2002, Uzarski *et al*. 2004, Boix *et al*. 2005, Davis *et al*. 2006, Solimini *et al*. 2008, Trigal *et al*. 2009). A commonality among all these studies is that they have developed indices which are applicable to a particular wetland type in a particular area (e.g. large depressional wetlands in Minnesota - Gernes and Helgen 2002, or flatland ponds of the North Iberian Plateau in Spain - Trigal *et al.* 2009), unlike the situation with regard to biotic indices in rivers, which are often applicable countrywide (e.g. SASS5 in South Africa: Dickens and Graham 2002; AUSRIVAS in Australia: Simpson and Norris 2000; RIVPACS in Britain: Wright *et al*. 1996). The natural (“least impaired”) state for a given wetland tends to vary considerably over small spatial scales due to environmental changes associated with the isolated hydrology of these discrete ecosystems (this is particularly so for small depressional wetlands). More connected river ecosystems, however, tend to display less spatial variation, which in turn generally allows for biotic indices in rivers to be more broadly applicable than for wetlands (Davis *et al.* 2006).

I argue that the spatial extent to which an invertebrate index can be applied is intimately linked to the inherent variability within the system being investigated. Less variable systems allow for more broadly applicable indices, more variable systems restrict indices to a specific area. This relates to one of the core findings of this study, that for a given scale, the strength of the invertebrate response pattern to anthropogenic activities was largely based on the strength of their response to natural variability. The ratio of these influences is expected to determine the effectiveness of invertebrates as indicators at a given scale; if the influence of natural variability on invertebrates largely outweighs the influence of anthropogenic-induced variability then the response signal from invertebrates is likely to be weak and an index will perform poorly, and *vice versa*. For example, the ratio of anthropogenic to natural influence on aquatic macroinvertebrates of permanent flatland ponds on the North Iberian Plateau in Spain was shown by Trigal *et al.* (2007) to be high (i.e. anthropogenic influence exceeded natural influence). They consequently decided to test a variety of macroinvertebrate metrics on an independent set of these ponds and successfully developed a multimetric index for their future ecological assessment (Trigal *et al.* 2009). Conversely, Tangen *et al.* (2003) investigated the feasibility of a macroinvertebrate index for ecological assessment of Prairie Pothole Region wetlands of North Dakota, USA, and found that the influence of natural variability on these macroinvertebrate assemblages far outweighed that of human-induced variability. They concluded that development of a biotic index for assessment of human impacts to these wetlands would not be viable. Although permanent, their study wetlands were found to be inherently variable and pronounced temporal fluctuations (including annual freezing over) indicated that these wetlands functioned more similarly to temporary wetlands than to relatively stable permanent ones. The results of Tangen *et al.* (2003) corroborate those of the current study, where high natural variability among temporary wetlands of the south-western Cape is also expected to hinder the establishment of an effective invertebrate index at the broad scale.

The proposed advantage of using metrics as summaries of assemblage composition is that they may elucidate patterns with human disturbance variables that might not be picked up when simply testing individual taxa as indicators (Helgen 2002, Teels and Adamus 2002). It was hoped that macroinvertebrate metrics would show better patterns of association with the human disturbance variables in this study, but this was not the case and the patterns were weak. Therefore one logically concludes that a multimetric index using macroinvertebrates to assess human disturbance around temporary wetlands in the region does not appear to be a feasible option, given that the metrics tested in this study cannot be used to infer (with any degree of reliability) levels of disturbance in or around wetlands. Three important caveats need to be appended to this statement. Firstly, a macroinvertebrate index is not recommended at the broad scale (i.e. the south-western Cape mediterranean-climate region). This is the practical scale at which such an index would be useful in that it would be broadly applicable and available for various users. At a smaller scale, for instance a single cluster of wetlands within a landscape, patterns between invertebrates and anthropogenic impacts may be easier to detect than at the broad scale because the amount of natural variation (noise) will be considerably reduced. Thus, biotic indices may be feasible at these smaller scales (this would require further investigation), but the practicality of such indices will be very low because they cannot be applied broadly enough.

Secondly, human disturbance in this study is proxied by levels of adjacent habitat transformation and a rapid-assessment index of disturbance based on several indicators scored within wetlands and in the adjacent landscape (< 500 m). There may be other variables that provide better proxies of human impacts on these wetlands and which in turn may relate better to the invertebrate metrics. For instance, one could have made a more detailed assessment of each wetland using an already-existing assessment method, such as the South African WET-Health index (based on wetland hydrology, geomorphology and vegetation, Macfarlane *et al*. 2008) and then regressed invertebrate metrics against these index scores. The WET-Health approach was however still being developed at the time when this study was being designed and furthermore it is an in-depth and time-consuming procedure. Due to the large scale of this study and limited time available for sampling at each wetland, the use of such a comprehensive index was not logistically feasible. The approach used in this study, whereby metrics and individual taxa were related to levels of habitat degradation around wetlands, as well as a rapid-assessment index of human disturbance in and around wetlands, is consistent with the majority of studies in the literature which have aimed to test feasibility of biotic indices in wetlands (e.g. Gernes and Helgen 2002, Tangen *et al*. 2003, Uzarski *et al*. 2004, Solimini *et al*. 2008, Trigal *et al*. 2009).

Thirdly, the gradients of habitat transformation and human disturbance scores against which metrics were regressed in this study, do not necessarily constitute gradients of "disturbance" *per se* for invertebrates. Rather these are simply measures used to represent or proxy disturbances to wetlands, but may not actually cause considerable disturbance from the perspective of invertebrates. To illustrate this point, consider that overall transformation of habitat around wetlands was shown in chapter 2 to be associated with physico-chemical conditions in the wetlands (albeit relatively weakly), but these physico-chemical alterations might not be considered a disturbance at all from the point of view of the "average" temporary wetland invertebrate. This is particularly so if members of the fauna are naturally resilient to mild or even moderate environmental fluctuations, as hypothesized at the outset of this study.

Only five microcrustacean metrics were significantly related to habitat transformation or the human disturbance scores (no metrics were significantly related to the predictor variable “Natural 500 m”) and these relationships reported slightly larger standardized regression slopes (β values) and partial r^2 values than for the macroinvertebrate metrics (Table 3.14). Although confidence in these microcrustacean models was better than for macroinvertebrates, the partial r^2 values were still low (maximum was 0.163), indicating that the power to make inferences regarding human disturbance was low. The two metrics which performed best were “total number of taxa” and “total number of ostracod individuals”. With regards to the latter metric, Külköylüoglu (2004) recommended the use of ostracods as bioindicators of human disturbance across a variety of different aquatic habitats in the Bolu region of Turkey. However their patterns were found using species-level identification of ostracods, which is well beyond the scope of a user-friendly bioassessment program in South Africa (and probably most countries).

Two studies could be sourced from the available literature that have successfully developed indices for assessment of wetland ecological condition using microcrustaceans (Boix *et al*. 2005 for flatland ponds of the north-east Iberian Peninsula, and Lougheed and Chow-Fraser 2002 for marshes of the Laurentian Great Lakes basin). Both studies involve permanent wetlands and once again require identification of taxa to genus- or species-level, which means that identification in the field would be inadequate. Such indices would not bode well for use in countries such as South Africa where financial resources and taxonomic knowledge are generally insufficient to support broad use of such assessment methods. The microcrustacean metrics developed and tested in this study are in no way considered an extensive set of metrics, but given the complete lack of pre-existing metrics in the literature only a crude set could be tested in this study. This simple set of metrics was based on the premise that a user-friendly bioassessment protocol should not entail identification of microcrustaceans beyond the level of Copepoda, Cladocera or Ostracoda, as this would require taking samples back to the laboratory for identification. In summary to the testing of microcrustacean metrics, the fact that only seven significant relationships were detected in total, and even these had low explanatory power (r^2), one cannot recommend the use of this fauna in multimetric indices for inferring human disturbance of temporary wetlands in the region. The sample size for testing microcrustacean metrics (n = 41) was however smaller than for macroinvertebrates (n = 90) and further studies testing the use of microcrustacean metrics in temporary wetlands might help to clarify this recommendation.

A weakness in common with most of the macroinvertebrate and microcrustacean metric and family-level relationships presented in this study is the tendency to provide information for making inferences about disturbance at one end of the regression plot only, whereas from the results of other studies that have established useful invertebrate metrics one can usually infer wetland condition at both ends of regression plots. To illustrate this point, hypothetical scenarios are produced in the figure below (Fig. 3.10). Scenario A depicts a useful metric with inferential power at both ends of the spectrum of a given human disturbance variable. Results for studies that have successfully developed indices using a multimetric approach, for example Gernes and Helgen (2002), are more aligned with the scenario A model than scenario B, the latter depicting the kind of results seen for even the most promising metrics or indicator taxa in this study. Figure 3.10B represents a positive correlation scenario, but reciprocal patterns for negative correlations were also observed in the results of this study (i.e. inferential power at the low end of the disturbance spectrum only). For example, consider that a given metric's numerical abundance (e.g. for macroinvertebrates: the "% Omnivores" metric, Fig. 3.8n) gives information on a wetland's disturbance state and the metric tends to score high in heavily impacted sites and low for minimally impacted sites. After gathering a sweep net sample from a wetland with an unknown disturbance history, it is established that this metric scores high for the sample. Given that this metric has shown a scenario A type of pattern when tested in other wetlands of the same type (and region), we can infer quite reliably that the wetland in question is likely to be in a disturbed state. Even the best metrics in the current study, however, conformed to a scenario B type of model and in this case only if the given metric scores low for a sample does it suggest one can infer the level of disturbance for the given wetland (minimal disturbance in this case), whilst a high score would present ambiguous information.

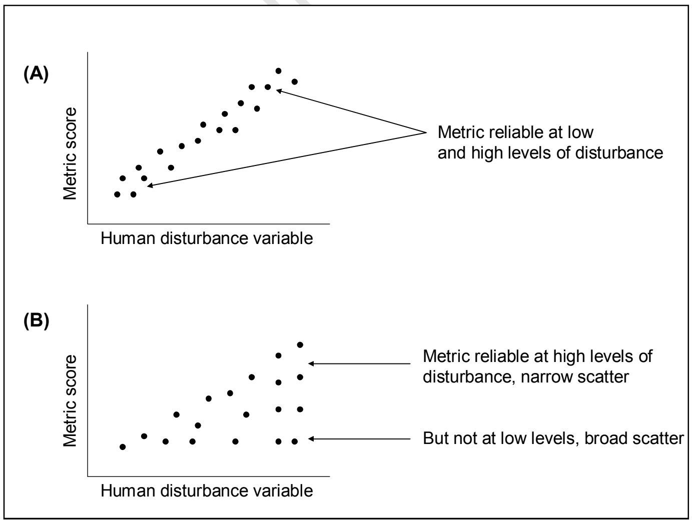


Figure 3.10. Hypothetical scenarios illustrating: A) regression plot with inferential power at both ends of the disturbance spectrum; B) regression plot with inferential power at one end of the disturbance spectrum only (in this case the upper end).

3.4.5. Conclusions

The role of habitat transformation in shaping the invertebrate assemblages of temporary wetlands in the region appears to be minor in comparison to the structuring effect of natural spatio-temporal and environmental factors. This small contribution from habitat transformation was however statistically significant in several cases (or at least very close to the significance level), indicating that human activities in the landscape do play a small but influential role. The scale of this study was fairly large, covering most of the south-western Cape coastal mediterranean-climate region of South Africa. At this scale, heterogeneity in environmental conditions was pronounced and explained much of the variation in invertebrate assemblage composition. The fact that several significant signals from habitat transformation were still detected over and above considerable “noise” (natural variation) in the data indicates that the effects on invertebrate assemblages of transforming natural habitats around temporary wetlands are not negligible. The evidence presented in this study ultimately leads to the hypothesis that invertebrate assemblages in temporary wetlands of the region are considerably more influenced by natural than by human-induced variation at broad spatial scales (when natural variation is high) and thus at smaller spatial scales (with reduced natural variation) one expects the relative influence of habitat transformation to increase, possibly matching or exceeding that of natural variation. This hypothesis will be addressed further in the next chapter. The patterns observed for assemblage composition, individual families and metrics do not lend themselves to creation of an invertebrate index of human disturbance for temporary wetlands of the region. This statement applies to both macroinvertebrates and microcrustaceans. The studies undertaken in the current and previous chapter have employed a broad-scale once-off sampling approach. The next chapter investigates the influence of habitat transformation on temporary wetland ecosystems (both environmental conditions and invertebrate assemblages) using a small-scale comparative study, with repeated sampling over two wet seasons.

CHAPTER 4

EFFECTS OF ALIEN VEGETATION INVASION ON TEMPORARY WETLANDS: A CASE STUDY OF THE KENILWORTH RACECOURSE CONSERVATION AREA, CAPE TOWN (SOUTH AFRICA)

ABSTRACT

According to the broad-scale studies of previous chapters in this thesis, the loss of indigenous vegetation habitat around temporary wetlands of the south-western Cape is weakly associated with environmental conditions and invertebrate assemblages in these wetlands and patterns appeared to be masked by the strong influence of natural environmental heterogeneity at this scale. This chapter investigates landscape-scale relationships between habitat transformation, in-wetland environmental conditions (physico-chemistry and biotope characteristics) and invertebrate assemblages (macroinvertebrates and microcrustaceans) across a gradient of alien vegetation invasion (primarily *Acacia saligna* and *Pennisetum clandestinum*) for a set of 12 temporary wetlands that were repeatedly sampled over two wet seasons (2008 and 2009) at the Kenilworth Racecourse Conservation Area, Cape Town (South Africa). The hypothesis is tested that the loss of indigenous vegetation around wetlands (due to alien vegetation invasion) is associated with changes in physico-chemical conditions in wetlands, which in turn mediates changes in the composition of invertebrate assemblages. With reduced influence from natural variation, it was further hypothesized that environmental and invertebrate response patterns to habitat transformation would be clearer at the fine scale of this study than observed for the broad-scale studies in previous chapters. Univariate linear regression models indicated that the replacement of indigenous "fynbos" habitat around wetlands with alien vegetation was strongly associated ($P < 0.001$, partial r^2 values ranging between ~30 - 55%) with increases in wetland pH, % cover of the macroalga *Cladophora* sp., % cover of the open water biotope, phosphate concentrations, % cover of the complex vegetation biotope and a decrease in the concentration of humic substances in wetlands. A variation partitioning procedure *sensu* Anderson and Gribble (2002) revealed that these environmental changes appeared to mediate highly significant ($P < 0.001$) changes in macroinvertebrate and microcrustacean assemblage composition, however the percentages of explained variation in response matrices according to multivariate regression models indicated weak-to-moderate strength relationships between invertebrates and habitat transformation (macroinvertebrates: 12.69%; microcrustaceans: 20.31%). As expected, environmental and invertebrate response patterns to habitat transformation were considerably stronger in this study than for the broad-scale studies (previous chapters). Although both study hypotheses were upheld, natural environmental heterogeneity among wetlands was nonetheless high, despite the small spatial scale of this study, and this natural variation appeared to exert the majority of influence on invertebrate assemblage composition in wetlands. Some implications of these findings for buffer size requirements around temporary wetlands and for biotic index development are discussed.

4.1. INTRODUCTION

4.1.1. Background and topic of investigation

The human-induced transformation of landscapes in which temporary wetlands are embedded is a conspicuous element of many lowland regions that support burgeoning human populations. The influence of this differential transformation of habitat around wetlands on their environmental conditions and invertebrate assemblages has thus far been investigated in this thesis across the south-western Cape mediterranean-climate region of South Africa. At this broad scale it appears that anthropogenic activities in the landscape (proxied by remaining levels of natural vegetation) may have a significant influence on physico-chemical conditions and invertebrate assemblage composition in temporary wetlands, but this influence is apparently far outweighed by that of natural variation (spatio-temporal factors, environmental factors, and their interaction). The need to observe ecological phenomena at various spatial and temporal scales has become well recognised since the seminal review paper by Levin (1992), who argued that the problem of pattern and scale is the central conceptual problem in all ecology. To quote Levin: "…there is no single natural scale at which ecological phenomena should be studied". With this in mind, it would appear that invertebrate response patterns to habitat transformation in adjacent landscapes, which were not clearly elucidated at a broad scale in previous chapters, may become clearer at a fine scale where natural heterogeneity is reduced.

Broad-scale patterns (across the south-western Cape region) were investigated in previous chapters because this was considered a practical scale for which a potential biotic index for these wetlands would be implemented. However, a large amount of "noise" in the data (natural variation) appeared to interfere with the detection of human-induced patterns at the broad scale and I hypothesized that the ratio of influence for habitat transformation relative to natural factors is likely to increase at a fine scale, due mostly to a reduction in natural variation at this scale. This broad hypothesis forms the backbone of the current chapter in which a cluster of 12 temporary wetlands, covering a spatial extent of approximately 1km^2, are investigated across a gradient of habitat transformation due largely to an invasion by alien vegetation. The reduced spatial extent and number of wetlands sampled compared to the work presented in previous chapters permitted an increase in replication through time and sites were visited on five repeated occasions over two wet seasons.

4.1.2. Habitat transformation in a south-western Cape context

Isolated depression wetlands in coastal lowland areas were historically embedded within a matrix of sclerophyllous heathland vegetation known broadly as fynbos. However over the last century this indigenous vegetation has largely been replaced by agriculture, urban development and alien invasive plants (Rouget *et al.* 2003). Prior to the work described in this thesis, it had not been established whether this transformation of the natural landscape has significantly altered the ecological character of those remaining temporary wetlands that have not been completely destroyed by infilling. This study investigates a cluster of wetlands occurring within a type of fynbos called Sand fynbos (*sensu* Rebelo *et al.* 2006), which was once widespread on highly leached oligotrophic sands of the south-western Cape lowlands, but the extent of which has been drastically reduced by human-induced habitat transformation (particularly urban development and alien invasive vegetation).

The specific type of habitat transformation addressed in this study is alien invasive vegetation, identified by Rouget *et al.* (2003) as the joint second-largest agent of habitat transformation (approximately equal to urban area, both preceded by agriculture) in the Cape Floristic Region (of which the south-western Cape forms a considerable fraction). Invasive alien vegetation, particularly in the form of woody *Acacia* spp. shrubs of Australian origin, is a serious threat to remaining indigenous vegetation habitat in the region. Using various modelling approaches, Rouget *et al.* (2003) predicted that between 27.2 and 30% of remaining untransformed habitat in the Cape Floristic Region is likely to be invaded by woody alien plants over the next 20 years (i.e. from the time of their study). Although a number of studies have addressed potential impacts of this invasion on the quantity of water available to aquatic ecosystems in South Africa (Enright 2000, Le Maitre *et al.* 2000, Görgens and van Wilgen 2004, Richardson and van Wilgen 2004), none have quantified impacts on surface water quality (e.g. physico-chemical conditions). The significant association between levels of alien vegetation and physico-chemical conditions in south-western Cape temporary wetlands, as demonstrated in chapter 2 (see Table 2.2), indicates that alterations to South African water resources associated with alien vegetation may not only be water quantity related. If indeed alien vegetation alters physico-chemical conditions in aquatic ecosystems (such as wetlands), this presents a possible mechanism by which the aquatic biota (such as invertebrates) may be altered. A strong relationship between physico-chemical conditions and invertebrate assemblage composition has been demonstrated in chapter 3 (see Table 3.7), suggesting that if alien vegetation stands around wetlands were to cause a sufficient shift in these conditions (from the natural state) then this could have knock-on effects on invertebrate assemblages. Data collected at the broad scale in chapter 3 did not however indicate a significant relationship between macroinvertebrate assemblages and levels of alien vegetation (see Table 3.5, microcrustaceans were not assessed in relation to each type of habitat transformation in chapter 3). It could be that physico-chemical changes associated with alien invasion were not sufficient at the broad scale to mediate a significant overall structuring effect on macroinvertebrate assemblages. This dynamic will be explored further in the current chapter using a small-scale, focussed comparative study of a set of naturally comparable wetlands that have recently become surrounded by differing levels of alien vegetation.

4.1.3. Mechanisms of ecosystem effects in transformed environments

An important caveat for any study assessing the effects of habitat transformation on aquatic ecosystems is that changes in ecosystem properties (such as physico-chemical conditions) associated with transforming the natural habitat around that waterbody could be due to the loss of indigenous vegetation that has been replaced, rather than the effect of the actual type of habitat transformation *per se* (e.g. alien vegetation). Realistically there is likely to be interplay of influence among the two factors (i.e. loss of indigenous vegetation *versus* the corresponding replacement by a new habitat type), the ultimate balance of which determines overall changes in measured ecosystem variables (environmental or biotic). This caveat applies to field-based studies of habitat transformation in general, in that detected effects on aquatic ecosystems may be due to the effects of the actual agent of transformation (e.g. urban development or agriculture) or due to changes associated with losing natural vegetation (e.g. the natural vegetation might have imparted a particular physico-chemical signature to the soil properties). To illustrate the above-mentioned interplay in the context of the current study, firstly consider the potential effects of replacing Sand fynbos with alien vegetation in the south-western Cape. Sand fynbos is dominated by sclerophyllous shrubs, which contain high levels of polyphenolic compounds (collectively known as tannins) in their tissues as an anti-herbivory adaptation for an evergreen lifestyle (Rebelo *et al.* 2006). This is a characteristic common to many vegetation types within the Fynbos Biome (though not all types, see Rebelo *et al.* 2006). Raubenheimer and Day (1991) experimentally demonstrated for two species of Sand fynbos plants that the leaching of these polyphenolic compounds into flasks filled with water caused an increase in the humic content of the water and a corresponding drop in pH levels. These authors and those of field-based studies (Gardiner 1988, Midgley and Schafer 1992) have reached the same conclusion that the darkly-stained waters of south-western Cape waterbodies surrounded by indigenous fynbos are caused by the leaching of humic compounds into these aquatic systems from the surrounding flora. It would therefore be reasonable to expect that the loss of fynbos vegetation around wetlands due to the human transformation of habitat will be associated with a decrease in the allochthonous input of humic compounds to these wetlands. This may have consequences for a variety of biota previously adapted to the blackwater state (Gardiner 1988). For instance, pH has often been cited as an important physico-chemical factor regulating biotic communities in aquatic environments (Harrison 1962, Feldman and Connor 1992, Jackson and Harvey 1993, Woodcock *et al.* 2005) and thus an increase in pH associated with a loss of fynbos vegetation around wetlands may well cause a significant change in wetland invertebrate assemblage composition.

Secondly, consider the potential impacts of alien invasive vegetation itself. The predominant invaders of Sand fynbos are various woody *Acacia* spp. shrubs (introduced from Australia) and various pioneer grasses (Milton 2004, Musil *et al.* 2005). Whilst acacias can directly outcompete established fynbos communities, grasses tend to invade after a disturbance has already cleared fynbos shrubs (Yelenik *et al.* 2004, Musil *et al.* 2005, Gaertner *et al.* 2011). Invasive pioneer grasses are often associated with acacias as nitrophilous opportunists, which make use of the increased soil nitrogen (N) availability caused by N_2-fixing acacias (Yelenik *et al.* 2004). Whilst the ecosystem effects of invasive grasses in the region are scarcely documented (but see Gaertner *et al.* 2011), various studies have documented the ability of invasive acacias to alter ecosystem structure and function in the south-western Cape (e.g. Witkowski and Mitchell 1987, Witkowski 1991, Musil 1993, Yelenik *et al*. 2004, Richardson and van Wilgen 2004). N_2-fixing acacias have high levels of tissue N, unlike the sclerophyllous fynbos it replaces. Invading acacias form dense stands which drop a large amount of high-N leaf litter into the previously nutrient-deficient soils, causing a shift in soil N-cycling from a low to high N-cycling regime, which then affects various other processes and biotic components of the ecosystem (Yelenik *et al.* 2004). Various studies have quantified higher levels of soil N in stands of acacias compared to fynbos control sites (e.g. Witkowski 1991, Musil 1993, Yelenik *et al.* 2004, Jovanovic *et al*. 2009, Gaertner *et al*. 2011). Furthermore, this may have implications for groundwater quality. For a Sand fynbos system in the south-western Cape (Riverlands Nature Reserve), Jovanovic *et al.* (2009) documented significantly elevated concentrations of NO_3+NO_2 (nitrate + nitrite) in groundwater of *A. saligna* (Port Jackson willow) stands compared to the Sand fynbos-dominated control. They suggested that elevated NO_3+NO_2 concentrations in groundwater due to invasion may have implications on water purification costs for municipalities that rely on groundwater as the main source of water supply (the nearby Atlantis settlement in their study case). A logical extension of their findings is that NO_3+NO_2 concentrations may become elevated for small waterbodies surrounded by extensive *Acacia* stands. This in turn may impact on aquatic ecosystem structure and function. For example, NO_3+NO_2 concentrations were significantly associated with macroinvertebrate assemblage composition in chapter 3 of this thesis (see Tables 3.8 and 3.9) and thus shifts in the regime of this variable in small temporary wetlands might be expected to have implications for the invertebrate biota.

Studies on the ecosystem effects of acacias in nutrient-deficient fynbos soils have concentrated on nitrogen effects and much less attention has been paid to the ability of acacias to influence phosphorus (P) levels in the system. Witkowski and Mitchell (1987) found that soil P status was altered by infestations of *A. saligna* and *A. cyclops* (rooikrans) in a Sand fynbos system (Fernwood, south-western Cape). They attributed the greatly elevated P levels in *Acacia*-dominated soils to the significantly higher leaf litterfall (and rapid turnover thereof) for acacias relative to fynbos. Because P concentrations of Sand fynbos soils are extremely low in their natural state (Witkowski and Mitchell 1987), elevation of soil P due to dense *Acacia* invasion may contribute to ecosystem effects on indigenous species that are adapted to naturally low levels of this variable. Potential effects of alien infestations on P levels in nearby waterbodies (in the form of phosphates) have not been investigated, but it is expected that if soil P is significantly elevated by alien vegetation then this would be reflected in higher concentrations of phosphates for small waterbodies surrounded by dense *Acacia* stands. Although the focus here has been on the nutrient effects of alien vegetation encroachment on aquatic ecosystems, various other knock-on effects could be associated with alien plants such as lower water temperatures due to increased shading caused by tall *Acacia* shrubs. Physico-chemical changes in aquatic environments caused by infestations of alien vegetation in adjacent terrestrial areas may have indirect effects on biota. For instance, aquatic macrophyte and macroalgal structure and abundance are key biotope factors determining invertebrate assemblage composition in wetlands (Williams 2006, Vanschoenwinkel *et al.* 2007, De Roeck 2008, Waterkeyn *et al.* 2008, Bilton *et al.* 2009). Changes in the structure and abundance of these biotopes associated with increases in nutrients and pH would be likely in turn to influence invertebrate assemblage composition in wetlands.

In summary, physico-chemical changes associated with habitat transformation around wetlands might be induced either by the effects of a particular agent of habitat transformation (e.g. alien invasive vegetation), or simply by losing the physico-chemical signature previously associated with indigenous habitat (irrespective of the particular type of habitat transformation). These physico-chemical changes could have direct (e.g. pH) or indirect (e.g. nutrient effects on biota *via* changes in biotope) impacts on wetland biota. The previous chapter of this thesis indicated a strong influence of physico-chemical conditions on temporary wetland invertebrate assemblages in the south-western Cape. Therefore if transformation of indigenous fynbos habitat around temporary wetlands induces a shift in the physico-chemical conditions in those wetlands, one expects this to influence aquatic invertebrate assemblages (directly or indirectly). Although wetland hydro-morphometry could be impacted by certain types of habitat transformation (e.g. berming associated with urban development), this is not a hypothesized effect for alien vegetation invasion in this study. To reduce the confounding effect of hydro-morphometrical variation, wetlands of similar size and depth were compared in this study.

4.1.4. Study aims and hypotheses

The aim of this study is to elucidate the effects of a single agent of habitat transformation (alien vegetation) on temporary wetlands environments and invertebrate assemblages using a small-scale analysis. I test the hypothesis that habitat transformation around temporary wetlands (induced by alien vegetation invasion) is associated with changes in physico-chemical conditions in these wetlands, which in turn mediates changes in aquatic invertebrate assemblages from the natural to transformed ecosystem state. If habitat transformation effects are negligible, I expect no significant relationship between the amount of indigenous vegetation around wetlands and physico-chemical conditions and/or invertebrate assemblages in wetlands. I further wish to test the overarching hypothesis that, due to the reduced influence of natural heterogeneity, patterns of environmental and biotic response to habitat transformation around temporary wetlands will be clearer at the fine scale of this study in comparison to patterns observed at the broad scale in previous chapters. In this regard, I expect the ratio of the influence of anthropogenic *versus* natural factors to increase at the fine scale relative to the very low ratio observed at a broader scale. Consequently it is expected that individual invertebrate taxa and metrics will present clearer patterns against the gradient of habitat transformation at the fine scale in this chapter than was observed in the previous chapter.

4.2. METHODS

4.2.1. Study area and site selection

The study took place in and around the Kenilworth Racecourse Conservation Area (KRCA) located in the suburb of Kenilworth in Cape Town, South Africa (Fig. 4.1). KRCA is situated on a flat sandy coastal plain known as the Cape Flats, which has become extensively transformed by the urban sprawl of Cape Town. The Cape Flats falls within the south-western Cape mediterranean-climate region of South Africa, receiving the majority of its 575 mm of mean annual precipitation (Rebelo *et al.* 2006) during the winter months (for further description of climate and biogeographical aspects of the region see chapter 2, section 2.2.1). KRCA has a total area of approximately 52 ha, consisting of a core conservation area with a history of minimal disturbance and a peripheral area that has received moderate levels of disturbance. A third area sampled was an extensively disturbed piece of vacant land (approximately 54 ha in size) at Youngsfield military base, which is separated from KRCA by a highway (Fig. 4.1). The core area of KRCA occurs inside Kenilworth Racecourse, the oldest horse racing track in South Africa (established in 1882). For most of its existence, the core conservation area was preserved inadvertently from degradation by the South African Turf Club (KRCA was only formally established in 2006), whilst areas surrounding the racecourse have mostly been developed for housing.

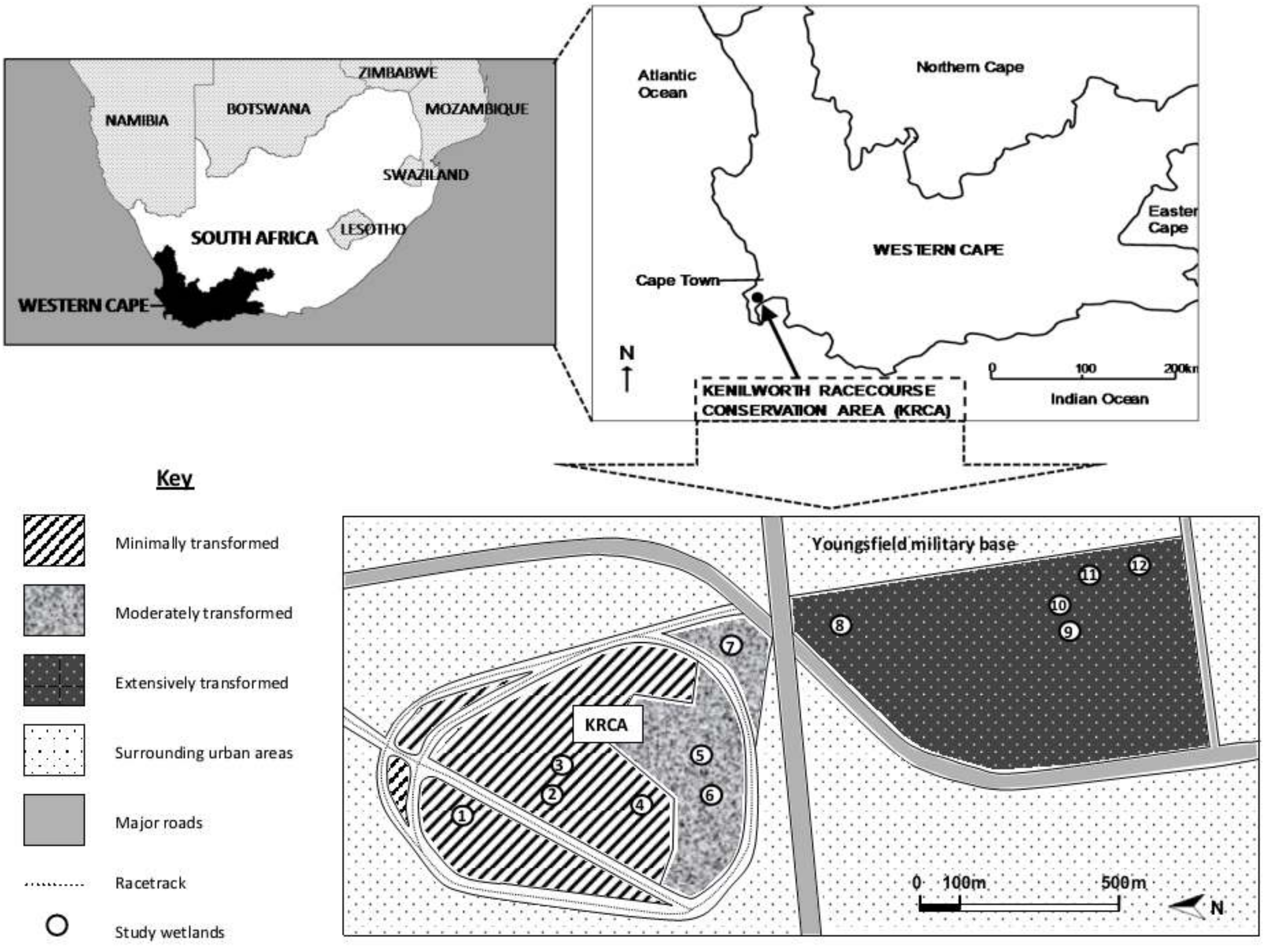


Figure 4.1. Position of the Kenilworth Racecourse Conservation Area (KRCA) within the Western Cape Province of South Africa (top). The layout of the 12 study wetlands is also depicted (bottom). See Appendix 11 for photographs of each wetland.

The specific type of Sand fynbos in and around KRCA is Cape Flats Sand fynbos (*sensu* Rebelo *et al.* 2006), which covers those parts of the Cape Flats with acid sandy soils of Tertiary origin (c.f. Cape Flats Dune strandveld occurring on calcareous sands of marine origin, Rebelo *et al.* 2006). Cape Flats Sand fynbos was once the most widespread lowland vegetation type around Cape Town. However, this habitat has largely been transformed by urban development (mostly housing) and is now Critically Endangered with only 15% remaining, although only 5% is considered to be in good condition and approximately 1% is statutorily conserved (Rebelo *et al.* 2006). KRCA contains the largest remaining and best condition fragment of Cape Flats Sand fynbos habitat and harbours a staggering 310 indigenous plant species within its 52 ha area (Gehrke *et al.* 2011). Cape Flats Sand fynbos is characterised by having an abundance of depression wetlands that fill during the winter when rising water tables manifest as surface water on the sandy coastal plain. Although these wetlands are still abundant across the Cape Flats, the majority now occur in a transformed urban landscape, primarily as depressions in vacant pieces of land invaded by alien vegetation and surrounded by residential and informal housing. Other than at KRCA, very few wetlands exist within the original fynbos habitat. It is likely that this widespread transformation of the landscape corresponds with a change in the environmental and biotic characteristics of these wetlands, but neither the extent of this change nor the resilience of these temporary wetland communities to change has previously been investigated.

During the 2008 and 2009 wet seasons, 12 temporary isolated depression wetlands (*sensu* Ewart-Smith *et al.* 2006) in and around KRCA were repeatedly sampled for various environmental constituents and aquatic invertebrate fauna. The chosen wetlands covered a gradient of habitat transformation, occurring across the three differentially transformed areas at Kenilworth running along a north-south axis. Four wetlands (sites 1-4, see Fig. 4.1 and Appendix 11) occurred within the core nature reserve area (surrounded by untransformed fynbos habitat), three wetlands (sites 5-7, see Fig. 4.1 and Appendix 11) occurred on the periphery of this area (surrounded by a mix of fynbos and transformed land) and five wetlands (sites 8-12, see Fig. 4.1 and Appendix 11) occurred immediately outside KRCA at Youngsfield (surrounded by extensively transformed land). Wetlands were sampled on five occasions in total, twice during 2008 (June and late August/early September) and three times during 2009 (late August/early September, October and November). Emphasis was placed on testing whether there were patterns of response to the gradient of habitat transformation that were consistent over time and not on testing for temporal differences between sampling events or between years.

Despite an abundance of isolated depression wetlands in the area, only those of similar overall size and depth were selected. This targeted approach was taken so as to minimise potentially confounding effects of total surface area and hydroperiod on invertebrate assemblage composition. It was also not expected that these variables would be affected by the predominant form of habitat transformation (alien vegetation invasion) at Kenilworth, given the lack of association between habitat transformation and hydro-morphometry reported at the broad scale (chapter 2, section 2.3.1). These hydro-morphometrical variables have been shown elsewhere to have a significant influence on wetland invertebrate assemblage composition and richness (Wiggins *et al*. 1980, Brooks 2000, Eitam *et al*. 2004, Williams 2006, De Roeck 2008; see chapter 3, section 3.3.2). The sites were selected *a priori* according to reconnaissance visits during the wet season of 2007. The chosen sites all had a similar inundation regime, filling up with the first heavy rains usually in early May and remaining inundated until late November or early December. Therefore their seasonal pattern of inundation is fairly predictable, being dry for roughly half the year during summer and autumn months and wet during winter and spring months. It was decided to choose sites that were not ephemeral in order to increase the likelihood of there being sufficient water for repeated sampling through the wet season. The direction of groundwater flow at Kenilworth is west to east across the study area and thus groundwater influence was not expected to confound the hypothesized biotic and abiotic patterns across the north-south gradient of habitat transformation.

4.2.2. Habitat transformation at Kenilworth

Land-use history

The three areas covered in this study are differentially invaded by alien vegetation due to differences in their land-use histories. First was the area sampled immediately outside the KRCA at Youngsfield. This piece of land was historically a buffer zone around an airfield landing strip (located where the military base now stands), which served aircraft during WWII. This buffer area (consisting of Cape Flats Sand fynbos) was relatively undisturbed during this time, but when the airfield was decommissioned in the 1960s and converted to a military base, much of the land was physically disturbed by dumping activities associated with its construction and much of the fynbos was lost. In the years following this, the area was zoned as a buffer region between the military base and the surrounding urban areas and was not used except for physical training exercises of South African Defence Force soldiers. Since the 1980s the land has remained a buffer zone for the military base and has not been used for human activities. Initial physical disturbance of the Youngsfield landscape allowed for the invasion of alien vegetation. This came in the form of the Port Jackson Willow *Acacia saligna*, originating from Australia, and the kikuyu grass *Pennisetum clandestinum* of East African origin. These two species have covered this vacant land for several decades (although the date of their first arrival here is unknown) and taken together constitute almost 100% areal cover of the land (other plant species are present, but are very sparsely represented in this area, see section 4.2.2).

The second area sampled falls within KRCA, but lies on the periphery of the core conservation area. This land has been moderately disturbed in the past for horse jumping activities and dumping of earth for building activities associated with the racetrack and surrounding grounds. Although the peripheral area in which wetlands were sampled has not been physically disturbed by humans for several decades (it was zoned for conservation prior to establishment of KRCA), the once-disturbed patches have been colonised by kikuyu grass and acacias, thus preventing the re-establishment of fynbos in these patches. This has resulted in a mosaic of alien invaded land mixed with fynbos habitat in roughly equal proportions.

Lastly, the area sampled within the core conservation zone of KRCA has a history of minimal disturbance. A particular alteration to the natural regime is the lack of fire experienced in this area in recent years, which can be considered a form of disturbance to the naturally serotinous Sand fynbos (Rebelo *et al.* 2006, Turner 2006). Whilst a controlled burn of part of the area was performed in 2005, most of the vegetation has not been burned for roughly one hundred years (Turner 2006). This remnant piece of Cape Flats Sand fynbos habitat is otherwise considered to be pristine and ecologically functional (Hitchcock 2006, Turner 2006, Hitchcock *et al.* 2008, Gehrke *et al.* 2011). Transformation of habitat within 100 m of wetlands within the core area includes the racetrack itself, which encloses the area (unfertilized, mowed kikuyu grass), and several gravel and sand roads.

Quantifying habitat transformation

Wetlands across the three adjacent areas described above were surrounded by differing levels of habitat transformation within approximately 500 m of their edges, but beyond this were all similarly surrounded by urban areas. The most pronounced differences in habitat cover were observed within approximately 100 m of wetlands. Thus it was decided to quantify habitat cover within 100 m of wetlands to best represent the gradient of habitat transformation in this study. To achieve this, four 100 m transects were assessed at each wetland, radiating north, east, south and west from each wetland edge. The habitat cover type was recorded every two metres along each transect at a point on the ground directly (perpendicularly) below each 2 m mark on the tape measure, producing 50 habitat cover points per transect and 200 per wetland. The categories of habitat cover recorded at each point were: indigenous vegetation (predominantly shrubs, but grasses and herbaceous forms were also recorded); alien shrub (*A. saligna*); alien kikuyu grass (*P. clandestinum*), other alien vegetation (very minimal cover, but other annual grasses and herbaceous species were present); racetrack (unfertilized kikuyu); mowed field (unfertilized); road (gravel and tar roads were present) and wetland (all surrounding wetlands were temporary). Upon completing transects, these variables were expressed in terms of their percentage cover across all four transects (200 points in total). Although the amount of wetland cover was recorded on transects, this variable does not represent a form of habitat transformation and thus did not contribute to the total percentage of habitat transformation calculated around wetlands. Transects were undertaken once off before commencement of sampling in 2008. Because no differences in terrestrial vegetation cover or human activities were noted during the study (nor were they expected), habitat transects were not repeated and thus the habitat transformation variables were considered static. All environmental variables (described in the sections following this) were considered labile variables and were sampled on each of the five occasions.

4.2.3. Sampling environmental variables

Hydro-morphometry

An effort was made to sample wetlands of comparable size and depth in this study, but because this was not a controlled experiment there was still a certain amount of variation in hydro-morphometry among wetlands. This was incorporated into analyses by measuring the maximum depth and total surface area of each wetland on each sampling occasion. Maximum depth (cm) was measured with a calibrated depth stick (approximately 0.5 cm accuracy). Total surface area (m^2) was estimated by mapping the perimeter of each wetland with a Garmin eTrex Vista handheld GPS (point accuracy of 3 m), using a minimum of 10 coordinate points to represent perimeter. Perimeter points were then used to calculate surface area (m^2) in Garmin MapSource v6.11.6 GIS software.

Biotope characteristics

Three major biotope types were sampled in this study and as per chapters 2 and 3, were based on habitat structural complexity, generally regarded as an important determinant of invertebrate abundance and assemblage composition in wetlands (Beckett *et al.* 1992, Cardinale *et al.* 1998, Batzer *et al.* 2006, Williams 2006, De Roeck 2008). These three structural habitat types were complex vegetation (generally submerged), simple vegetation (generally emergent) and open water (no vegetation). Complex vegetation was typically inter-woven and with fine dissected leaves and was dominated by the aquatic macrophyte *Isolepis rubicunda*, endemic to the south-western Cape (van Ginkel *et al.* 2011). However, various other vegetation species also formed a complex submerged habitat, including flooded semi-aquatic and terrestrial grasses. Simple-structured vegetation habitat was further divided into two types: reeded form (consisting of two species in this study, namely *Typha capensis* and *Phragmites australis*) and sedge-like form (consisting mostly of the sedge species *Bulboschoenus maritimus* and the restio *Elegia tectorum*). Reeded structure involved broad strap-like stems, whereas a sedge-like form entailed finer stems typical of the Cyperacae and Restionacae, but which had a considerably simpler structure than observed for complex vegetation types such as *I. rubicunda*. Open water habitat was defined as surface water devoid of vegetation, being at least 30 cm in depth above the bottom substrate (this was practical for sweep-netting purposes, see section 4.2.4). All 12 wetlands had vegetated bottom substrates and thus the fourth category represented in chapters 2 and 3, benthic un-vegetated habitat, was not sampled in this study.

At each wetland, two orthogonal transects crossing the centre of the wetland were used to quantify the cover of each aquatic habitat type. Along each transect, ten points were selected at evenly spaced intervals and at each point the habitat type was recorded. The presence of the macroalga *Cladophora* sp. (herein referred to as the variable “macroalgae”) was also noted at each point. Although this macroalga did not form a habitat type for invertebrate sampling (the dense mats could not be sampled as they clog the sweep net), its cover was quantified for use as a variable in further analyses. The twenty habitat points per wetland were used to estimate percentage cover of the three major habitat types and macroalgae per wetland.

Physico-chemistry

Several physico-chemical variables were measured *in situ* for each of the three major habitat types per wetland. The following measurements were taken in each habitat: pH was measured using a Crison pH25 meter; dissolved oxygen was recorded using a Crison OXI45 oxygen meter; electrical conductivity was recorded using a Crison CM35 conductivity meter; and turbidity was measured using a Hach 2100P turbidimeter. Temperature was recorded on the pH, oxygen and conductivity meters, although for analysis purposes an average of the readings across all three instruments was used. All physico-chemical readings were taken at a standardized depth of 30 cm across all habitats. Exceptions to this were for readings taken from the conductivity meter, which floats on the water surface, and for any readings taken in habitats < 30 cm deep.

In order to analyse water column nutrients, chlorophyll *a* and humic concentrations, samples from the water column of each wetland were collected and further analysed in the laboratory. Five 1L surface water samples were collected from each wetland, with the aim of covering the full spatial extent of each site, and pooled to form a bulk 5L sample. This pooled sample was then thoroughly mixed and a 1L sub-sample was taken for analysis of chlorophyll, nutrients and humics in the laboratory. This sub-sample was stored immediately in the dark at 4°C before being filtered in the laboratory within 24 hours. In the laboratory, each 1L sub-sample was filtered through a Whatman GF/F 0.7 µm glass fibre filter. The filter paper was stored in a petri dish, which was then wrapped in foil and frozen at -18°C for further analysis of chlorophyll *a* concentration. Using the 1L filtrate, four 50 ml sub-samples were taken (three for nutrients and one for humics) and stored frozen at -18°C for further analysis. All frozen samples were analysed for nutrients, chlorophyll *a* and humics within 30 days.

NO_3^-+NO_2^-–N, PO_4^{3+}–P and NH_4^+–N concentrations were estimated using a Lachat Flow Injection Analyser, as follows: NH_4^+-N was measured using Lachat's QuikChem® Method 31-107-06-1, based on the Berthelot reaction in which indophenol blue is generated; NO_3^- and NO_2^- were estimated using Lachat's QuikChem® Method 31-107-04-1-E, in which NO_3^- is converted to NO_2^- and diazotized with sulfanilamide to form an azo dye; PO_4^{3+} was measured by forming an antimony-phospho-molybdate complex using QuikChem® Method 31-115-01-1. Approximate detection limits are: for PO_4^{3+} 15µg.L^{-1} P; for NO_3^- and NO_2^- 2.5µg.L^{-1} N; and for NH_4^+ 5µg.L^{-1} N. These variables are herein referred to in the text as phosphates, nitrates + nitrites and ammonium respectively. Chlorophyll *a* was extracted from filter papers using 90% ethanol and concentrations were measured using the spectrophotometric method of Sartory and Grobbelaar (1984), as summarised by Biggs and Kilroy (2000). Absorbance (665nm and 750nm) was measured using a Merck Spectroquant Pharo 100 spectrophotometer. The relative concentration of humic (polyphenolic) substances among wetlands was proxied using ultraviolet absorbance (UVA) at 254nm. Specific UVA at 254nm ($SUVA_{254}$) is considered a standard technique for estimation of absolute concentrations of humic compounds in surface waters (Thebe *et al.* 2000, Weishaar *et al.* 2003, Mamba *et al.* 2009). $SUVA_{254}$ incorporates the concentration of dissolved organic carbon (DOC) in its calculation, but could not be undertaken in this study as a DOC analyser was not available. However, interest lay in determining the relative quantities of humic substances among wetlands and thus absolute concentrations from the $SUVA_{254}$ technique were not considered critical for this study. Furthermore, several authors have reported strong correlations between UVA readings, DOC concentration and total concentration of humic compounds in surface waters of the Western Cape (Midgley and Schafer 1992, Thebe *et al.* 2000). Therefore straightforward UVA at 254nm can be considered a useful proxy for relative concentrations of dissolved humic substances among wetlands in this study. All UVA readings for humics were performed in a 1 cm quartz cuvette using a Beckman DU-64 spectrophotometer.

4.2.4. Invertebrate sampling

Wetlands were sampled for aquatic invertebrates using a square-framed, long-handled sweep net with a 235 mm mouth and 80 µm mesh. The sweep net approach to sampling invertebrates in wetlands is generally regarded as the single most efficient technique for this purpose (Cheal *et al.* 1993, Turner and Trexler 1997, Gernes and Helgen 2002, Bowd *et al.* 2006), but does have certain limitations (e.g. highly motile taxa may evade the net and dense macroalgae will usually clog the net). As for chapter 3 (section 3.2.1), the goal of sampling in this study was to capture a representative sample of the overall wetland assemblage and thus a multi-habitat strategy was adopted. The three major biotopes present in the Kenilworth wetlands were complex vegetation, simple vegetation and open water (see section 4.2.3 for further description) and each of these biotopes was sampled for aquatic invertebrates within each wetland and on each sampling occasion.

The sweep sampling protocol was the same as employed in the previous chapter (section 3.2.1), but for ease of reference is repeated here:

Samples were standardized and made as quantifiable as possible through a strict sampling method. The goal of the sweep net sampling in this study was to collect a representative sample of the aquatic invertebrate fauna from each wetland. The sampling method follows that of Rundle *et al.* (2002) who established that five replicate 1 m sweeps from each biotope using a standard sweep net (area 200 x 250 mm) consistently sampled 60 – 80% of the total invertebrate species pool from that biotope for a set of 16 temporary isolated depression wetlands in Cornwall, south-west Britain. I modified the protocol of Rundle *et al.* (2002) by sampling each biotope within three different areas of each wetland so as to maximise spatial representation of each biotope. The method for each 1 m sweep follows that prescribed by various authors for sampling temporary and permanent wetland invertebrates (Gernes and Helgen 2002, Rundle *et al.* 2002, Bowd *et al.* 2006), whereby each sweep constituted dragging the net down from the water surface at a 45^{o} angle until nearly touching the bottom and then completing the sweep arc by returning the net back to the surface at a 45^{o} angle, covering a distance of one metre with each full sweep. For cases where habitats were shallow (< 30 cm) this method had to be modified to a straight one metre sweep keeping the net immediately above the bottom substrate. Each biotope sample comprised 3 × 1 m sweeps for three different areas of the wetland, so that one sample was a pooled combination of 9 × 1 m sweeps from three different areas where the habitat was found in the wetland. This method thus provided three replicate sweeps at each location in a wetland as well as three replicates across different areas in the wetland where the habitat was found. The nine sweeps per biotope is likely to represent the majority of the invertebrate assemblage for that biotope, given the recommendation of Rundle *et al.* (2002) of five sweeps per biotope. As the procedure was in turn repeated three times per wetland (to obtain three biotope samples), this produced a total effort of 27 × 1 m sweeps evenly divided over the spatial area of each wetland and representing the major biotopes that were present. The three samples from each wetland were preserved and stored separately.

4.2.5. Invertebrate sample processing

All samples were fixed on site in buffered 10% formalin and replaced with a 70% ethanol solution after 24-48 hours for longer-term preservation. For each wetland, the separate biotope samples were combined in the laboratory and invertebrates were picked from the pooled samples. Although it would have been preferable to pick each habitat sample separately, this was not achievable given the time constraints. Vegetation and pieces of macroalgae were carefully removed from samples prior to picking invertebrates, making sure that invertebrate loss with the removed material was minimised. Macroinvertebrates (defined as taxa > ~1 mm in size and visible to the naked eye) and microcrustaceans (defined as copepods, ostracods and cladocerans) were identified and enumerated using a sub-sampling procedure. First, the whole sample was scanned for five minutes in a tray and large rare (LR) macroinvertebrate and microcrustacean taxa (defined as taxa with large easily visible specimens represented by < 10 individuals per sample) were picked out in accordance with recommendations made by Vinson and Hawkins (1996) and King and Richardson (2002). The sample was then emptied into a rectangular tray divided into a grid of 35 equal-sized square cells numbered 1 to 35 and macroinvertebrates were picked out. To achieve this, a series of random numbers from 1 to 35 was generated for each sample, and sub-sampling proceeded in that sequence. Every macroinvertebrate individual in each cell was picked and counted, before proceeding to the next one. Sub-sampling stopped when 200 individuals had been counted, after first completing the cell in which the 200th individual was counted. Macroinvertebrate abundances were extrapolated to whole sample estimates in order to standardise final abundances. Samples with < 200 individuals were completely picked (i.e. all 35 cells were completed). After picking out macroinvertebrates, samples were preserved in 70% ethanol before being sub-sampled for microcrustaceans at a later stage.

A more efficient and potentially more accurate technique for sub-sampling microcrustaceans is employed in this study compared to that used in chapter 3. Following the highly useful approach of McCallum (1979) for sub-sampling freshwater zooplankton, each sample was drained of ethanol and then made up to 500 ml with tap water in a glass beaker. The sample was then homogenised by blowing bubbles into it for 5 seconds through a straight-sided, graduated pipette with a wide bore aperture of 5 mm. After homogenisation, a 1 ml sub-sample was sucked into the pipette and emptied into a Bogorov tray and individuals were enumerated under a dissection microscope. This process was repeated until 200 individuals were counted (upon first completing the sub-sample in which the 200th individual was counted). As the maximum total volume sub-sampled to obtain 200 individuals was 20 ml, microcrustacean abundances across all samples were standardised to a 20 ml estimate by extrapolation. The choice of 200 individuals as a stopping point for sub-sampling of macroinvertebrates and microcrustaceans was adapted from the recommendations of various bioassessment studies in rivers (Barbour and Gerritsen 1996, Somers *et al.* 1998, Barbour *et al.* 1999), and more recently from a study in wetlands (King and Richardson 2002). Although these studies involved macroinvertebrates, the results were generalised to microcrustaceans in this study. Sub-sampling trials in chapter 3 (see section 3.2.2) indicated that in almost all cases the full complement of microcrustacean taxa was encountered within a 200-organism count.

All macroinvertebrate and microcrustacean taxa were identified to genus- or species-level. Exceptions to this were for certain dipteran, coleopteran and trichopteran larvae, where identification could only be made to family level. The major reference source for keying out invertebrate taxa was the series of “Guides to the Freshwater Invertebrates of Southern Africa“ (Day *et al.* 1999, Day *et al.* 2001a, Day *et al.* 2001b, Day and de Moor 2002b, a, Day *et al.* 2003, de Moor *et al.* 2003b, a, Stals and de Moor 2007). Problematic taxa which required expertise (e.g. chironomids and ostracods) were identified by a specialist taxonomist for the given taxon. The final lists of macroinvertebrate and microcrustacean taxa recorded in this study are presented in Appendices 12 and 13 respectively.

4.2.6. Data analysis

Data preparation

Invertebrate analyses in this study were conducted using relative abundance data. Macroinvertebrate abundances for all wetlands were standardised to an estimated number per whole sample, whilst microcrustaceans were standardised to an estimated number per 20 ml subsample. As for analyses in chapter 3, the focus for comparisons in this study was among wetlands (rather than biotopes) and the unit of replication was thus individual wetlands, represented by standardised relative abundances (as expressed above). Raw data matrices were ln(x+1) transformed for both macroinvertebrates and microcrustaceans. Fluctuations in microcrustacean abundances were not as severe as for chapter 3 and thus ln(x+1) transformation was chosen over 4th root. Macroinvertebrate and microcrustacean data were once again analysed separately due in part to the different scales at which relative abundances were estimated, but largely because they are ecologically differentiated assemblages in terms of organism size, total abundance, life history patterns and dispersal (e.g. microcrustaceans are passive dispersers whereas macroinvertebrates are often active dispersers). Thus it was of interest to assess the separate responses of these two assemblages to habitat transformation gradients. Note that three invertebrate samples collected during September 2008 were spilt during laboratory processing and the contents were lost. These three samples are not included in the analyses.

Physico-chemical variables measured *in situ* were averaged across the three biotopes to produce mean values per wetland. All other environmental variables were measured at the individual wetland scale and were used as such for analyses. Environmental variables were $\log_{10}$ transformed where appropriate to improve normality. Percentage variables were arcsine square root transformed to improve normality, with the exception of one of the habitat transformation variables (% kikuyu grass cover), which did not benefit from this transformation. The raw environmental data for each wetland are presented in Appendices 14 (static variables) and 15 (labile variables).

Analysis approach

The approach to analysing data in this study follows a similar framework to that of previous chapters and incorporates multiple linear regression models to assess relationships between the habitat transformation gradient, environmental conditions in wetlands and invertebrate assemblages. The focus is on relationships between various response variables (both biotic and environmental) and the gradient of habitat transformation around wetlands. This gradient is represented by several quantitative variables (namely % indigenous vegetation cover, % kikuyu grass cover, % *Acacia* shrub cover and % road cover) and thus a regression approach suited the aim of relating invertebrate assemblages and environmental conditions to the habitat transformation predictor variables. Detrended Correspondance Analysis (DCA) indicated that gradient lengths in the macroinvertebrate, microcrustacean and environmental datasets were all best suited to linear rather than unimodal analyses (gradients lengths were all < 3, Lepš and Šmilauer 2003), as was the case for data in chapters 2 and 3. Both multivariate and univariate response data were analysed in context of the habitat transformation gradient at Kenilworth. The multivariate data analysed were invertebrate assemblage composition (both macroinvertebrates and microcrustaceans) and environmental conditions in wetlands. In certain analyses the environmental response matrix was further sub-divided into sets of variables, namely physico-chemical conditions, biotope characteristics and hydro-morphometry. Univariate response data analysed were the following: (1) individual environmental variables; (2) invertebrate taxon richness and diversity; (3) invertebrate family-level abundance data (testing for indicator taxa); and (4) invertebrate metrics. The following sections provide more details on these multivariate and univariate analyses.

Multivariate analyses

a) Unconstrained ordinations

The ln(x+1) transformed abundance data for macroinvertebrates and microcrustaceans was converted to a resemblance matrix using the Bray-Curtis similarity coefficient (Bray and Curtis 1957). Non-metric multidimensional scaling ordination (MDS, *sensu* Kruskal 1964) was used to visualize multivariate patterns in assemblage composition on a two-dimensional plane. Principal Components Analysis (PCA) was used to visualize multivariate patterns in the transformed, normalized environmental data on a two-dimensional plane. For both MDS and PCA ordinations, wetland sites were coded according to ordinal categories representing surrounding habitat transformation. The percentage cover of indigenous vegetation within

100 m of wetlands was used to represent overall levels of habitat transformation around wetlands. The range of this variable was trisected, resulting in three ordinal categories based on the amount of surrounding indigenous vegetation cover. The resulting categories were: no cover; moderate cover; and extensive cover. Although the habitat transformation data were expressed as quantitative percentage variables, visual depiction of the transformation gradient on ordination plots was best represented using ordinal categories coded as factors. Wetlands were individually labelled according to site code and time of sampling, which allowed a multivariate visualisation of invertebrate and environmental differences among individual sites and also within individual sites over successive sampling occasions.

b) Constrained ordinations

Multivariate linear regressions of invertebrate assemblage composition and environmental conditions on habitat transformation were performed using distance-based redundancy analysis (dbRDA, Legendre and Anderson 1999, McArdle and Anderson 2001). dbRDA is a non-parametric multiple regression procedure implemented on a multivariate resemblance measure of the users choice (for more information see chapter 2, section 2.2.5). In this study, macroinvertebrate and microcrustacean assemblage composition was represented using Bray-Curtis similarity matrices and environmental conditions using Euclidean distance matrices. In terms of the latter, the response matrices of interest were: 1) environmental conditions in wetlands (all environmental variables in one matrix); 2) physico-chemical conditions (represented by pH, conductivity, average temperature, turbidity, dissolved oxygen, phosphates, nitrates + nitrites, ammonium, chlorophyll *a* and humics); and 3) biotope characteristics (represented by % complex vegetation, % reeded simple vegetation, % sedge-like simple vegetation, % open water and % macroalgae). Resemblance matrices were all regressed on the variables representing habitat transformation. First, matrices were regressed on the percentage indigenous vegetation cover within 100 m of wetlands to assess relationships with the gradient of overall habitat transformation. Next, separate regressions were run for each response matrix on each of the variables representing the different types of habitat transformation within 100 m of wetlands at Kenilworth (namely “% kikuyu grass cover”, “% *Acacia* shrub cover” and “% road cover”). Although the cover of alien vegetation species other than *A. saligna* and *P. clandestinum* was recorded in this study, the data for this variable was too sparsely represented to be included in analyses.

The multivariate regressions described above were aimed at determining which form of terrestrial habitat transformation around wetlands was best associated with biotic and environmental conditions within these wetlands. A further form of dbRDA ordination undertaken in this study was the regression of macroinvertebrate and microcrustacean resemblance matrices on the full array of environmental predictor variables. Results were depicted using dbRDA ordination plots (two dimensional), with the aim of showing which environmental variables were associated with the habitat transformation gradient at Kenilworth, and in turn had an influence on the invertebrate faunal composition of these wetlands. The MDS and PCA plots described earlier present unconstrained ordinations, whilst the dbRDA plots described here are constrained by a set of predictor variables and provide information on which factors may have mediated changes in assemblage composition at Kenilworth. To reduce the number of redundant variables in these dbRDA models, step-wise selection of the environmental variables was first performed using dbRDA with an adjusted R^2 criterion. Significant variables from the step-wise procedure were retained as predictor variables in the final model. Once again the habitat transformation gradient on ordination plots was represented using three ordinal categories coded as factors.

Categorical variables were used to represent the five sampling occasions in this study (namely June 2008, September 2008, September 2009, October 2009 and November 2009 – see Appendix 15) and were coded using five dummy variables, although the fifth variable is obsolete for statistical purposes and does not contribute to the degrees of freedom. These variables represented time in this study and were included as covariables in all regressions because it was required that their influence first be taken into account before assessing the influence of the variables of interest, such as those representing habitat transformation. The influence of the seasonal variables was expected to be considerable given the well established role of succession in temporary wetland invertebrate assemblages (see for review Williams 2006). It was thus considered vital to quantitatively account for invertebrate variation due to temporal factors in all regressions. The role of succession itself is not examined in this study, but rather its effects are taken into account in the partial regressions.

c) Variation partitioning

As for chapter 3, a key component of the multivariate analyses conducted in this study involved the partitioning of variation among sets of predictor variables in order to assess the relative influence of natural and anthropogenic factors in structuring invertebrate assemblages at Kenilworth. A variation partitioning procedure was applied to the macroinvertebrate and microcrustacean compositional datasets to partition variability among three broad sets of explanatory variables, namely habitat transformation, environmental conditions and temporal covariables. The technique estimates the unique contribution of each set of explanatory variables that is independent of the other sets. It also estimates components of variation that are shared between variable sets (interactive effects). The procedure is fully explained in chapter 3 (section 3.2.4) and is not repeated here. However, a major difference between the two approaches is that the spatial variables are not included here. This is because both latitude and longitude displayed strong linear correlation with the gradient of habitat transformation at Kenilworth (r = 0.93 and -0.86 respectively). Thus the transformation gradient also essentially represents a spatial gradient, and habitat transformation (% indigenous vegetation cover as a measure of overall transformation) provides a proxy for the spatial variables. Variation of invertebrate assemblages attributed purely to habitat transformation can be interpreted as being due to pure spatial and pure habitat transformation effects, which overlap strongly and cannot be disentangled. However, of more interest in these analyses was the interactive (shared) effect of habitat transformation and environmental variables, as this represents variation in assemblage composition along the gradient of habitat transformation that was mediated by environmental factors.

The method for these data results in eight different components of variation, which have different labels to the components in chapter 3. These are: (1) Pure environmental (E); (2) pure temporal (T); (3) purely due to habitat transformation (H); (4) pure temporal component of environmental (TE), this is the overlap in the variation explained by temporal and environmental variables; (5) pure habitat transformation component of environmental (HE), similar to (4), this is the overlap in the variation explained by transformation of habitat and environmental variables; (6) pure combined temporal and habitat transformation component (TH), which is the fraction of the variation in the invertebrate data that is not related to the environmental variables, but which can be attributed to the pure combination of temporal and habitat transformation patterns; (7) combined temporal and habitat transformation component of environmental (THE), the fraction of the variation that can be explained by the combined action of temporal, habitat transformation and environmental variables; (8) Unexplained (U), the remaining variation in the invertebrate data that cannot be explained by the environmental, temporal or habitat transformation variables that have been recorded in this study. Although the components of variation have different labels to those in chapter 3, the format of the partitioning procedure is the same. The reader is referred to chapter 3, section 3.2.4 for further details.

Univariate analyses

a) Environmental variables associated with habitat transformation

Univariate multiple linear regression models were used to test for relationships between each of the environmental variables (as response variables) and the gradient of habitat transformation ("% indigenous vegetation cover within 100 m" as a single predictor). The influence of time from repeated sampling was represented by four categorical dummy variables (the fifth variable not being required for computation), which were specified as covariables in all models. These linear regression models provide an exploratory approach for testing which wetland environmental variables were associated with the gradient of habitat transformation at Kenilworth. The parametric nature of these models required that standard assumptions regarding normality and heterogeneity of spread in residuals were examined. These assumptions were inspected for models yielding significant results. Relationships between the habitat transformation predictor variable and response variables were visualized using partial residual plots, introduced in chapter 2 (see section 2.2.5 for more information on these plots). The partial residual plots also provide a means for assessing whether assumptions of normality and heterogeneity of variance in the residuals were upheld in regression tests, as well as allowing for the identification of outliers.

b) Taxon richness and diversity

Relationships between taxon richness/diversity and the gradient of habitat transformation at Kenilworth were investigated using univariate multiple linear regression models. The predictor variable of interest here was "% indigenous vegetation cover within 100 m" and the four categorical covariables representing time were included in all models. Partial residual plots were used to visualize relationships between response variables and the habitat transformation predictor variable. The terms "taxon" richness and "taxon" diversity are used here, because the level of taxonomic resolution in this study was not always to species. Five commonly used measures of richness or diversity were incorporated into these analyses:

First was taxon richness (S), represented simply by the total number of taxa;

Second was Margalef's index (d), a richness index, given by the equation:

$d = (S - 1) / \log N$;

Third was the Shannon diversity index (H'), given by the equation:

$H' = - \sum_i p_i \log (p_i)$, where p_i is the proportion of the total count arising from the ith taxon;

Fourth was Pielou's index of evenness (J'), given by the equation:

$J' = H' / \log S$;

Fifth was the Simpson diversity index ($1 - \lambda$), which once again expresses evenness and is given by the equation:

$1 - \lambda = 1 - (\sum p_i^2)$.

c) Family-level tests for indicator taxa

This study tests for linear relationships between individual invertebrate taxa (at the family level) and the gradient of habitat transformation. The human disturbance scores incorporated into the analyses of chapter 3 are not employed in this study because the gradient of habitat transformation at Kenilworth would effectively proxy a gradient of disturbance produced from a rapid-assessment index. The design of this study ensured that there were minimal differences among wetlands, other than for habitat transformation due to alien vegetation invasion. Impacts in the nearby landscape (< 500 m) and within wetlands were largely controlled for and a rapid assessment index of human disturbance would therefore present redundant information given the gradient of habitat transformation. Several other key differences exist between the indicator taxa and metric testing employed in this study compared to that of chapter 3. Firstly, the scale of the study has been reduced from a broad (across a geographical region) to a fine (within a single landscape) scale and, secondly, taxa are related to habitat transformation within 100 m of wetlands (the variable "% indigenous vegetation cover within 100 m") and not within 500 m as was done in chapter 3. Patterns were once again visualized using partial residual plots and, as for the previous section (*Taxon richness and diversity*), the temporal covariables were included in all regressions. It was decided to exclude those families that were present in five or fewer samples (< 10% of samples) as these low occurrences would present unreliable patterns for regression analysis. Macroinvertebrate and microcrustacean family abundances were ln(x+1) transformed prior to analysis.

d) Testing potential metrics

The same list of metrics tested at the broad scale in chapter 3 (section 3.2.4, Appendix 10) was tested in this study, bearing in mind the hypothesis that metrics would present clearer patterns at this smaller spatial scale. As with previous univariate analyses in this study, multiple linear regression was the preferred method to assess relationships between each metric and the habitat transformation gradient (represented by the variable "% indigenous vegetation cover within 100 m"). Models included the temporal covariables and results were visualized using partial residual plots. Following the explanation given in the previous paragraph, metrics were only regressed against the gradient of habitat transformation and human disturbance scores were not employed in this study. As noted in chapter 3, the terms "tolerant" and "intolerant" used for certain macroinvertebrate metrics are derived from Hicks and Nedeau (2000) for New England (USA) and are not based on levels of known tolerance for taxa in the south-western Cape study region.

Software used

DCA ordinations were performed using CANOCO for Windows v4.5 (Ter Braak and Šmilauer 2002). All dbRDA models were implemented using the DISTLM routine of the PERMANOVA+ software package (Anderson *et al.* 2008). P values for dbRDA models were tested by 9999 permutations of residuals under the reduced model. Univariate multiple linear regressions (including step-wise models and partial residual plots) were performed using STATISTICA v10 software (Statsoft Inc. 2010, Tulsa, Oklahoma, USA). MDS and PCA ordinations were performed using PRIMER v6 software (Clarke and Warwick 2001, Clarke and Gorley 2006). The significance level for all regression tests in this study was $\alpha = 0.05$.

4.3. RESULTS

4.3.1. Multivariate analyses: unconstrained ordinations

Macroinvertebrate (Fig. 4.2) and microcrustacean (Fig. 4.3) assemblages were clearly differentiated among those sites with extensive surrounding indigenous vegetation (within 100 m) and those with none (i.e. between minimally and extensively transformed sites respectively). The separation was also apparent for environmental conditions among wetlands (Fig. 4.4). This pattern of difference between least and most transformed sites appeared to be consistent over time, as reflected by separation of the two groups in MDS and PCA plots over the five successive sampling occasions in this study covering two wet seasons. Microcrustacean assemblages showed the clearest pattern of differentiation among the habitat transformation categories, as depicted by the gradational change in assemblage composition along the gradient of habitat transformation (Fig. 4.3). In this regard, moderately transformed sites are positioned roughly between least and most transformed sites on the MDS plot. The macroinvertebrate assemblages of moderately transformed sites appeared to be differentiated from those with minimal transformation, but showed considerable overlap with the extensively transformed sites (Fig. 4.2). The 2D stress values are reasonably high on the MDS plots (0.22), indicating that some distortion may have occurred when projecting the points onto two dimensions. However, the patterns described above are very similar for MDS plots in three dimensions (with a lower 3D stress value of 0.16), thus increasing confidence in the validity of these multivariate patterns.

Environmental conditions in the moderately transformed wetlands were generally similar to those of extensively transformed wetlands (as evidenced by the overlap of these sites on the PCA plot, but there was considerable scatter among the moderate category sites and some overlap was shared with the least transformed sites (Fig. 4.4). From the PCA plot it appears that there was considerable environmental variation among individual wetlands, even within each of the three transformation categories. Despite a large amount of variation among individual sites, PC1 appears to be associated with the loss of indigenous vegetation. Variables that are positively correlated with this axis ($r > | 0.3 |$) are pH, phosphates, % open water and % macroalgae (Table 4.1a). Negatively correlated variables are humics and % complex vegetation (Table 4.1a). The first two axes of the PCA ordination captured a fairly small proportion of the variation in environmental conditions at Kenilworth (42%, Table 4.2b) and thus do not necessarily present a clear picture of environmental gradients in the dataset. Environmental correlates of habitat transformation are explored further in section 4.3.4.

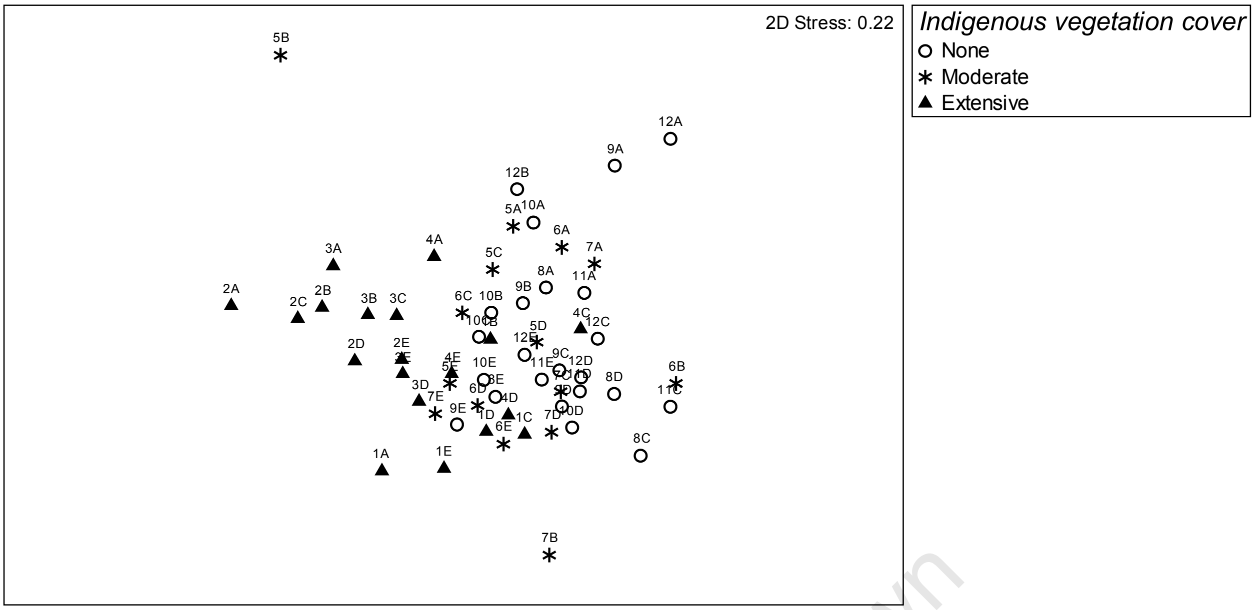


Figure 4.2. Multidimensional scaling (MDS) plots of macroinvertebrate assemblage composition, represented by the Bray-Curtis similarity among sites (n = 57). The level of habitat transformation around wetlands is proxied by the remaining amount of indigenous vegetation within 100 m. Sites are coded according to three broad levels of surrounding indigenous vegetation cover: none; moderate (ranging between 33 and 51% cover); and extensive (ranging between 75 and 99% cover). These categories represent extensive, moderate and minimal levels of habitat transformation respectively. Sites 1-12 are labelled according to the date of each sampling occasion (A: June 2008; B: August/September 2008; C: August/September 2009; D: October 2009; E: November 2009 – see Appendix 15 for details).

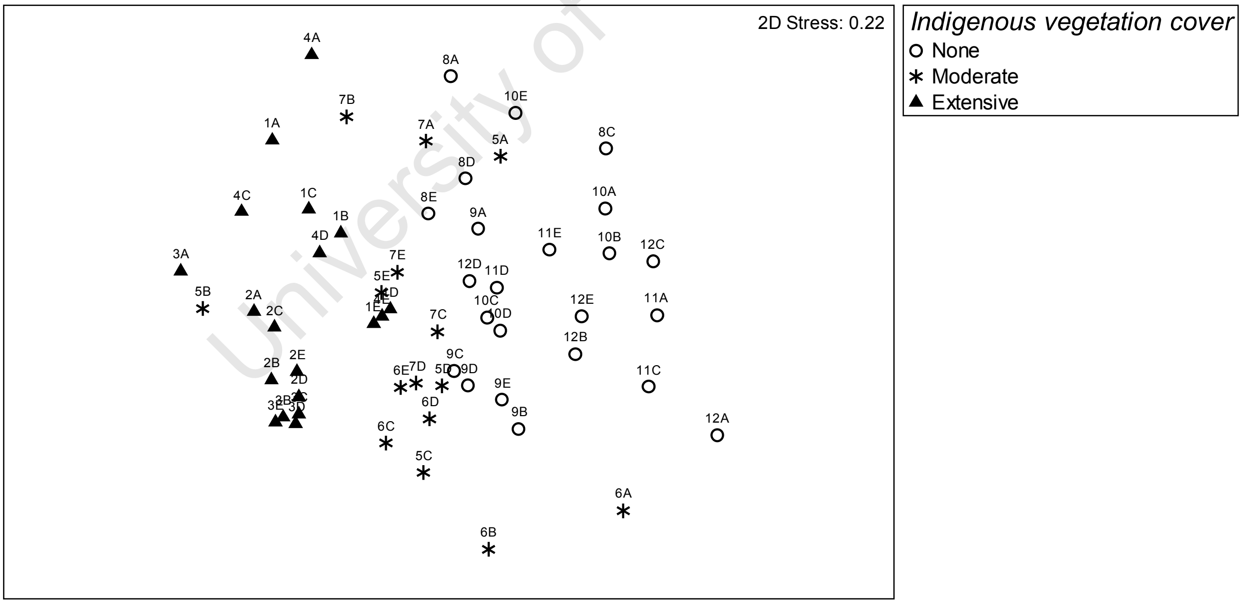


Figure 4.3. Multidimensional scaling (MDS) plots of microcrustacean assemblage composition, represented by the Bray-Curtis similarity among sites (n = 57). The level of habitat transformation around wetlands is proxied by the remaining amount of indigenous vegetation within 100 m. Sites are coded according to three broad levels of surrounding indigenous vegetation cover: none; moderate (ranging between 33 and 51% cover); and extensive (ranging between 75 and 99% cover). These categories represent extensive, moderate and minimal levels of habitat transformation respectively. Sites 1-12 are labelled according to the date of each sampling occasion (A: June 2008; B: August/September 2008; C: August/September 2009; D: October 2009; E: November 2009 – see Appendix 15 for details).

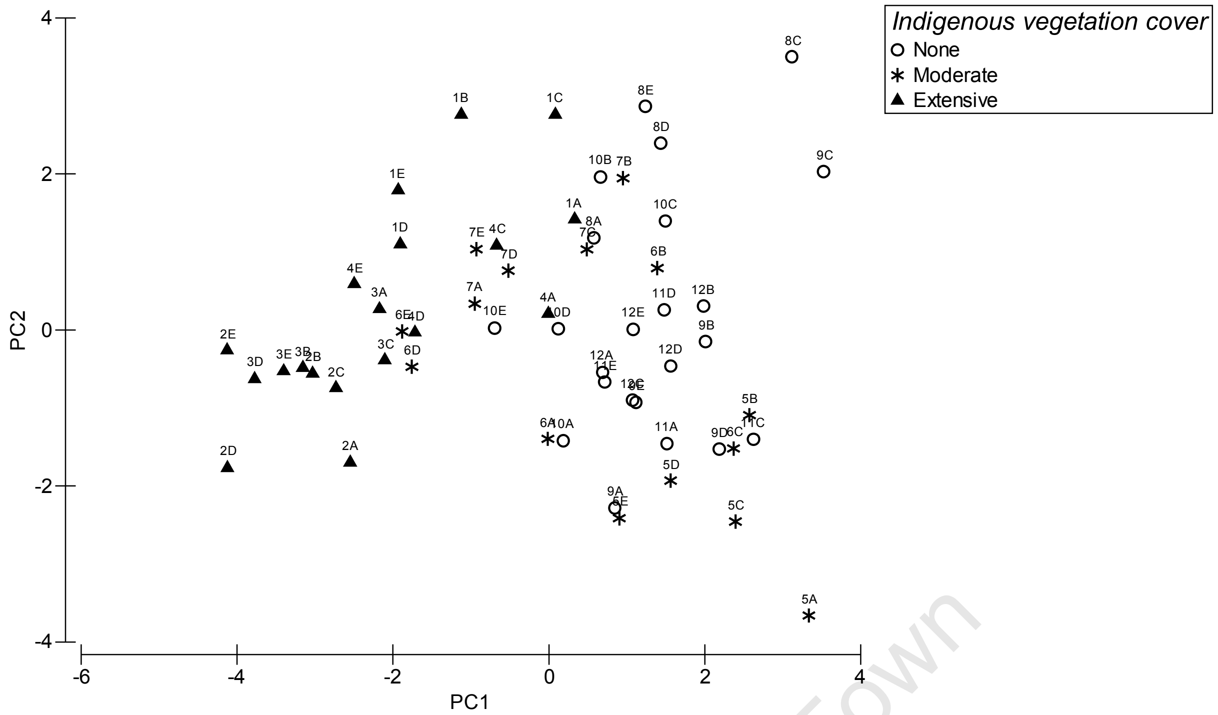


Figure 4.4. Principal components analysis (PCA) on the normalized set of environmental variables, showing the ordination of sites sampled at Kenilworth (n = 57). The first two principal component axes are displayed. The level of habitat transformation around wetlands is proxied by the remaining amount of indigenous vegetation within 100 m. Sites are coded according to three broad levels of surrounding indigenous vegetation cover: none; moderate (ranging between 33 and 51% cover); and extensive (ranging between 75 and 99% cover). These categories represent extensive, moderate and minimal levels of habitat transformation respectively. Sites 1-12 are labelled according to the date of each sampling occasion (A: June 2008; B: August/September 2008; C: August/September 2009; D: October 2009; E: November 2009 – see Appendix 15 for details).

Table 4.1. (a) Coefficients in the linear combinations of environmental variables making up principal component axes 1 and 2. Variables with correlation of r > ┃ 0.3 ┃ are indicated with an asterisk. (b) Eigenvalues and corresponding percentage of environmental variation explained by each of the first five principal component axes.

	a) Eigenvectors			*b) Eigenvalues*			
	Variable	PC1	PC2	PC	Eigenvalues	% Variation	Cumulative % variation
*	pH	0.351	0.323	1	4.03	26.8	26.8
	Conductivity	0.127	-0.094	2	2.27	15.2	42.0
	Turbidity	0.253	-0.375	3	2.01	13.4	55.4
	Oxygen	0.075	0.445	4	1.38	9.2	64.6
	Average temperature	-0.135	0.088	5	1.26	8.4	73.0
	Nitrates + nitrites	-0.049	-0.236				
	Ammonium	0.174	0.084				
*	Phosphates	0.328	-0.227				
	Chl *a*	-0.143	-0.157				
*	Humics	-0.331	-0.361				
	% Simple veg. (sedge)	0.083	0.266				
	% Simple veg. (reed)	0.280	-0.414				
*	% Open water	0.356	0.112				
*	% Complex veg.	-0.419	0.120				
*	% Macroalgae	0.340	-0.052				

4.3.2. Multivariate analyses: constrained ordinations

dbRDA multivariate regressions

The multivariate regression results (Table 4.2) offer quantitative confirmation of the gradient patterns observed in the unconstrained ordination plots (Figs 4.2 - 4.4). Invertebrate assemblages and environmental conditions showed highly significant ($P < 0.001$) linear relationships with overall levels of habitat transformation as proxied by the percentage cover of indigenous vegetation around wetlands. According to the amounts of explained variation in each response matrix, microcrustacean assemblage composition was more strongly related to the habitat transformation gradient than was macroinvertebrate assemblage composition (20.31% *versus* 12.69% respectively). The amounts of explained variation across the invertebrate and environmental matrices were not particularly high (ranging between 12.69% and 25.42%), despite being highly statistically significant. The invertebrate and environmental response matrices were strongly related ($P < 0.001$) to each of the two types of alien vegetation cover (% kikuyu and % *Acacia*), although % kikuyu consistently explained slightly larger amounts of variation in the response matrices. The percentage road cover explained small (2.42% to 5.50%), but mostly significant ($P < 0.05$) amounts of variation in the invertebrate assemblages and environmental conditions. In terms of environmental conditions in wetlands, biotope characteristics showed stronger association with the gradient of habitat transformation (25.42% explained variation) than did physico-chemical conditions (14.77% explained variation).

dbRDA ordination plots

Fairly little of the fitted and total variation of invertebrate assemblage composition is explained by the first two axes in the dbRDA plots (Figs 4.5 and 4.6), although certain patterns are apparent. Axis 1 in both plots appears to be associated with a loss of indigenous vegetation around wetlands (as in the PCA plot earlier), although neither axis fully captures the gradient of habitat transformation because it tends to follow an oblique angle from bottom left to top right in each plot. dbRDA ordination plots are interpreted similarly to standard redundancy analysis (RDA) plots (except the response matrix is based on Bray-Curtis resemblance rather than Euclidean distance), and thus vector lengths in the diagrams represent the strength of each environmental correlate in explaining invertebrate assemblage composition (constrained ordination). Only the % macroalgae and pH vectors in

Figure 4.5 display some degree of association with the gradient of habitat transformation, whilst in turn having a discernable correlation (r > 0.2) with macroinvertebrate assemblage composition. A suite of physico-chemical and biotope factors appear to be positively associated with extensively transformed sites and are in turn related to microcrustacean assemblage composition (Fig. 4.6). These factors are % macroalgae, phosphates, nitrates + nitrites, pH, % simple vegetation (reeded) and % open water. Humics was not included as a predictor variable in the dbRDA plots due to strong collinearity with pH (r = -0.86). The ecological effects of pH and humic concentrations on aquatic invertebrates are difficult to disentangle from each other in this study.

Table 4.2. dbRDA regressions of the multivariate response matrices of interest in this study on variables representing the habitat transformation gradient (predictors). Response matrices used Bray-Curtis similarity for invertebrates and Euclidean distance for environmental variables (normalized to the same scale). % Indigenous vegetation is used as a proxy for overall amount of habitat loss, whereas the remaining three predictor variables represent the different agents of habitat transformation at Kenilworth. % Var - the percentage of variation in each response matrix that is explained by the respective predictor variable in each model. The four variables representing time were included as covariables in all regressions, resulting in 51 residual degrees of freedom in each model. All relationships were significant (α = 0.05), with one exception (see *).

PREDICTORS		MULTIVARIATE RESPONSE MATRICES				
		Macroinvertebrates	Microcrustaceans	Environmental conditions	Physico-chemical conditions	Biotope characteristics
% Indigenous vegetation	F	9.933	16.126	12.986	13.183	19.005
	P	<0.001	<0.001	<0.001	<0.001	<0.001
	% Var	12.69	20.31	16.35	14.77	25.42
% Kukuyu	F	9.375	13.284	13.075	14.350	15.262
	P	<0.001	<0.001	<0.001	<0.001	<0.001
	% Var	12.09	17.47	16.44	15.79	21.57
% *Acacia*	F	6.703	11.020	7.748	7.570	11.109
	P	<0.001	<0.001	<0.001	<0.001	<0.001
	% Var	9.04	15.02	10.63	9.29	16.75
% Road	F	2.636	3.550	2.235	2.465	1.352
	P	0.005	0.002	0.033	0.028	*0.247
	% Var	3.83	5.50	3.38	3.31	2.42

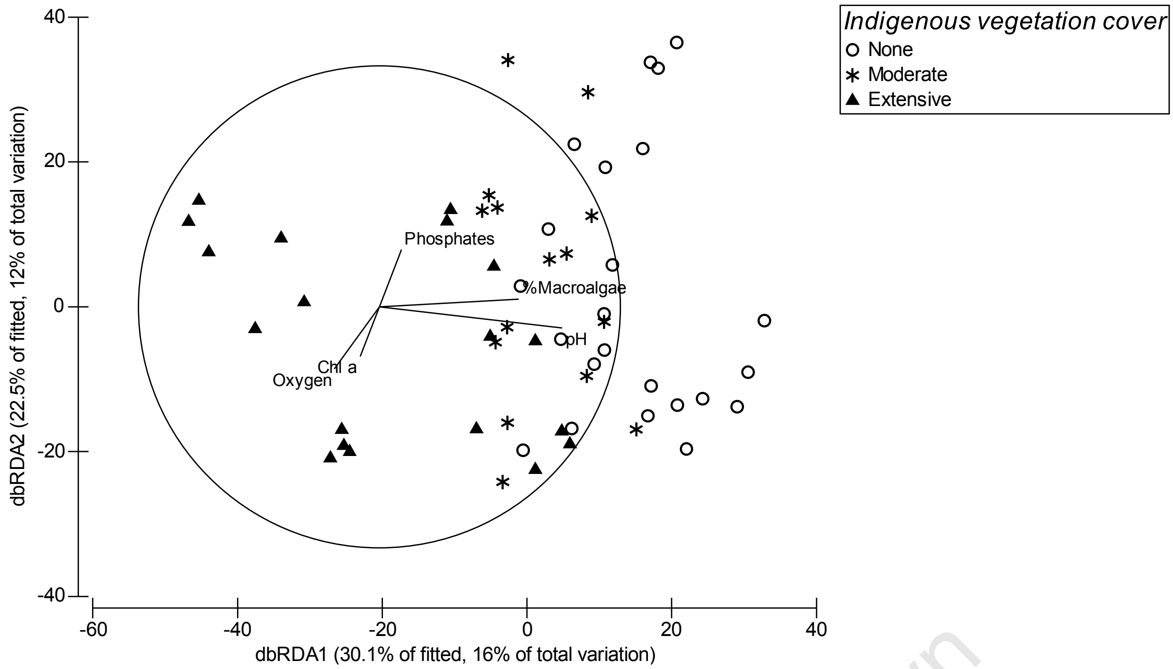


Figure 4.5. dbRDA ordination of macroinvertebrate assemblage composition among sites (Bray-Curtis similarity), constrained by the environmental variables. Explained variation in the fitted model and total explained variation is indicated for each axis. The subset of environmental variables used here were those which were pre-selected using dbRDA step-wise selection. Variables with a correlation of r < 0.2 are not displayed on the plot. The four variables representing time were included as covariables in the step-wise and final model. The level of habitat transformation around wetlands is proxied by the remaining amount of indigenous vegetation within 100 m. Sites are coded according to three broad levels of surrounding indigenous vegetation cover: none; moderate (ranging between 33 and 51% cover); and extensive (ranging between 75 and 99% cover). These categories represent extensive, moderate and minimal levels of habitat transformation respectively.

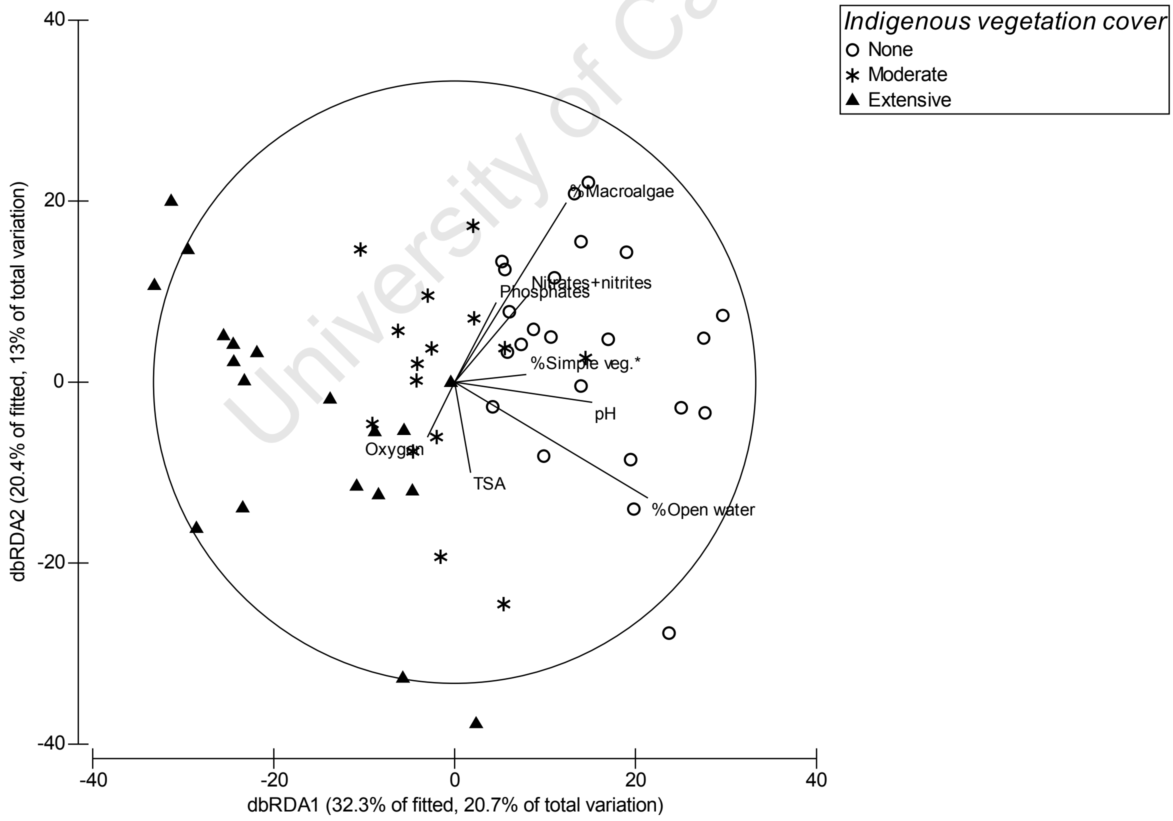


Figure 4.6. dbRDA ordination of microcrustacean assemblage composition among sites (Bray-Curtis similarity), constrained by the environmental variables. Explained variation in the fitted model and total explained variation is indicated for each axis. The subset of environmental variables used here were those which were pre-selected using dbRDA step-wise selection. Variables with a correlation of r < 0.2 are not displayed on the plot. The four variables representing time were included as covariables in the step-wise and final model. The level of habitat transformation around wetlands is proxied by the remaining amount of indigenous vegetation within 100 m. Sites are coded according to three broad levels of surrounding indigenous vegetation cover: none; moderate (ranging between 33 and 51% cover); and extensive (ranging between 75 and 99% cover). These categories represent extensive, moderate and minimal levels of habitat transformation respectively. TSA – total surface area.

4.3.3. Multivariate analyses: variation partitioning

Environmental changes in wetlands along the gradient of habitat transformation at Kenilworth explained 10.4% and 17.1% of the variation in macroinvertebrate and microcrustacean assemblage composition respectively (segment “e” in Figs 4.7 and 4.8). Accordingly, microcrustaceans appeared to be more affected by environmental changes associated with the habitat transformation gradient than were macroinvertebrates. These “habitat transformation x environment” contributions were considerably larger than those from habitat transformation only, independent of environmental changes (segment “b” in both plots). However, the small unique contributions from habitat transformation alone to explained variation were statistically significant (P = 0.004 and 0.002 for macroinvertebrates and microcrustaceans respectively). Microcrustacean assemblage composition was, on the whole, slightly better explained by the predictor variables collected in this study than was the case for macroinvertebrates, as reflected by the smaller total amount of explained variation in Fig. 4.7 (65.5%) compared to Fig. 4.8 (72.1%). Invertebrate assemblage composition in the wetlands at Kenilworth was largely explained by the unique influence of environmental factors (i.e. independent of the habitat transformation gradient and temporal or spatial factors). This environmental contribution (segment “c” in Figs 4.7 and 4.8) was slightly more than twice that of any other contribution to explained variation in both plots. Consequently the influence of natural variation in environmental factors on wetland invertebrate assemblages was considerably higher than that from anthropogenic-induced variation in environmental conditions due to habitat transformation around wetlands.

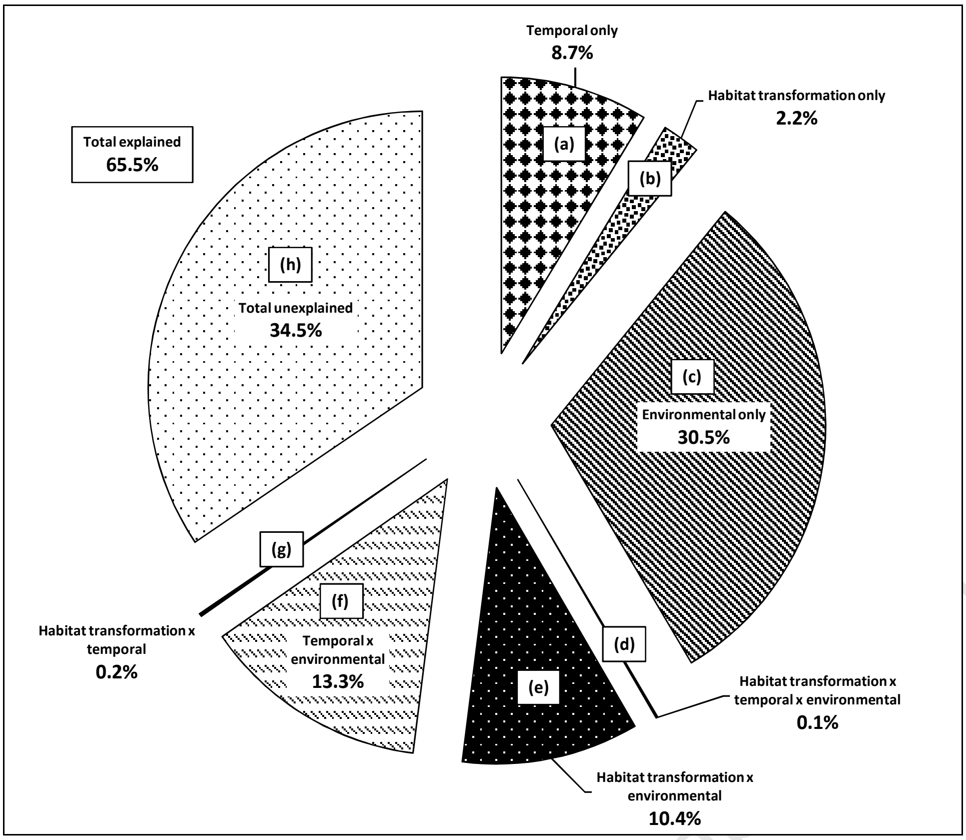


Figure 4.7. Results of the variation partitioning procedure for multivariate regressions of macroinvertebrate assemblage composition on temporal, environmental and habitat transformation variables. The contributions to explained variation in assemblage composition are represented by segments of the pie chart (a – h).

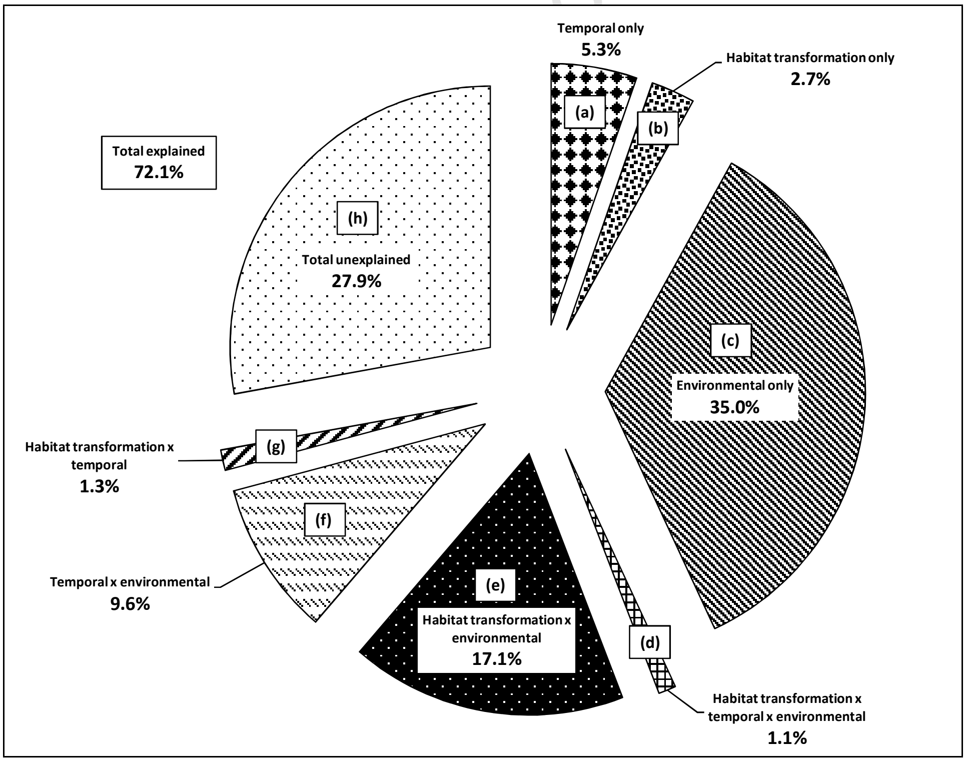


Figure 4.8. Results of the variation partitioning procedure for multivariate regressions of microcrustacean assemblage composition on temporal, environmental and habitat transformation variables. The contributions to explained variation in assemblage composition are represented by segments of the pie chart (a – h).

4.3.4. Univariate analyses

Environmental variables associated with habitat transformation

Table 4.3 presents the linear relationships between each of the environmental variables measured in this study (response variables) and the percentage cover of indigenous vegetation around wetlands as a proxy for habitat transformation. Eight environmental variables describing the physico-chemistry or biotope characteristics of wetlands were significantly associated ($P < 0.05$) with the habitat transformation gradient at Kenilworth (variables a – h). Six of these relationships (a – f) were highly significant ($P < 0.001$). The standardised β coefficients for the six highly significant relationships were all > | 0.5 |, indicating strong linear slopes for these relationships. Of the eight significant relationships, pH, % macroalgae, % open water, phosphates, % simple vegetation (reeded) and turbidity in wetlands all displayed negative relationships with the amount of indigenous vegetation around wetlands. The concentration of humics and % complex vegetation in wetlands were positively related to the amount of surrounding indigenous vegetation. The amount of explained variation (partial r^2) in each of the response variables that could be attributed to the amount of indigenous vegetation (i.e. habitat transformation) ranged from 8.6% (turbidity) to 55.1% (pH) for those relationships that were statistically significant. Nine variables (i – q in Table 4.3) were not significantly related to the gradient of habitat transformation and the percentage of explained variation for these variables ranged from < 0.01% (dissolved oxygen) to 2.8% (maximum depth). The two variables representing the hydro-morphometry of wetlands (maximum depth and total surface area) were not significantly related to the habitat transformation gradient, as was expected given that an effort was made to compare wetlands of similar size and depth in this study so as to minimise the confounding effects of these factors.

Partial residual plots (Fig. 4.9) depicting the eight significant relationships in Table 4.3 reveal generally strong linear patterns between these environmental variables and the gradient of habitat transformation. The first six plots (a – f), which formed the highly significant relationships in Table 4.3 ($P < 0.001$), showed the strongest patterns with minimal scatter and few outliers. Three clear outliers are present in plot “b” (% macroalgae) at point zero on the x axis (no surrounding indigenous vegetation), but otherwise the negative linear relationship remains intact for this variable. Plot “f” (% complex vegetation) has five outlying points occurring at the ~35% mark on the x axis, but otherwise presents a positive linear trend. The amount of simple vegetation (reeded) in wetlands did not show a clear linear decrease with the amount of indigenous vegetation surrounding wetlands and instead showed a unimodal pattern of response with moderately transformed wetlands having the highest amounts of simple vegetation (plot “g”). Turbidity, despite being significantly related to amount of surrounding indigenous vegetation ($P < 0.05$), displayed a weak negative trend in plot “h”. This is also reflected by its relatively shallow slope (β coefficient) in relation to the other significant variables (Table 4.3). Despite one clear outlier in plot “h”, the amount of scatter is minimal for this relationship. Sites with no or moderate surrounding cover of indigenous vegetation appear to have similar turbidity levels, whereas wetlands surrounded by extensive indigenous vegetation appear to be less turbid than those with no or moderate surrounding indigenous vegetation cover. Therefore the low turbidity of wetlands surrounded by extensive indigenous vegetation appears to be the driving the overall linear trend in plot “h”. In summary, six environmental variables (a – f) show clear linear relationships with the amount of indigenous vegetation cover around wetlands (as a proxy for habitat transformation), whilst the amount of simple structured vegetation habitat (reeded) showed a unimodal rather than linear response to habitat transformation and turbidity was only noticeably lower for untransformed sites, but similar among moderately and extensively transformed sites.

Table 4.3. Multiple linear regressions of environmental response variables (a - q) regressed against the amount of indigenous vegetation cover within 100 m of wetlands (universal predictor variable). The four variables representing time were included as covariables in all regressions, resulting in 51 residual degrees of freedom in each model. Results are listed in decreasing order of relationship strength based on P values. Only partial relationships between the response variables and amount of indigenous vegetation are reported here, not the full model results. β – standardized regression coefficient; SE – standard error of regression coefficient; Partial r^2 – coefficient of partial determination. Significant P values ($P < 0.05$) are presented in boldface.

RESPONSE VARIABLES	β	SE	Partial r^2	t(51)	P
a) pH	-0.734	0.093	0.551	-7.913	**<0.001**
b) % Macroalgae	-0.670	0.098	0.477	-6.826	**<0.001**
c) % Open water	-0.663	0.096	0.482	-6.890	**<0.001**
d) Humics	0.651	0.105	0.428	6.179	**<0.001**
e) Phosphates	-0.623	0.101	0.425	-6.144	**<0.001**
f) % Complex vegetation	0.518	0.112	0.297	4.637	**<0.001**
g) % Simple vegetation (reeded)	-0.342	0.129	0.122	-2.658	**0.010**
h) Turbidity	-0.279	0.127	0.086	-2.192	**0.033**
i) Maximum depth	-0.157	0.131	0.028	-1.205	0.234
j) Nitrates + nitrites	-0.133	0.137	0.018	-0.970	0.336
k) Conductivity	-0.131	0.110	0.027	-1.201	0.235
l) Chlorophyll *a*	0.089	0.125	0.010	0.717	0.476
m) Total surface area	0.087	0.135	0.008	0.647	0.521
n) Average temperature	-0.082	0.051	0.049	-1.627	0.110
o) Ammonium	-0.057	0.060	0.018	-0.953	0.345
p) % Simple vegetation (sedge)	0.039	0.138	0.002	0.285	0.776
q) Dissolved oxygen	0.008	0.117	<0.001	0.069	0.945

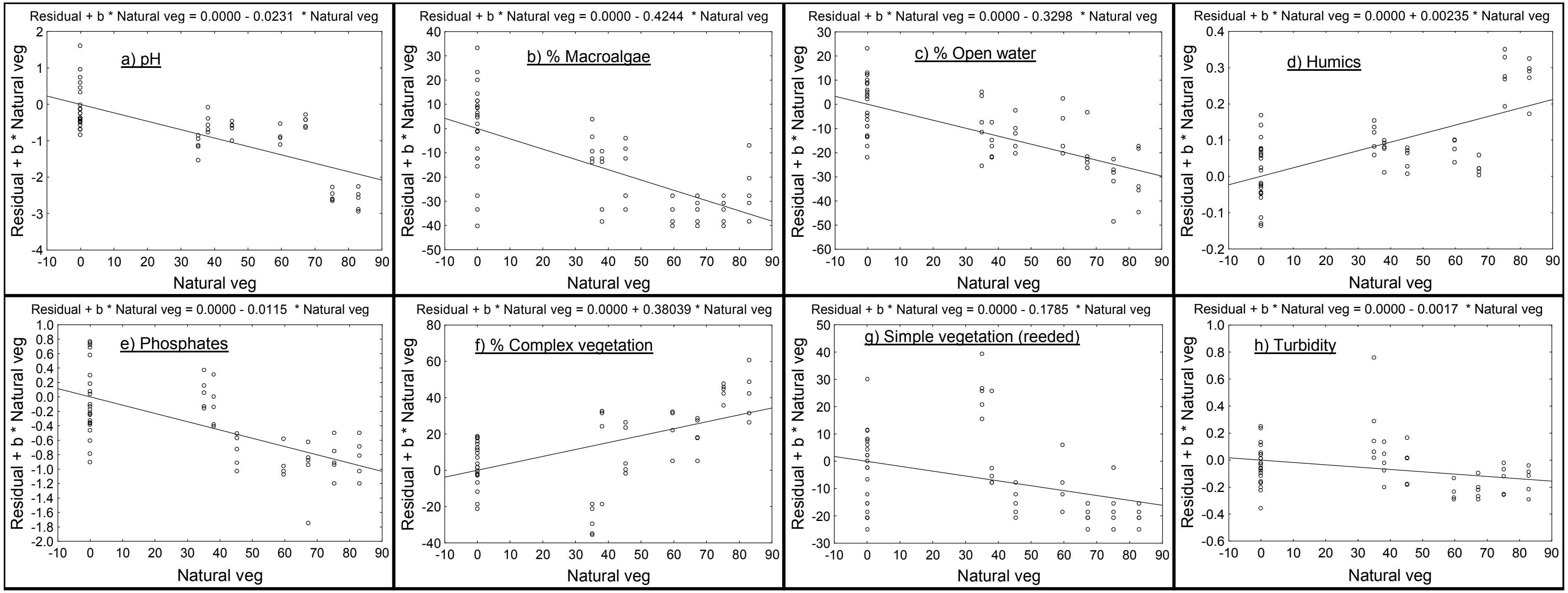


Figure 4.9. Partial residual plots displaying relationships between individual environmental response variables (a - h) and the percentage cover of indigenous vegetation within 100m of wetlands ('Natural veg'), holding the temporal covariables constant. Percentage variables were arcsine square root transformed. Humics, phosphates and turbidity were $\log_{10}(x+1)$ transformed.

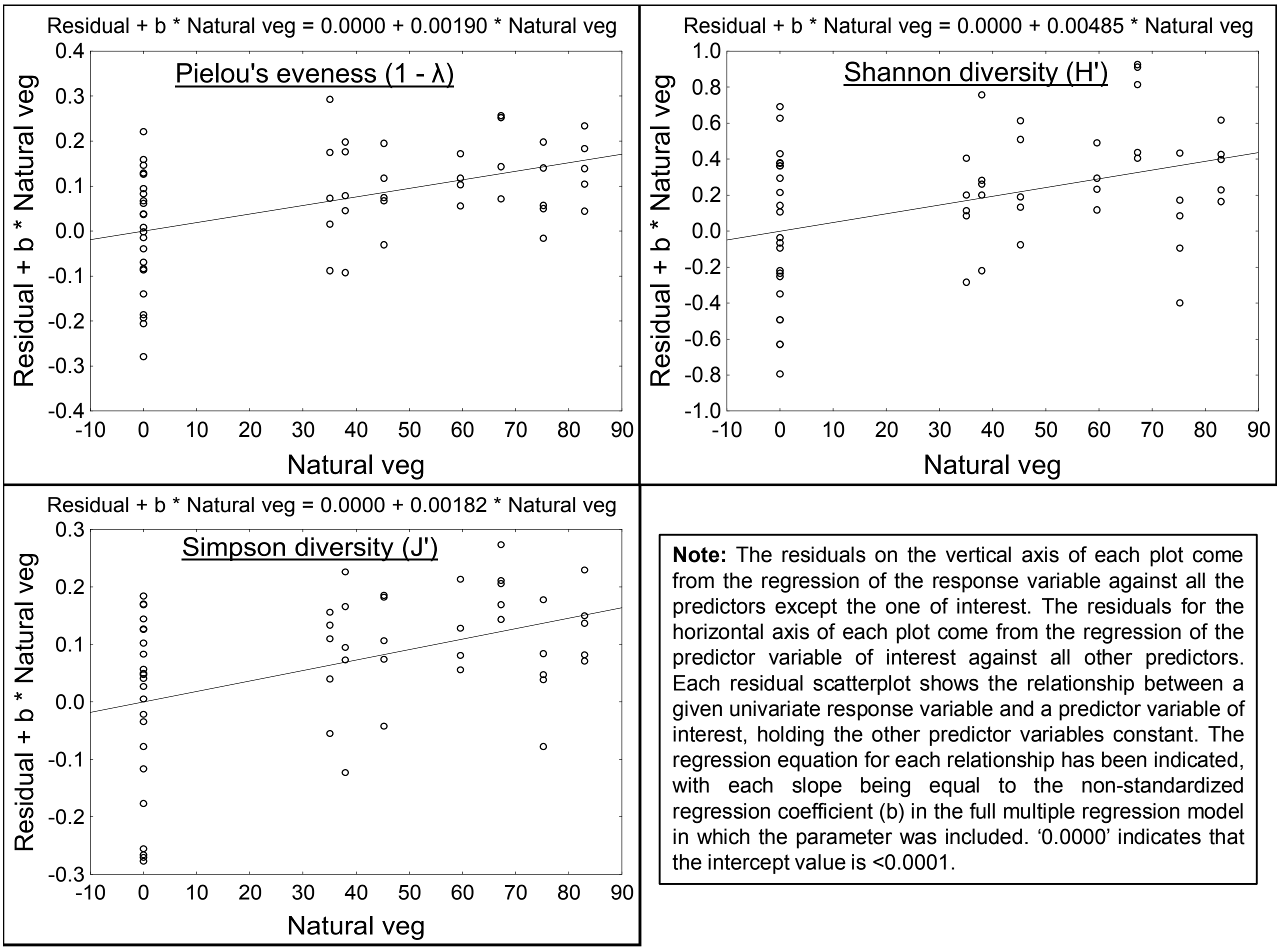
Note: The residuals on the vertical axis of each plot come from the regression of the response variable against all the predictors except the one of interest. The residuals for the horizontal axis of each plot come from the regression of the predictor variable of interest against all other predictors. Each residual scatterplot shows the relationship between a given univariate response variable and a predictor variable of interest, holding the other predictor variables constant. The regression equation for each relationship has been indicated, with each slope being equal to the non-standardized regression coefficient (b) in the full multiple regression model in which the parameter was included. '0.0000' indicates that the intercept value is <0.0001.

Taxon richness and diversity

The three diversity measures for macroinvertebrates (Pielou's evenness, Shannon diversity and Simpson diversity) were significantly and positively related to levels of indigenous vegetation around wetlands, but no significant relationships were found for the two richness measures (number of taxa and Margalef's richness, Table 4.4a). Visual assessment of the significant relationships between macroinvertebrate diversity measures and percentage cover of surrounding indigenous vegetation (Fig. 4.10) indicates that these results are not particularly reliable due to the large amount of spread in the residuals for wetlands with no surrounding indigenous vegetation within 100 m (point zero on the x axis). Although there is otherwise a positive relationship in the distribution of the partial residual points, the large amount of vertical scatter in residuals at point zero on the x axis prevents the pattern from being clear or reliable. None of the microcrustacean richness or diversity measures was significantly related to levels of indigenous vegetation around wetlands (Table 4.4b).

Table 4.4. Multiple linear regression models for macroinvertebrate (a) and microcrustacean (b) richness and diversity measures regressed against the amount of indigenous vegetation within 100 m of wetlands (universal predictor variable). The four variables representing time were included as covariables in all regressions, resulting in 51 residual degrees of freedom in each model. Only partial relationships between the response variables and amount of indigenous vegetation are reported here, not the full model results. β – standardized regression coefficient; SE – standard error of regression coefficient; Partial r^2 – coefficient of partial determination. Significant P values ($P < 0.05$) are presented in boldface.

RESPONSE VARIABLES	β	SE	Partial r^2	t(51)	P
a) Macroinvertebrates					
S: number of taxa	0.009	0.120	0.000	0.077	0.939
d: Margalef's richness	0.160	0.135	0.027	1.183	0.242
J': Pielou's evenness	0.430	0.111	0.227	3.873	**<0.001**
H': Shannon diversity	0.360	0.118	0.154	3.051	**0.004**
1-λ: Simpson diversity	0.402	0.116	0.189	3.449	**0.001**
b) Microcrustaceans					
S: number of taxa	-0.202	0.129	0.046	-1.564	0.124
d: Margalef's richness	-0.232	0.132	0.057	-1.752	0.086
J': Pielou's evenness	-0.030	0.129	0.001	-0.236	0.814
H': Shannon diversity	-0.172	0.127	0.035	-1.353	0.182
1-λ: Simpson diversity	-0.175	0.126	0.036	-1.388	0.171



Note: The residuals on the vertical axis of each plot come from the regression of the response variable against all the predictors except the one of interest. The residuals for the horizontal axis of each plot come from the regression of the predictor variable of interest against all other predictors. Each residual scatterplot shows the relationship between a given univariate response variable and a predictor variable of interest, holding the other predictor variables constant. The regression equation for each relationship has been indicated, with each slope being equal to the non-standardized regression coefficient (b) in the full multiple regression model in which the parameter was included. ‘0.0000’ indicates that the intercept value is <0.0001.

Figure 4.10. Partial residual plots displaying relationships between the three univariate measures of macroinvertebrate taxon diversity and the amount of indigenous vegetation within 100 m of wetlands (“Natural veg”), holding the temporal covariables constant. These three measures were significantly related to the habitat transformation gradient (Table 4.4). Measures of richness or diversity that were not significant in Table 4.4 are not reported here.

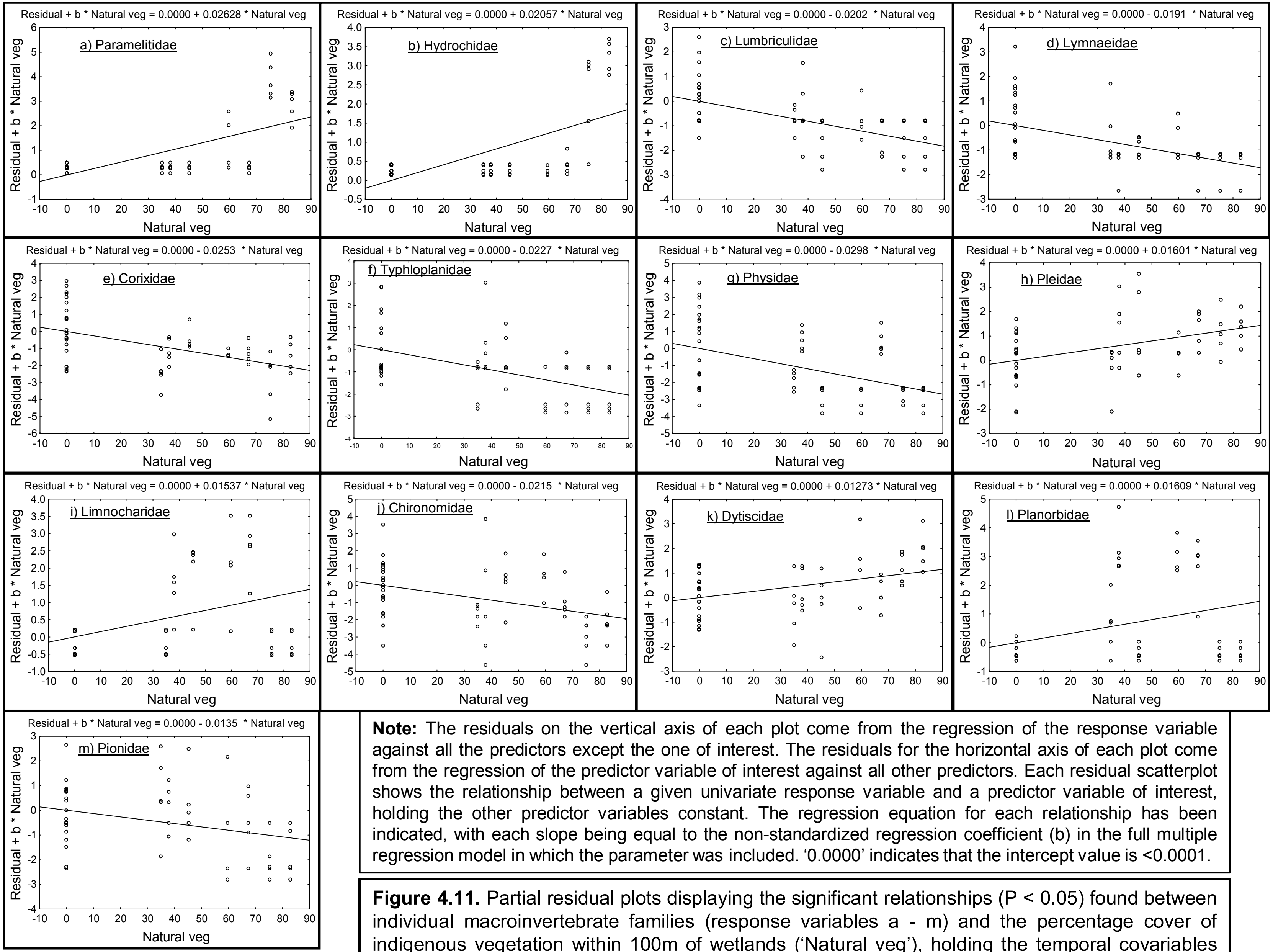
Family-level tests for indicator taxa

Twenty-nine macroinvertebrate families (i.e. excluding those present in < 10% of samples) were tested as potential indicators of habitat transformation. Thirteen of these families presented significant relationships when regressed on the amount of indigenous vegetation within 100 m of wetlands (Table 4.5). The relationships are visually depicted in the partial residual plots of Figure 4.11. Non-significant relationships between macroinvertebrate or microcrustacean families and the gradient of habitat transformation are not presented here. Eleven of the relationships were strongly significant ($P < 0.01$) and of these seven were highly significant ($P < 0.001$). This contrasts with the mere three families that were significantly related to habitat transformation (within 100 m) at the broad scale (Table 3.13 in chapter 3). Figure 4.11 depicts vastly stronger relationships than those presented by macroinvertebrate families at the broad scale (Fig. 3.7 in chapter 3). The amount of scatter is considerably reduced in the former plots and hence confidence for inferring levels of disturbance is improved. Although the plots in Figure 4.11 are an improvement on those of Figure 3.7, they still display a moderate degree of scatter and their reliability for inferring levels of transformation in the landscape does not appear to be high. The first eight plots (a – h) in Figure 4.11 show the clearest patterns with considerably less scatter than plots i – m. Variation explained (partial r^2) in the response variables due to habitat transformation within 100 m of wetlands is considerably higher in this study (Table 4.5) than observed at the broad scale in chapter 3 (Table 3.13).

Of the eight microcrustacean families tested for linear relationships with the habitat transformation gradient, five were found to be significant (Table 4.6). Of these, four had P values <0.01 and three had P values <0.001. These results are in contrast to those of chapter 3, where only one microcrustacean family (Daphniidae) showed a significant relationship with habitat transformation (Table 3.13). Visual representation of the relationships in this study (Fig. 4.12) indicates that the Chydoridae, Cyprididae and Cypridopsidae (plots a – c) show the best linear patterns of response to the habitat transformation gradient, despite the three outliers present in each of plots “a” and “c” (at point zero on the x axis). The Lymnocytheridae and Macrothricidae relationships (plots “d” and “e”) show considerable scatter and are dominated by outliers, thus not presenting these families as good indicator taxa.

Table 4.5. Multiple linear regressions of individual macroinvertebrate families (response variables a - m) regressed against the amount of indigenous vegetation cover within 100 m of wetlands (universal predictor variable). Only significant relationships ($P < 0.05$) are presented here. Results are listed in decreasing order of relationship strength based on P values. The four variables representing time were included as covariables in all regressions, resulting in 51 residual degrees of freedom in each model. Only partial relationships between the response variables and amount of indigenous vegetation are reported here, not the full model results. β – standardized regression coefficient; SE – standard error of regression coefficient; Partial r^2 – coefficient of partial determination.

RESPONSE VARIABLES	β	SE	Partial r^2	t(51)	P
a) Paramelitidae	0.654	0.105	0.433	6.236	<0.001
b) Hydrochidae	0.627	0.108	0.396	5.781	<0.001
c) Lumbriculidae	-0.451	0.095	0.308	-4.769	<0.001
d) Lymnaeidae	-0.453	0.107	0.258	-4.213	<0.001
e) Corixidae	-0.406	0.099	0.249	-4.115	<0.001
f) Typhloplanidae	-0.417	0.102	0.246	-4.078	<0.001
g) Physidae	-0.438	0.119	0.211	-3.691	<0.001
h) Pleidae	0.327	0.097	0.182	3.371	0.001
i) Limnocharidae	0.379	0.125	0.152	3.026	0.004
j) Chironomidae	-0.327	0.114	0.139	-2.870	0.006
k) Dytiscidae	0.310	0.114	0.126	2.712	0.009
l) Planorbidae	0.336	0.131	0.114	2.566	0.013
m) Pionidae	-0.258	0.117	0.088	-2.219	0.031

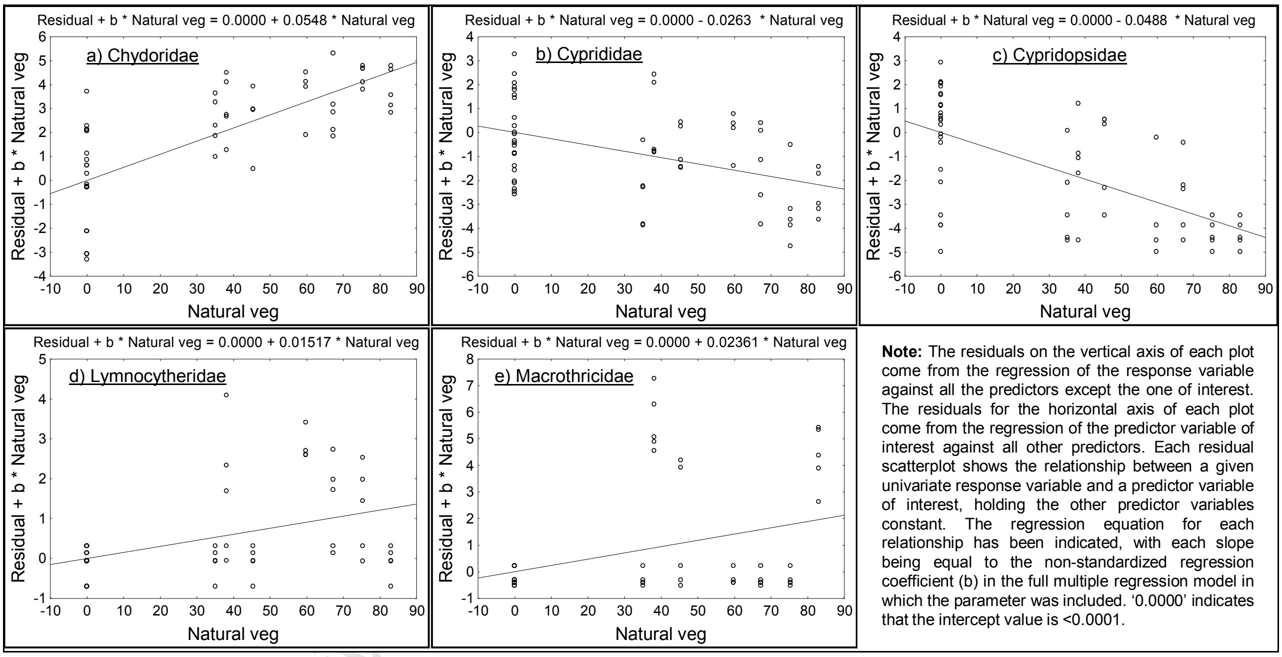


Note: The residuals on the vertical axis of each plot come from the regression of the response variable against all the predictors except the one of interest. The residuals for the horizontal axis of each plot come from the regression of the predictor variable of interest against all other predictors. Each residual scatterplot shows the relationship between a given univariate response variable and a predictor variable of interest, holding the other predictor variables constant. The regression equation for each relationship has been indicated, with each slope being equal to the non-standardized regression coefficient (b) in the full multiple regression model in which the parameter was included. '0.0000' indicates that the intercept value is <0.0001.

Figure 4.11. Partial residual plots displaying the significant relationships ($P < 0.05$) found between individual macroinvertebrate families (response variables a - m) and the percentage cover of indigenous vegetation within 100m of wetlands ('Natural veg'), holding the temporal covariables constant. Percentage cover has been arcsine square root transformed.

Table 4.6. Multiple linear regressions of individual microcrustacean families (response variables a - e) regressed against the amount of indigenous vegetation cover within 100 m of wetlands (universal predictor variable). Only significant relationships ($P < 0.05$) are presented here. Results are listed in decreasing order of relationship strength based on P values. The four variables representing time were included as covariables in all regressions, resulting in 51 residual degrees of freedom in each model. Only partial relationships between the response variables and amount of indigenous vegetation are reported here, not the full model results. β – standardized regression coefficient; SE – standard error of regression coefficient; Partial r^2 – coefficient of partial determination.

RESPONSE VARIABLES	β	SE	Partial r^2	t(51)	P
a) Chydoridae	0.670	0.084	0.557	8.001	<0.001
b) Cypridopsidae	-0.615	0.107	0.396	-5.777	<0.001
c) Cyprididae	-0.423	0.121	0.193	-3.492	<0.001
d) Lymnocytheridae	0.399	0.122	0.173	3.266	0.002
e) Macrothricidae	0.340	0.131	0.117	2.605	0.012



Note: The residuals on the vertical axis of each plot come from the regression of the response variable against all the predictors except the one of interest. The residuals for the horizontal axis of each plot come from the regression of the predictor variable of interest against all other predictors. Each residual scatterplot shows the relationship between a given univariate response variable and a predictor variable of interest, holding the other predictor variables constant. The regression equation for each relationship has been indicated, with each slope being equal to the non-standardized regression coefficient (b) in the full multiple regression model in which the parameter was included. '0.0000' indicates that the intercept value is <0.0001.

Figure 4.12. Partial residual plots displaying the significant relationships ($P < 0.05$) found between individual microcrustacean families (response variables a - e) and the percentage cover of indigenous vegetation within 100 m of wetlands ("Natural veg"), holding the temporal covariables constant. Percentage cover has been arcsine square root transformed.

Testing potential metrics

Thirty macroinvertebrate metrics (Appendix 10) were tested against the gradient of habitat transformation in this study. Sixteen of these presented significant relationships (Table 4.7), whilst in chapter 3 only five of these metrics were significantly related to habitat transformation within 100 m (Table 3.14). Of the 16 linear regression models presented in Table 4.7, nine relationships were strong ($P < 0.01$) and of these six were highly significant ($P < 0.001$). Non-significant relationships between macroinvertebrate or microcrustacean metrics and the gradient of habitat transformation are not presented here. Depicting the significant relationships with partial residual plots (Fig. 4.13) revealed vastly improved patterns in comparison to those presented at the broad scale in chapter 3 (Fig. 3.8), but shows that there is still considerable scatter in the plots. Thus, although the inferential power has increased from the broad to fine scale, the patterns are still only moderately reliable. Scatter among residual points in the plots of Figure 4.13 is generally most pronounced at the zero point on the x axes, where wetlands had no surrounding indigenous vegetation cover. Taken on the whole, however, macroinvertebrate metric relationships with habitat transformation are considerably clearer at the small scale of this study than for the coarse scale examined in chapter 3. This is further reinforced by comparison of the standardised β and partial r^2 values among the two studies, which are both noticeably higher for the metrics reported in the current study. Five of the 15 microcrustacean metrics tested against the gradient of habitat transformation in this study presented significant relationships (Table 4.8). Following the same reasoning as described above for macroinvertebrate metrics, patterns appeared to be stronger at the fine scale of this study than for those observed for the significant metrics presented in chapter 3 (c.f. Fig. 4.14 *versus* Fig. 3.9). Once again, there was a reasonable amount of scatter among residuals at point zero on the x axis of plots in this study (Fig. 4.14).

Table 4.7. Multiple linear regressions of macroinvertebrate metrics (response variables a - p) regressed against the amount of indigenous vegetation cover within 100 m of wetlands (universal predictor variable). Only significant relationships ($P < 0.05$) are presented here. Results are listed in decreasing order of relationship strength based on P values. The four variables representing time were included as covariables in all regressions, resulting in 51 residual degrees of freedom in each model. Only partial relationships between the response variables and amount of indigenous vegetation are reported here, not the full model results. β – standardized regression coefficient; SE – standard error of regression coefficient; Partial r^2 – coefficient of partial determination.

RESPONSE VARIABLES	β	SE	Partial r^2	t(51)	P
a) Corixidae (as % of Coleoptera and Hemiptera)	-0.578	0.111	0.348	-5.218	<0.001
b) % Coleopteran individuals (of total sample count)	0.537	0.105	0.338	5.101	<0.001
c) Total number of “intolerant” individuals (All “intolerant” taxa)	0.418	0.101	0.251	4.138	<0.001
d) Average score per taxon (SASS)	0.467	0.115	0.244	4.060	<0.001
e) Total number of “intolerant” individuals (AAA - Acarina+Aeshnidae+Amphipoda)	0.410	0.106	0.228	3.879	<0.001
f) Total number of Gastropod individuals	-0.432	0.119	0.206	-3.638	<0.001
g) % “Intolerant” individuals (AAA) of total sample count	0.379	0.114	0.179	3.329	0.002
h) % “Intolerant” individuals (All “intolerant” taxa) of total sample count	0.373	0.113	0.175	3.289	0.002
i) % Scrapers	-0.352	0.119	0.147	-2.967	0.005
j) Total number of “tolerant” Coleopteran individuals	0.300	0.112	0.123	2.672	0.010
k) Total number of Coleopteran individuals	0.293	0.117	0.110	2.516	0.015
l) Total number of individuals in dominant taxon	-0.286	0.117	0.105	-2.446	0.018
m) Total number of individuals in dominant three taxa	-0.267	0.111	0.102	-2.411	0.020
n) Sum (Physidae + Planorbidae individuals)	-0.299	0.128	0.097	-2.335	0.023
o) Total number of individuals	-0.240	0.107	0.090	-2.243	0.029
p) % Predators	0.273	0.125	0.085	2.182	0.034

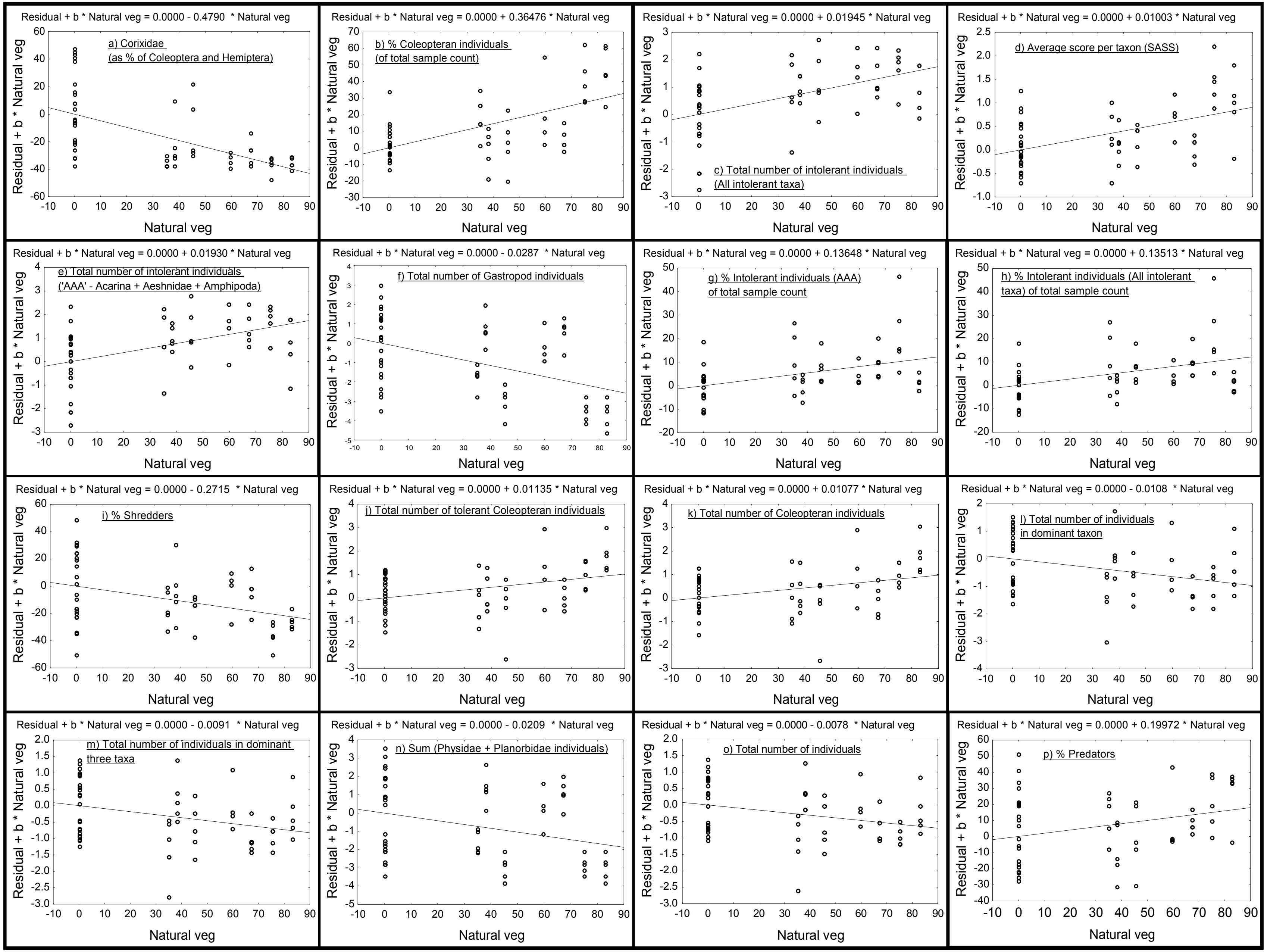
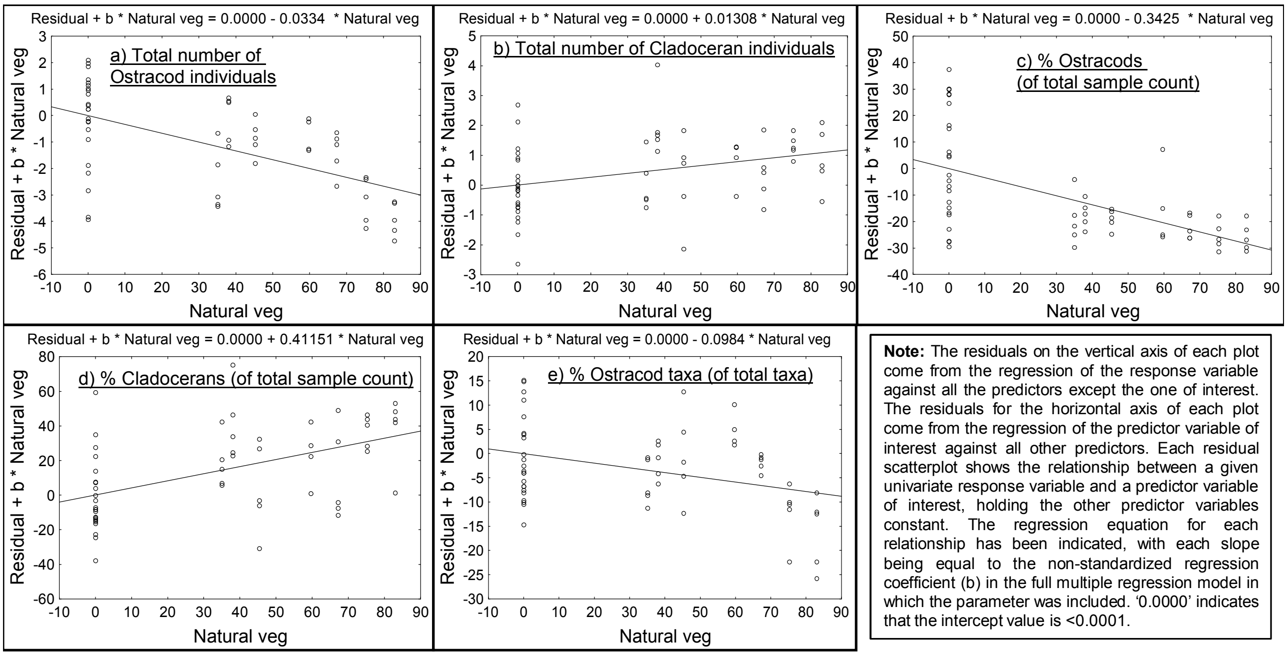


Figure 4.13. Partial residual plots displaying the significant relationships ($P < 0.05$) found between macroinvertebrate metrics (response variables a - p) and the percentage cover of indigenous vegetation within 100 m of wetlands ('Natural veg'), holding the temporal covariables constant. Percentage cover has been arcsine square root transformed.

Note: The residuals on the vertical axis of each plot come from the regression of the response variable against all the predictors except the one of interest. The residuals for the horizontal axis of each plot come from the regression of the predictor variable of interest against all other predictors. Each residual scatterplot shows the relationship between a given univariate response variable and a predictor variable of interest, holding the other predictor variables constant. The regression equation for each relationship has been indicated, with each slope being equal to the non-standardized regression coefficient (b) in the full multiple regression model in which the parameter was included. '0.0000' indicates that the intercept value is <0.0001.

Table 4.8. Multiple linear regressions of microcrustacean metrics (response variables a - e) regressed against the amount of indigenous vegetation cover within 100 m of wetlands (universal predictor variable). Only significant relationships ($P < 0.05$) are presented here. Results are listed in decreasing order of relationship strength based on P values. The four variables representing time were included as covariables in all regressions, resulting in 51 residual degrees of freedom in each model. Only partial relationships between the response variables and amount of indigenous vegetation are reported here, not the full model results. β – standardized regression coefficient; SE – standard error of regression coefficient; Partial r^2 – coefficient of partial determination.

RESPONSE VARIABLES	β	SE	Partial r^2	t(51)	P
a) % Ostracods (of total sample count)	-0.569	0.108	0.350	-5.244	<0.001
b) Total number of Ostracod individuals	-0.527	0.104	0.336	-5.081	<0.001
c) % Cladocerans (of total sample count)	0.439	0.105	0.255	4.183	<0.001
d) % Ostracod taxa (of total taxa)	-0.350	0.129	0.126	-2.714	0.009
e) Total number of Cladoceran individuals	0.280	0.110	0.113	2.544	0.014



Note: The residuals on the vertical axis of each plot come from the regression of the response variable against all the predictors except the one of interest. The residuals for the horizontal axis of each plot come from the regression of the predictor variable of interest against all other predictors. Each residual scatterplot shows the relationship between a given univariate response variable and a predictor variable of interest, holding the other predictor variables constant. The regression equation for each relationship has been indicated, with each slope being equal to the non-standardized regression coefficient (b) in the full multiple regression model in which the parameter was included. '0.0000' indicates that the intercept value is <0.0001.

Figure 4.14. Partial residual plots displaying the significant relationships ($P < 0.05$) found between microcrustacean metrics (response variables a - e) and the percentage cover of indigenous vegetation within 100 m of wetlands ("Natural veg"), holding the temporal covariables constant. Percentage cover has been arcsine square root transformed.

4.4. DISCUSSION

4.4.1. Environmental changes associated with habitat transformation

Environmental conditions within wetlands were significantly related to the amount of terrestrial indigenous fynbos vegetation within 100 m (Table 4.2). Sites in the extensively invaded landscape at Youngsfield showed clear and consistent environmental separation from those in the core conservation area at KRCA, whilst sites in a moderately transformed setting generally overlapped with the extensively transformed sites in terms of their environmental conditions (Fig. 4.4). These findings suggests that the characteristic environmental "signature" of temporary wetlands occurring within lowland sand fynbos can be altered by even moderate transformation of the surrounding landscape. This has conservation implications for remaining wetlands in lowland areas such as the Cape Flats in that it appears to be crucially important to maintain an extensive cover of fynbos vegetation within 100 m of wetlands in order to prevent significant alteration of the environmental template that characterises these systems. This is an extension of the recommendation stated in chapter 2, whereby it was suggested that conserving a natural vegetation buffer within 100 m of wetlands would be an effective step towards maintaining natural environmental conditions in temporary wetlands. The findings in the current chapter extend this by suggesting that it is required to conserve an extensive cover of indigenous fynbos within 100 m of wetlands and that conserving only a moderate cover of fynbos may not be enough to ensure the maintenance of natural environmental conditions in these wetlands. From a restoration perspective, the environmental character of wetlands occurring in moderately transformed landscapes (such as vacant land that has been partly invaded by alien vegetation) could potentially be restored if land within a 100 m radius is cleared of alien vegetation and re-planted with fynbos vegetation.

To my knowledge, no other quantitative studies have addressed the conservation of temporary wetland environmental conditions in lowland areas of the south-western Cape and the current study provides important baseline data in this regard. Although these recommendations are drawn from the results of a study on a single area, the wetlands inside the racetrack at Kenilworth are likely to be representative of the natural state for temporary wetlands across vast lowland areas that were once naturally vegetated with Sand fynbos. Thus, it is expected that the findings at Kenilworth are more broadly applicable than to just the Kenilworth system itself. Further studies on temporary wetlands in other areas of Sand fynbos are expected to confirm these generalities, but the difficulty will lie in finding suitable study areas that have sufficient numbers of unimpaired wetlands, given that extremely few wetlands in these lowland areas remain within untransformed fynbos.

Within the broad category of “environmental conditions”, both physico-chemical and biotope characteristics in wetlands were significantly related to the habitat transformation gradient at Kenilworth and the amounts of explained variation in these multivariate matrices due to habitat transformation within 100 m (physico-chemical: 14.77%; biotope: 25.42%; Table 4.2) were considerably higher in comparison to the reported values in chapter 2 (physico-chemical: 2.62%, Table 2.2; biotope: 2.26%, Table 2.3). Despite these relationships being stronger at the fine scale of this study, there was still a large amount of environmental variation between individual wetlands independent of the habitat transformation gradient, as signalled by the high dispersion among sites within each of the transformation categories in Figure 4.4. This indicates that temporary depression wetlands can display high levels of environmental heterogeneity even at small spatial scales, irrespective of human disturbance levels in the landscape.

A suite of environmental factors in wetlands were significantly related to the habitat transformation gradient at Kenilworth (Table 4.3). Six environmental variables presented strong, clear relationships ($P < 0.001$, > 20% explained variation) and are discussed further here. The strongest response appeared to be for pH (55.1% explained variation), which was generally low for wetlands in the core conservation area and increased linearly as indigenous vegetation around wetlands was replaced by alien species. There was fairly strong leverage in this trend from the two sites surrounded by > 90% indigenous vegetation (sites 2 and 3, Appendix 14), which had pH values between 4 and 5 throughout the study (Appendix 15). Examination of the partial residual plot for pH in Figure 4.9 indicates a strong overall trend with no real outliers or strongly leveraged values, however. The relative concentration of humic compounds in wetlands showed an inverse relationship to that of pH, as expected, and displayed a strong positive correlation with the amount of indigenous vegetation around wetlands. There was more scatter in this relationship compared to that for pH (Fig. 4.9) and the amount of explained variation was lower (42.8%, Table 4.3), but the overall trend was still strong. The evidence gathered in this study thus firmly suggests that the loss of sclerophyllous Sand fynbos vegetation around wetlands results in decreased input of humic substances to the system (due to decreased leaching of tannins from surrounding vegetation), which in turn results in elevated pH levels. Although this is not a novel concept for aquatic ecosystems of the fynbos biome (see Gardiner 1988, Midgley and Schafer 1992), the relationship has never been quantified across a gradient of habitat transformation. This fundamental change in aquatic physico-chemical conditions due to the loss of fynbos habitat around waterbodies has not received any comment in the conservation literature, but is expected to result in cascading ecosystem impacts considering the important ecological role of humic compounds (and closely related pH levels) in aquatic environments (Gardiner 1988).

The strong negative relationship between phosphate concentrations in wetlands and amount of surrounding indigenous vegetation (42.5% explained variation, see Appendix 15 for raw phosphate values recorded at each wetland) is in line with the findings of Witkowski and Mitchell (1987). In brief, they found greatly elevated soil P levels in areas infested by *A. cyclops* (rooikrans) and *A. saligna* in comparison to an undisturbed Sand fynbos area and attributed this to the significantly higher leaf litterfall (and rapid turnover thereof) for acacias relative to fynbos. It was thus expected in the current study that phosphate levels in wetlands draining P-rich soils infested with *A. saligna* would be higher than for those draining oligotrophic fynbos soils. A comment on the data limitations is necessary here. Although results indicate a positive association between phosphate levels in wetlands and amount of surrounding alien vegetation, the correlative nature of these results cannot elucidate the mechanisms governing this trend. For instance, soil P levels were not measured in this study and thus the difference in soil P across the habitat transformation gradient was not quantified, although it has been assumed to mediate the relationship between amount of surrounding alien vegetation and phosphate levels in wetlands. Furthermore, alien vegetation invasion was represented by both *Acacia* and kikuyu species, and the relative influence of these species on soil P are not known. The presence of acacias might explain the relationship observed in this study, but other potential sources of nutrients are possible in an urban area such as this one. Confounding sources of nutrient input are possible given the context of this study in an urban area. However, this is unlikely given that all sites occurred on vacant land with no obvious sources of nutrients nearby. The racetrack itself consists of mowed unfertilized kikuyu and thus does not present a nutrient input source, and anyway if it did, it would be expected to mostly affect the undisturbed sites within the core conservation area. Slow groundwater flow at Kenilworth moves from west to east across the study area (McDowell 1989). A potential nutrient source occurs at the eastern end of KRCA in the form of a quarantine area for race horses, but wetland sites in this study were chosen so as to occur upstream (west) of groundwater flow emanating from this potential contaminant source (with the possible exception of site 7, situated approximately 500 m north-east of the quarantine area). The entire study area was equally surrounded by urban area at the broader scale (>500 m), and if anything, sites surrounded by fynbos tended to occur closer to the urban area boundary than moderately and extensively disturbed sites. Contamination of groundwater from surrounding urban areas is possible, but should equally affect all sites. There was no reason to expect elevated groundwater nutrient input from external urban sources for sites that were invaded by alien vegetation. However this is always a possibility and only a controlled, manipulative experiment focussing specifically on nutrient input of individual alien plant species into soil and water could establish causality of the relationship.

A surprising result from this study was that alien invasion did not appear to result in higher concentrations of nitrates + nitrites in wetland surface water (see Appendix 15 for raw nitrate + nitrite values recorded at each wetland), as was expected following the findings of Jovanovic *et al.* (2009) for groundwater quality in a comparable Sand fynbos system (Riverlands Nature Reserve). This could possibly be explained by the general predominance of kikuyu over acacias as the invasive species in the current study (for relative areal coverage see Appendix 14). Gaertner *et al.* (2011) found no elevation of nutrient levels in the soil of a kikuyu-dominated landscape in comparison to an adjacent Sand fynbos landscape, suggesting that perhaps kikuyu does not alter ecosystem nutrient levels as has been observed for acacias. For instance, whilst acacias are N_2-fixers and thus able to export N to the ecosystem, kikuyu is a nitrophilous opportunistic species, which makes use of the increased soil N made available by acacias, thereby reducing soil N levels (Gaertner *et al.* 2011). Unravelling the specific relationships between these two invasive species and ecosystem chemistry requires manipulative experimental work and would be a useful future research avenue towards understanding the impacts of these key invaders on both terrestrial and aquatic ecosystems.

After pH, the next strongest environmental association with the habitat transformation gradient at Kenilworth was the strong positive relationship with the percentage cover of macroalgae in wetlands. Although macroalgae may be treated as a biotic variable in ecological studies, it is treated as an environmental variable here, describing biotope cover in the wetlands. In this study, macroalgae was represented solely by the mat forming *Cladophora* sp. Although capable of surviving slightly acidic waters (pH 6 - 7), members of the genus *Cladophora* are generally regarded as indicator organisms for alkaline conditions (Prescott 1951, Whitton 1970, Dodds and Gudder 1992). *Cladophora* spp. are also well known as indicators of elevated nutrient levels, particularly phosphorus, but they can also be abundant in habitats where nitrogen supply limits primary production (see Dodds and Gudder 1992 for a review). The alga was absent from all sites in this study that were surrounded extensively by indigenous fynbos and proliferated in most wetlands at Youngsfield that had no surrounding fynbos. Given the rise in wetland pH levels and phosphate concentrations accompanying habitat transformation, this proliferation was most likely an opportunistic response to human-induced environmental changes in the transformed wetlands.

The decrease in the amount of complex vegetation habitat in wetlands associated with increasing levels of habitat transformation, and corresponding increase in open water habitat, suggests a general simplification of in-wetland habitat structure with increasing levels of habitat transformation around wetlands. This seemed to be linked to the predominance in the least and moderately transformed sites of the aquatic macrophyte *Isolepis rubicunda*, which formed the bulk of the complex vegetation habitat in these wetlands, but was absent from the extensively transformed sites at Youngsfield (sites 8 – 12). Complex submerged vegetation was present in the Youngsfield sites, but mostly in the form of submerged terrestrial vegetation, particularly in shallow areas. Conversely, all the minimally transformed sites in the core conservation area of KRCA (sites 1 – 4) possessed extensive cover of *I. rubicunda* beds and two of the three moderately transformed sites (6 and 7) had extensive cover of these beds (it was absent from site 5). This pattern is probably linked to the natural distribution of this species, being endemic to sandy temporary depression wetlands of the south-western Cape (Muasya and Simpson 2002, van Ginkel *et al.* 2011). It is thus adapted to the typically oligotrophic, humic-rich conditions characteristic of these wetlands. The changes in physico-chemical conditions associated with extensive habitat transformation at Kenilworth (discussed above) were thus a likely cause for the loss of this species. The increase in the percentage cover of open water habitat associated with increasing habitat transformation thus appears to be due to the loss of *I. rubicunda*.

The decrease in structural complexity of habitats in wetlands associated with surrounding transformation of the landscape at Kenilworth is not consistent with the findings of chapter 2, where no clear association between biotope characteristics in wetlands and habitat transformation was observed at the broad scale. As discussed in the paragraphs above, the mechanisms potentially underlying the habitat changes at Kenilworth are most likely specific to Sand fynbos areas in that increases in pH and nutrients were associated with the loss of Sand fynbos around wetlands and replacement with alien vegetation, and this in turn was likely to have affected the abundance of the complex vegetation *I. rubicunda* and the macroalga *Cladophora* sp. in wetlands. These biotope changes are not expected to be found consistently throughout the south-western Cape and thus at the broad scale the overall relationship may not have been clear. Furthermore, the reduced amount of natural variation at Kenilworth in comparison to that at the broad scale is likely to have elucidated clearer detection of the relationship between biotope characteristics and the gradient of habitat transformation at this fine scale, as was the general trend for physico-chemistry and invertebrate assemblages in this study. The negative association between biotope complexity and land use reported by Declerck *et al.* (2006) for small permanent ponds in Belgium was attributed largely to increases in wetland turbidity with cropland agriculture and trampling by cattle. As discussed earlier, the mechanisms underlying changes in biotope structure at Kenilworth are not likely to be linked to turbidity and thus although a similar trend in biotope complexity has been observed among the results of this study and those of Declerck *et al.* (2006), the causal factors are almost certainly unrelated.

4.4.2. Response of invertebrate assemblages to habitat transformation

Assemblage composition

Macroinvertebrate and microcrustacean assemblage composition displayed highly significant relationships with the habitat transformation gradient at Kenilworth. As with environmental conditions in wetlands, differentiation of invertebrate assemblages appeared to be most marked between least and extensively transformed sites, as evidenced by their temporally consistent separation in the MDS plots (Figs 4.2 and 4.3). The environmental overlap between moderately and extensively transformed sites in the PCA plot (Fig. 4.4) was reflected by a similar pattern of overlap in the MDS plots for macroinvertebrates, but not for microcrustaceans, which showed fairly clear separation among sites with least, moderate and extensive levels of transformation. Microcrustaceans were also more strongly related to the habitat transformation gradient than were macroinvertebrates according to the relative percentages of explained variation from the multivariate regressions (Table 4.2) and variation partitioning results (c.f. segment “e” in Fig. 4.7 *versus* segment “e” in Fig. 4.8). It therefore appears that microcrustacean assemblage composition was more affected by habitat transformation than was the case for macroinvertebrates. A similar pattern was found at the broad scale in chapter 3, although the differences were less marked than at the fine scale in this study and were only really apparent in relation to habitat transformation within

100 m (and not 500 m) of wetlands. As commented on in chapter 3 (section 3.4.1), these findings differ from those of Batzer *et al.* (2004), who observed stronger relationships between motile insect taxa and environmental gradients than for passive dispersing taxa such as leeches. They argued that sedentary taxa would be expected to show more homogenous distributions among different environmental conditions because they are forced to adapt to environmental changes rather than escape them for more favourable conditions. As with the findings in chapter 3, these results suggest that passive dispersing microcrustaceans in temporary wetlands are not necessarily resilient to moderate levels of environmental change induced by human transformations of the landscape.

Macroinvertebrates must colonise from another nearby water source, and given an increasingly transformed landscape, they may have also had a prolonged exposure to stresses resulting from habitat transformation. Macroinvertebrates could be considered to some extent "pre-adapted" to environmental variability because they are forced to encounter a variety of habitats. The sedentary lifestyle of microcrustaceans is likely to expose remnant natural populations to changing wetland environmental conditions (e.g. physico-chemistry) associated with increasing transformation of the landscape. Although certain wetland microcrustacean populations may have been decimated by the environmental effects of habitat transformation during the observation period of this study, it is also likely that a diversity remains in the sediment, given the well known ability of this fauna to survive prolonged harsh periods as dormant cysts (Wiggins *et al.* 1980, Williams 1997, Williams 2006). This would suggest the likely success of rehabilitation measures, given that the return of natural environmental conditions to a wetland would be expected to trigger the restoration of long-dormant (possibly for decades or more) populations of microcrustaceans.

Nearby large waterbodies such as dams present a potential confounder of macroinvertebrate composition or richness/diversity patterns observed in this study. At both KRCA and Youngsfield there were two dams that occurred within 500 m of study wetlands and which might have influenced the composition or richness/diversity of macroinvertebrate assemblages in the temporary wetlands. The dams at KRCA were considerably larger and closer to wetlands than were those at Youngsfield and thus more likely presented a permanent source pool of macroinvertebrate colonists to the temporary wetlands at KRCA than for those at Youngsfield. Given the lack of richness and diversity patterns observed across the habitat transformation gradient in this study (see following section: *Taxon richness and diversity*) it is not likely that the differential proximity and size of dams to study wetlands exerted any obvious effect on richness/diversity along the north-south transformation gradient. Although the spatial arrangement and size of nearby dams presented a potential interference with macroinvertebrate composition patterns in this study, they were not likely to have exerted any significant structuring effect on microcrustacean assemblages given the relatively sedentary nature of this fauna. The highly significant relationships between habitat transformation and microcrustacean assemblage composition were thus highly unlikely to have been caused by a confounding influence of nearby dams. For both macroinvertebrate and microcrustacean assemblages, I argue that the significant relationships with habitat transformation observed in this study are far more likely to be explained by the corresponding gradient of environmental conditions in wetlands (as discussed in section 4.4.1 above) than by differential proximity to dams. The role of physico-chemical and biotope factors in mediating habitat transformation effects on invertebrate assemblages is further discussed below (see following paragraph, but particularly section 4.4.3).

From a biogeographical perspective, the freshwater invertebrates of southern Africa belong to two major phylogenetic groups: the ancient palaeorelictual fauna, originating from the landmass of Gondwanaland that split into the southern continents between 150 and 65 million years ago; and the more recent Pan-Ethiopian fauna of tropical African origin (although the actual timeframes of origin are unknown, Harrison 1978, Allanson *et al*. 1990). The Pan-Ethiopian fauna makes up the bulk of the southern African invertebrate fauna, whilst Gondwanan relicts are restricted mostly to the south-western Cape, where they are adapted to physico-chemical condition characterised by high humic concentrations, low pH and very low nutrient concentrations (Harrison 1978, Day *et al.* 2005). It is interesting to note in the current study that such physico-chemical conditions characterised wetlands that were extensively surrounded by fynbos vegetation and that the genus *Paramelita* (Amphipoda: Paramelitidae) was only represented in these humic-rich wetlands surrounded by fynbos. The genus *Paramelita* has been listed as being of Gondwanan palaeorelictual origin (Day *et al.* 2005) and thus its presence in a wetland would be indicative of a Gondwanan assemblage. Unfortunately information could not be sourced on the phylogenetic origins of the other taxa recorded in this study, but the presence of *Paramelita* individuals only in the untransformed wetlands at Kenilworth certainly suggests that at least a part of the assemblages inhabiting these wetlands is of palaeorelictual origin. This has implications for understanding the effects of habitat transformation on lowland wetlands in Sand fynbos. Gondwanan relicts in the south-western Cape are adapted to humic-rich waters with low pH and nutrients, as is typical of waters draining fynbos-vegetated catchments (Day *et al.* 2005).

The removal of fynbos vegetation around wetlands due to anthropogenic habitat transformation is likely to be associated with a rise in pH and nutrients (following results of the current chapter) and is thus expected to lead to a loss of Gondwanan relicts from these wetlands. No baseline data exists on the distribution of palaeorelictual taxa in lowland wetlands of the south-western Cape (although more extensive records exist for mountain stream taxa: Picker and Samways 1996, Day *et al*. 2005) and explicitly addressing the hypothesis that these taxa are being lost to transformation of fynbos habitat around wetlands would require the collection of such taxonomic information. This is a recommended avenue for further research and is particularly important in the context of conserving biodiversity, given that palaeorelictual taxa are largely endemic to the region (Picker and Samways 1996, Day *et al.* 2005).

Taxon richness and diversity

There is some evidence for a decrease in the diversity of macroinvertebrate assemblages with increasing levels of habitat transformation at Kenilworth, but as commented on in section 4.3.4, this trend was weak due to the large amount of scatter in the partial residual plots (Fig. 4.10). No significant associations between macroinvertebrate richness and habitat transformation were detected, nor for the microcrustacean richness and diversity measures. At the broad scale (see chapter 3, section 3.4.2), certain measures of microcrustacean richness and diversity were significantly related to the habitat transformation gradient for the microcrustacean fauna only, and no significant relationships were detected for macroinvertebrates. Furthermore, microcrustacean richness and diversity were positively associated with increasing levels of habitat transformation, thus presenting an opposite trend to that observed for macroinvertebrates in the current study. Taken as a whole, results across the two spatial scales in this thesis indicate no clear and consistent effect of habitat transformation in nearby landscapes on the richness or diversity of invertebrate assemblages in temporary wetlands. Other studies in temporary wetlands have found either no effect (Mahoney *et al.* 1990, Bagella *et al.* 2010) or a negative effect (Euliss and Mushet 1999, Lahr *et al.* 2000, Angeler and Alvarez-Cobelas 2005) of anthropogenic disturbance in the landscape on invertebrate richness and/or diversity. There is thus a general lack of consensus as to the effects of habitat transformation around temporary wetlands on richness and diversity of their invertebrate biotas and results vary depending on the region being investigated and the nature and intensity of the habitat transformation.

4.4.3. Habitat transformation effects mediated *via* environmental changes

The variation partitioning results (Figs 4.7 and 4.8) indicate that the influence of habitat transformation on invertebrate assemblage composition was mostly due to environmental differences associated with habitat transformation. That said, the differences in environmental conditions between individual wetlands that were independent of temporal, spatial and habitat transformation factors explained the bulk of the variation in assemblage composition (slightly more than twice that of any other explained component) for both macroinvertebrates and microcrustaceans. This finding is most likely linked to the heterogeneous environmental conditions among wetlands in this study, even independent of the transformation gradient (as evidenced in Fig. 4.4, see section 4.4.1). This environmental heterogeneity appears to have a considerable structuring effect on the invertebrate assemblages. A similar finding was reported at the broader scale in chapter 3 (see section 3.4.1), suggesting that intrinsic heterogeneity among temporary depression wetlands in the south-western Western Cape plays a major role in structuring invertebrate assemblages across multiple scales of study. This strong influence of environmental heterogeneity as a structuring agent of invertebrate assemblage composition in temporary wetlands, even at the small spatial scale of this study, indicates that they are indeed responsive to even moderate environmental gradients. This adds to the growing body of literature reporting a strong influence of among-wetland environmental variation on temporary wetland invertebrates, both within the south-western Cape study region (De Roeck 2008) and other regions of the world (Mahoney *et al.* 1990, Eitam *et al.* 2004, Bilton *et al.* 2006, Vanschoenwinkel *et al.* 2007, Waterkeyn *et al.* 2008, Bagella *et al.* 2010).

Yet other studies have not been able to establish clear evidence of environmental influences on temporary wetland invertebrates (Wissinger *et al.* 1999, Battle and Golladay 2001, Spencer *et al.* 2002, Batzer *et al.* 2004, Studinski and Grubbs 2007, Ganguly and Smock 2010). The reasons for this disparity in results among different study areas have not been established, but are probably due to the relative strength of the environmental gradients being investigated. For example, heterogeneous terrestrial landscapes such as the south-western Cape present strong variation in environmental conditions over small spatial scales (see Rebelo *et al.* 2006 for a review), as reflected by the marked environmental heterogeneity among the wetlands covered this thesis. Yet studies such as Batzer *et al.* (2004), who argue for a lack of environmental control on temporary wetland invertebrates, deal with wetlands in landscapes that are almost certainly more environmentally homogenous (e.g. the Minnesota region in the case of Batzer *et al*. 2004 is mostly forested, whilst the south-western Cape has a high patchiness of different vegetation types over small spatial scales). Irrespective of the reasons behind the above-mentioned inconsistency in results among different studies, there is strong evidence that for the south-western Cape environmental variation among temporary wetlands has a marked structuring effect on invertebrate assemblages in these wetlands.

The unique influence of habitat transformation independent of correlated environmental factors was relatively minor, but was a statistically significant contribution. One cannot disentangle the unique influence of habitat transformation from purely spatial effects because of the inter-correlated nature of the spatial variables (latitude and longitude) and the habitat transformation gradient in this study. Regardless of the relative contributions of these factors, one can conclude that both the pure (unique) spatial and pure habitat transformation contributions played a minor (albeit significant) role in structuring invertebrate assemblages in this study. The above comments indicate firstly that habitat transformation effects were predominantly mediated *via* environmental changes, as hypothesized at the outset of this study, and secondly, that the pure spatial arrangement of wetlands across the study landscape (distances between individual wetlands) had a fairly negligible influence on assemblage composition. As discussed in the previous paragraph, the most important influence on invertebrate assemblage composition in this study came from environmental factors independent of the habitat transformation gradient or the spatial arrangement of wetlands. The predominance of environmental influence over spatial factors in small-scale studies on temporary wetland invertebrates have been mirrored elsewhere, for example, by Vanschoenwinkel *et al.* (2007). These authors studied a set of 36 temporary rock pools over an area of ± 9000 m^2 in the eastern Free State Province of South Africa and found very similar results to the current study in that although the unique influence of spatial factors on invertebrate assemblages was significant, it was relatively weak in comparison to the unique effects of environmental factors. Their study presents evidence that environmental variation can have an important influence on invertebrate assemblages even at very small spatial scales.

As already mentioned, the effects of habitat transformation on invertebrate assemblage composition appeared to be mediated by changes in environmental variables that were associated with the gradient of habitat change. The dbRDA plots were used to establish important environmental factors in this regard. Results indicated that pH and cover of macroalgae were the key factors associated with the habitat transformation gradient that in turn had an impact on the composition of macroinvertebrate assemblages (Fig. 4.5). A broader suite of environmental factors appeared to mediate the effects of habitat transformation on microcrustacean assemblages (e.g. % macroalgae, % open water, pH, phosphates, nitrates + nitrites, % reeded simple vegetation, Fig. 4.6). The findings are consistent with my hypothesis that the effects of habitat transformation on aquatic invertebrate assemblages in temporary wetlands are mediated through physico-chemical factors, although the effects were shared with biotope factors, which played an equally or possibly more important role than physico-chemistry. The methods of this study cannot quantify if habitat transformation had a direct effect on biotope structure, or whether effects on biotope were in turn mediated by physico-chemical changes associated with the transformation of adjacent habitats. As discussed in section 4.4.1, it is likely that changes in the amount of complex vegetation (and correspondingly the amount of open water) were linked to physico-chemical changes associated with the loss of fynbos vegetation along the transformation gradient. Similarly, changes in the abundance of macroalgae were almost certainly linked to the rise in pH and phosphates associated with increasing transformation of fynbos habitat.

The patterns observed in this study therefore strongly suggest that the effects of habitat transformation on invertebrate assemblages were initiated through changes in physico-chemical conditions, either directly or indirectly through associated changes in biotope characteristics. This mechanism is likely to be applicable beyond the spatial context of the current study and to a variety of wetland types and forms of habitat transformation, because the transformation of indigenous fynbos habitat around wetlands of the Western Cape is expected to often involve the loss of the characteristic physico-chemical signature of fynbos-associated aquatic ecosystems. Harrison (1962) and Gardiner (1988) both reported a strong association between aquatic invertebrate assemblages and pH levels in south-western Cape lowland depressional wetlands. These studies reported differences in assemblage composition among wetlands with low, moderate and high humic content and attributed these differences largely to the changes in pH associated with the different levels of humics. My work extends their results by providing quantitative evidence of the positive association between fynbos cover around wetlands and the concentrations of humic substances (and hence pH levels) in these wetlands, which in turn appears to mediate changes in the invertebrate assemblages. Although linear relationships are explored in this study, it is acknowledged that a rise in pH may not have linear impacts on wetland biota owing to the potential buffering mechanisms of individual taxa. Potential non-linear thresholds of individual taxa to changes in wetland pH would warrant interesting further study, but was beyond the scope of the current work.

It should be noted that in certain cases one will expect the major effects of habitat transformation to be hydrological (e.g. if wetland basin morphometry is altered by the predominant land use) or even direct physical effects (e.g. ploughing of temporary wetlands in cultivated fields during the dry season). These hydrological and physical effects were not a feature of the current study, but may be important for other areas where human land-use impacts differ.

4.4.4. Testing potential indicator families and metrics

There was a vast improvement in the ability to assign family-level indicator taxa at the fine scale of this study in comparison to the broad-scale results presented in chapter 3. This statement holds true for both macroinvertebrates and microcrustaceans. At the fine scale, patterns in the partial residual plots displayed considerably less scatter and better explanatory power than for plots presented at the broad scale. Comparison of metric patterns observed in chapters 3 and 4 revealed similar improvements in metric performance for both macroinvertebrates and microcrustaceans from the broad to fine scale. Despite these improvements, the patterns could still only be considered moderately reliable due to the considerable amount of vertical scatter of residual points that was often observed for sites that had no surrounding indigenous vegetation within 100 m (i.e. extensively transformed sites). The families and metrics presented in this study were thus generally better indicators of "pristine" conditions in the landscape than of extensive transformation.

The observations described above are in line with the broad hypothesis (see chapter 3, section 3.4.4) that the spatial extent to which an invertebrate index can be applied is intimately linked to the natural variability within the system being investigated. The large amount of environmental heterogeneity among temporary depression wetlands of the south-western Cape hinders the formation of a broad-scale biotic index of disturbance using invertebrates because their distribution and abundance is largely in response to natural factors rather than anthropogenic disturbances in the landscape. It was expected that the performance of potential indicator taxa and families would improve in situations where the ratio of anthropogenic influence to natural influence on invertebrate assemblages increases.

This ratio of influence did show a noticeable increase from the broad scale in chapter 3 to the fine scale in this study (see discussion in section 4.4.3), and a corresponding improvement in the performance of families and metrics as indicators of habitat transformation was observed. Even at the small spatial scale of the current study, however, natural heterogeneity in environmental conditions has been shown to be large and appears to be the primary determinant of invertebrate assemblage structure, rather than anthropogenic transformation of the landscape. This appears to be the reason for the moderate levels of reliability indicated by the family and metric patterns at Kenilworth. If the variation partitioning procedure (section 4.4.3) had revealed more anthropogenic than natural influence on invertebrate assemblages at Kenilworth, then one would expect to observe higher levels of reliability in the family and metric patterns in terms of inferring levels of habitat transformation in the surrounding landscape.

As discussed in chapter 3 (section 3.4.4), feasibility studies for wetland indices in other regions of the world (see Tangen *et al*. 2003, Trigal *et al*. 2007) have reported results that are in line with my general expectation that the ratio of anthropogenic to natural influence on invertebrate assemblages is the key determinant of whether a biotic index of disturbance will be reliable, and at what spatial scale. This ratio will in turn be primarily determined by the natural levels of environmental heterogeneity among wetlands. If levels are low, then anthropogenic influences on wetland biota are likely to be more meaningful and provide more reliable indices than for cases where natural environmental heterogeneity is pronounced.

4.4.5. Conclusions

The results of this study indicate that habitat transformation in the landscape, in this case due largely to invasion by alien vegetation, had a significant influence on environmental conditions in temporary wetlands. In-wetland environmental changes associated with the loss of surrounding fynbos vegetation appeared to mediate significant changes in the composition of invertebrate assemblages in wetlands. The hypothesis that habitat transformation would alter invertebrate assemblage composition through associated changes in physico-chemical conditions was partially met in that both physico-chemical and biotope factors varied with habitat transformation and in turn appeared to exert an influence on invertebrate assemblages. These results contribute to wetland ecological theory by providing evidence that temporary wetland invertebrates are not necessarily resilient habitat generalists (as originally hypothesized in chapter 3) and are affected by human activities in the landscape. Environmental conditions and invertebrate assemblages in wetlands showed a substantially clearer response to the gradient of habitat transformation in this study than was observed at the broad scale (chapter 2). Although there was a considerable improvement in the strength of the aforementioned relationships from the broad to fine scales of study, natural environmental heterogeneity among the wetlands at Kenilworth was pronounced, despite the small scale of the study. This natural environmental heterogeneity (independent of the influence of habitat transformation) appeared to explain the bulk of the variation observed for invertebrate assemblage composition at Kenilworth. The stronger relationships between invertebrate assemblages and habitat transformation gradients observed at the fine scale than at the broad scale was reflected in the improvement of metric and family-level relationships with these gradients at the fine scale. The following chapter provides an integrated summary of the findings across the three data chapters of this thesis, including some theoretical and applied ramifications of this work.

CHAPTER 5

SYNTHESIS

5.1. Introduction

This thesis aimed to improve our understanding of how human transformation of coastal plains in the south-western Cape has impacted on the ecology of temporary wetlands embedded in these landscapes. Although many temporary wetlands in the region have been lost or dramatically altered by agriculture or urban development, a large number still remain in low-lying areas and form a salient feature of the wet-season landscape. Prior to the work reported in this thesis, no information existed on the extent to which these remaining temporary wetlands ecosystems have been altered by human activities in surrounding landscapes. My work has focussed on the impacts of habitat transformation within immediately surrounding landscapes (within 100 m and 500 m of wetlands) on temporary isolated depression wetlands. The specific focus was on environmental constituents (physico-chemistry, biotope characteristics and hydro-morphometry) and invertebrate assemblages (macroinvertebrates and microcrustaceans) and whether these characteristic ecological components of temporary wetlands have been altered by the predominant agents of habitat transformation in the region (alien invasive vegetation, agriculture and urban development). This chapter discusses the important findings from each chapter of the thesis.

5.2. Chapter 2. Temporary wetland environments in transformed landscapes: a broad-scale perspective

Chapter 2 involved a broad-scale investigation of relationships between habitat transformation and environmental conditions in 90 temporary isolated depression wetlands on coastal plains of the south-western Cape. The hypothesis was tested that the degree of habitat transformation around wetlands is associated with in-wetland physico-chemical conditions, and this in turn mediates changes in the structural complexity of vegetation biotopes. It was found that the physico-chemistry of these wetlands was significantly associated with the overall extent of habitat transformation in surrounding landscapes, but this anthropogenic influence appeared to be weak in comparison to that of natural spatio-temporal factors, which accounted for the majority of the explained variation in the environmental response variables. There was, however, no evidence for an influence of habitat transformation on biotope characteristics or hydro-morphometry of wetlands. Natural spatio-temporal influences appeared to mask anthropogenic influences at this broad scale of analysis. The fact that a significant signal was still detected for relationships between habitat transformation and physico-chemistry, over and above the strong spatio-temporal influence, indicates that the transformation of landscapes around temporary wetlands has induced meaningful changes in physico-chemical conditions in these wetlands. The nature and strength of these alterations appears to depend on factors that operate at a small scale (within individual landscapes), such as the geological context of a wetland and natural vegetation type of the area, which appear to determine whether a specific type of habitat transformation will influence wetland physico-chemistry. To illustrate this, an interesting finding from chapter 2 was the significant relationship between physico-chemical conditions in wetlands and surrounding cover of alien invasive vegetation within 100 and 500 m, which appeared to be driven largely by a rise in wetland pH associated with alien vegetation invasion. This was probably a result of the replacement of tannin-rich fynbos with invasive vegetation species that do not leach humic substances into the soil (and in turn into nearby wetlands). This mechanism is only expected to affect wetlands in areas that are naturally vegetated by tannin-rich fynbos species, such as those occurring within Sand fynbos vegetation. Therefore, this relationship between alien invasive vegetation (or any other agent of habitat transformation) and temporary wetland pH is not expected to be consistent across the region, but rather would only be apparent for certain clusters of wetlands historically surrounded by Sand fynbos or similar vegetation types that naturally leach humic substances into wetlands. There are likely to be other ecological mechanisms governing habitat transformation effects on wetland environmental variables that are not apparent at the broad scale, but are clear for specific clusters of wetlands. Further discussion of fine-scale associations between habitat transformation and wetland environmental conditions is provided in the synopsis of chapter 4 (see section 5.4).

Chapter 2 also revealed that the association between wetland physico-chemistry and the cover of indigenous vegetation within 100 m of wetlands was slightly stronger than for indigenous cover within 500 m. From an applied perspective, these results indicate that the conservation of natural vegetation buffers within 100 m radii of temporary wetlands would assist in maintaining natural physico-chemical conditions in these wetlands. Given that these wetlands occur predominantly in prime areas for human activities (particularly agriculture and urban development), the conservation of natural vegetation buffers of 500 m radii around wetlands is probably not realistic considering the abundance of temporary wetlands in the region. Striving for 100 m buffers around as many wetlands as possible appears to be a good trade-off between human needs and conserving wetland physico-chemical conditions. Of course, there are other criteria for deciding on the size and spatial arrangement of wetland buffers (for reviews see Castelle *et al*. 1994, Goates *et al*. 2007), such as creating corridors of indigenous vegetation to allow the connection of species metapopulations; my data only allows comment on what would be useful for conserving wetland physico-chemical conditions. No studies could be sourced from the literature that have addressed the buffer requirements of temporary wetlands, and one is required to draw on studies for small permanent wetlands as the best available comparison. A study by Declerck *et al*. (2006) on 99 small permanent wetlands scattered across agricultural landscapes in Belgium investigated relationships between environmental conditions in wetlands and surrounding land use. Their study measured land use at multiple spatial scales ranging from 50 m of the wetland edge to 3.2 km and found the relationships with physico-chemical conditions in wetlands to be strongest between 100 and 200 m (depending on the type of land use). This lead to their recommendation that natural vegetation buffers of at least 200 m radius around wetlands should be striven for in order to maintain water quality in these wetlands. Akasaka *et al.* (2010) similarly found the strongest associations between physico-chemical conditions and land use at radii between 100 and 250 m of the wetland edge for a set of 55 permanent wetlands differentially affected by rice paddy agriculture in western Japan.

Using a set of 73 large permanent wetlands in south-eastern Ontario, Houlahan and Findlay (2004) reported quite different results to those of the aforementioned studies. They found relationships between land use and the water and sediment quality of wetlands (mostly nutrient measures) to be strongest at 2 – 4 km distances from wetlands and recommended that natural vegetation buffers around wetlands be correspondingly large. The wetlands investigated in their study were however fundamentally different to the small wetlands covered in the current study and those of Declerck *et al.* (2006) and Akasaka *et al*. (2010), primarily because they were mostly large palustrine wetlands with an average surface area of tens of hectares. Thus, they would be expected to receive drainage from considerably larger catchments than for small isolated temporary or permanent wetlands. Although my study does not actually measure land use at spatial scales larger than 500 m (due to the lack of GIS covers for alien vegetation), isolated temporary wetlands in the region are nonetheless expected to receive drainage from small areas. This assumption is supported by the findings of Davies *et al*. (2008) who compared the catchment areas associated with five waterbody types (ditches, ponds, rivers, lakes and streams) in an agricultural area of lowland England and found that isolated ponds (< 2ha in size) received drainage from very localised catchment areas, on average just 18ha. The findings from this thesis (chapter 2), and those of Declerck *et al*. (2006), Davies *et al*. (2008) and Akasaka *et al*. (2010), provide evidence that associations between habitat transformation in the landscape and the physico-chemistry of small isolated wetlands are strongest at small spatial scales (< ~250 m from the wetland edge). From a “water quality” or physico-chemical perspective, it appears that the conservation of localised natural vegetation buffers around small isolated wetlands (whether they be temporary or permanent) is likely to be an effective step towards maintaining natural conditions in these wetlands.

5.3. Chapter 3. Temporary wetland invertebrates in transformed landscapes: a broad-scale perspective

Aquatic invertebrates, particularly macroinvertebrates, are widely used as indicators of anthropogenic disturbance in rivers and lakes (see chapter 3, section 3.1.3). They also show potential for use as indicators of wetland condition (see chapter 3, section 3.1.3), although results appear to vary by region and not all studies show promise in this regard (see Tangen *et al*. 2003). Their use as biological indicators of disturbance in temporary wetlands has not been investigated prior to this thesis. The key aims of chapter 3 were, firstly, to assess whether the structure of aquatic invertebrate assemblages in temporary wetlands of the south-western Cape have been altered by changes around wetlands induced by habitat transformation; secondly, to determine the relative influence of the measured natural *versus* anthropogenic factors in explaining the composition of invertebrate assemblages; and thirdly, to investigate the feasibility of aquatic invertebrates as indicators of disturbance in and around temporary wetlands of the region. I hypothesized that the invertebrates inhabiting these temporary wetlands are naturally adapted to fluctuating environments and thus are expected to show resilience to the weak, or at most, moderate environmental changes in wetlands caused by the transformation of surrounding habitats. Consequently, it was not expected that temporary wetland invertebrates would constitute effective biological indicators for use in a biotic index of wetland disturbance for the region. This study took a broad-scale sampling approach and collected once-off samples from the same set of 90 wetlands investigated in chapter 2 (although a subset of 41 wetlands was analysed for microcrustaceans). At this scale of analysis, it was apparent that natural heterogeneity in environmental conditions among wetlands was far more influential in structuring invertebrate assemblages than were anthropogenic factors, represented by gradients of habitat transformation around wetlands. Temporary wetland invertebrates did not however appear to be unaffected to the influence of habitat transformation around wetlands, as evidenced by several low P values (either significant or very close to the 5 % significance level) for multivariate regression relationships between invertebrate assemblage composition and the habitat transformation variables. The sets of environmental factors measured in this study (physico-chemistry, biotope characteristics and hydro-morphometry) in almost all cases explained significant components of variation in the composition of invertebrate assemblages among wetlands. Although the amounts of explained variation in this regard were not high (< 25%), the results were strongly significant and corroborate the findings of De Roeck (2008) that temporary wetland invertebrates in the south-western Cape are indeed structured by environmental gradients. A fundamental difference between her findings and mine was the relatively minor role of hydro-morphometry (represented by maximum depth and total surface area) as a determinant of invertebrate assemblage composition in the current study, whereas De Roeck found hydro-morphometry to be a key structuring agent of invertebrate assemblages. Given these ambivalent findings, it is recommended that further work be conducted focussing on the relative role of different environmental factors as determinants of invertebrate assemblage structure in temporary wetlands of the region. Irrespective of the relative order of importance of the environmental variables in the current study, there was strong evidence that aquatic invertebrates inhabiting these temporary wetlands are structured by environmental gradients among wetlands. This appears to refute the suggestion of Batzer *et al.* (2004) that temporary wetland invertebrates are resilient to natural environmental gradients. This topic is revisited in the following section (5.4).

With the possible exception of the Daphniidae (positively associated with disturbance), regressions of individual macroinvertebrate and microcrustacean families against gradients of habitat transformation and scores from the human disturbance index displayed very weak trends, indicating that these families do not show potential as indicators of human disturbance. Similarly, the array of macroinvertebrate and microcrustacean metrics regressed against the human disturbance variables in this study displayed poor relationships and gave strong indication that a multimetric index of disturbance using aquatic invertebrates is not a feasible option for the south-western Cape region. The poor performance of invertebrate families and metrics in this study as indicators of human disturbance appears to mirror the generally weak (albeit sometimes significant) multivariate relationships observed between invertebrate assemblage composition and habitat transformation gradients (discussed above). A general theme across the results of chapters 2 and 3 was the prominent role of natural heterogeneity as a driver of environmental conditions and invertebrate assemblage composition among temporary wetlands. At the broad scale of analysis (chapters 2 and 3) this natural influence on temporary wetland ecosystems appears to override that from anthropogenic factors. Although temporary wetland invertebrates were expected to display a certain degree of resilience to anthropogenic disturbances in and around wetlands, the evidence from this study suggests that the poor performance of indicator taxa and metrics in relation to anthropogenic disturbance gradients is more likely to be a reflection of the strong role of natural spatio-temporal and environmental factors as determinants of assemblage composition, which masks the relationships with variables representing anthropogenic disturbance. Regardless of the mechanisms involved, an invertebrate index of human disturbance for isolated temporary depression wetlands of the region cannot be recommended given the results of this study.

5.4. Chapter 4. Ecological impacts of alien vegetation invasion on temporary wetlands: a fine-scale perspective

Chapter 4 reports on the effects of habitat transformation on temporary wetlands within a single landscape. The aim of this study was to elucidate the effects of a single agent of habitat transformation (alien vegetation) on temporary wetlands environments and invertebrate assemblages using a small-scale analysis. Sampling was replicated in time to assess the consistency of trends. A cluster of 12 wetlands occurring in and around the Kenilworth Racecourse Conservation Area in Cape Town were repeatedly sampled over two wet seasons for various environmental constituents, macroinvertebrates and microcrustaceans. These wetlands were chosen because they would be comparable in their natural state, but recently have become invaded by *Acacia saligna* (Port Jackson willow) and *Pennisetum clandestinum* (kikuyu grass) to different degrees. This set up a “natural experiment” in that a gradient of habitat transformation around wetlands (< 100 m periphery) presented itself across a small spatial area (~ 100 ha). This gradient encompassed wetlands surrounded extensively by Sand fynbos habitat (minimal or no alien vegetation), those surrounded by moderate cover of alien vegetation and those that have become extensively invaded by alien vegetation (complete loss of indigenous fynbos cover). I hypothesized that changes in wetland physico-chemistry would accompany the replacement of indigenous Sand fynbos habitat around wetlands with alien vegetation. Given the strong relationships between physico-chemistry and invertebrate assemblage composition in chapter 3, it was expected that physico-chemical changes across the gradient of habitat transformation at

Kenilworth would mediate changes in the composition of invertebrate assemblages. It was further hypothesized that the influence of habitat transformation on environmental conditions and invertebrate assemblages in temporary wetlands would be more apparent at this small spatial scale than for the broad-scale studies of chapters 2 and 3.

The replacement of indigenous Sand fynbos habitat around wetlands with alien vegetation was strongly associated with increases in pH, % cover of the macroalga *Cladophora* sp., % cover of the open water biotope, phosphate concentrations, % cover of the complex vegetation biotope and a decrease in the concentration of humic substances in wetlands. The cumulative influence of these environmental changes on temporary wetland invertebrates was highly significant for both macroinvertebrates and microcrustaceans. As expected, environmental and invertebrate response patterns to the habitat transformation gradient in this study were considerably clearer than for those observed at the broad scale and this improvement in detecting anthropogenic influences was attributed to reduced interference from natural variation at the fine scale. That said, natural environmental variation (independent of the habitat transformation gradient) among the wetlands at Kenilworth remained high, despite the small spatial scale of this study. This environmental heterogeneity appeared to be considerably more important in structuring invertebrate assemblages than was the influence of habitat transformation. This suggests that temporary wetland environments, at least in this region, are intrinsically heterogeneous (which has also been reported elsewhere, see for example Magnusson and Williams 2006), and this heterogeneity appears to drive biotic community structure and cause interference in detecting anthropogenic influences across multiple spatial scales of analysis.

Chapter 4 details a scenario where a gradient of anthropogenic-induced change around temporary wetlands has resulted in a corresponding gradient of environmental change within wetlands, which in turn is reflected by a gradational difference in the composition of invertebrate assemblages. The study provides strong evidence that temporary wetland invertebrate assemblages are structured by environmental gradients, even those involving moderate environmental differences as is the case in this study. These results offer clarification of the trends observed in chapter 3, where invertebrate responses to habitat transformation gradients were significant or close to significant, but weak. Chapter 4 thus strongly suggests that invertebrate assemblage composition can indeed be structured by the influence of habitat transformation and that temporary wetland invertebrates are responsive to human changes in the nearby landscape. As was mentioned, invertebrate assemblages appeared to be even more strongly structured by environmental changes among wetlands independent of human influences, offering further evidence that temporary wetland invertebrates are not necessarily resilient to environmental controls. The results across chapters 3 and 4 of this thesis counter those of other studies (Wissinger *et al*. 1999, Battle and Golladay 2001, Spencer *et al*. 2002, Batzer *et al*. 2004, Studinski and Grubbs 2007, Ganguly and Smock 2010) that have found a lack of response from temporary wetland invertebrates to environmental gradients. Batzer *et al.* (2004) specifically argued that temporary wetland invertebrate assemblages are unlikely to be responsive to moderate environmental changes among wetlands due to the generalist lifestyle of most temporary wetland invertebrate taxa. The study at Kenilworth revealed that approximately a quarter to a third of the total variation of invertebrate assemblage composition was unexplained by the factors measured during the study. Thus, there is some indication of a “generalist” response from these invertebrate taxa, given that some of the variation was unexplained. On the whole, however, invertebrate distribution and abundance among wetlands was largely explained by the environmental and anthropogenic variables recorded in this study. Other studies have similarly reported among-wetland environmental variation as playing an important role in structuring temporary wetland invertebrate assemblages (Mahoney *et al*. 1990, Eitam *et al*. 2004, Bilton *et al*. 2006, Vanschoenwinkel *et al*. 2007, Waterkeyn *et al*. 2008, Bagella *et al.* 2010), despite the contrasting findings of other authors (listed above). The reason for this discrepancy in results among different regions is unknown, but may well be linked to the level of environmental heterogeneity intrinsic to wetlands of the area being investigated. Although my thesis has not resolved the global debate as to how resilient temporary wetland invertebrates are to environmental controls, I have presented evidence to disprove any universal claims that invertebrate assemblages in temporary wetlands are habitat generalists that are not structured by prevailing environmental conditions. In the south-western Cape, the environment presented by a given temporary wetland, particularly aspects related to its physico-chemistry, appears to be an important determinant of which invertebrate taxa are likely to inhabit that wetland.

The results of chapter 4 indicated that even moderate levels of habitat transformation within 100 m of wetlands can cause marked changes in the environmental conditions within wetlands. This extends the findings of chapter 2 by suggesting that although 100 m buffers around temporary wetlands may be useful for maintaining environmental conditions within wetlands, it may be required to maintain an extensive cover of indigenous vegetation within this 100 m radius if buffers are to be effective. This has only been demonstrated within one area (Kenilworth), and thus may not be widely applicable in the region. Further research is recommended to clarify the role of natural vegetation around temporary wetlands of the south-western Cape as a means to preserve wetland environments and invertebrate assemblages. This thesis provides evidence that transforming natural vegetation habitat within 100 m of wetlands is likely to alter environmental conditions in wetlands, predominantly through physico-chemical changes, and this in turn mediates changes in the composition of invertebrate assemblages in wetlands. Given that these wetlands are expected to drain small areas (Davies *et al*. 2008), and given the results presented in this thesis, it appears crucial to maintain indigenous vegetation habitat within the immediate (<~100 m) vicinity of temporary wetlands in the region if one wishes to maintain their ecological integrity. There are however other factors to incorporate into management decisions regarding buffer width and design, such as the inclusion of corridors of indigenous vegetation linking metapopulations of wetland species across landscapes (Semlitsch and Bodie 1998). Another important consideration is the influence of buffers of varying size on other biotic components in wetlands (e.g. amphibians, see Lehtinen *et al*. 1999). The results of this thesis only allow comment on the role of individual circular buffers and their potential influence on environmental conditions and invertebrate assemblages in wetlands.

There was a vast improvement in the performance of individual families and metrics as indicators of human disturbance at Kenilworth in comparison to patterns observed at the broad scale of analysis in chapter 3. Despite this improvement, relationships were still only of moderate strength, as evidenced by the low or moderate partial r^2 values in the regression models and the moderate amount of scatter in the partial residual plots, particularly for sites that had no surrounding indigenous vegetation within 100 m (i.e. extensively transformed sites). The families and metrics presented in this study thus appeared to be better indicators of “pristine” conditions in the landscape than of extensive transformation. The moderate scatter observed for some of these trends was attributed to natural interference from environmental factors (independent of the habitat transformation gradient), which as previously discussed played a strong role in determining invertebrate assemblage composition even at the small spatial scale of this study. From an applied perspective, the family and metric patterns in chapter 4 indicate that a biotic index of disturbance using invertebrates may well be a feasible option at Kenilworth, but this would not be a practical spatial scale to apply such an index. As demonstrated in chapter 3, an invertebrate index reflecting human disturbances in and around temporary wetlands does not appear to be a feasible option at the relevant spatial scale (i.e. the south-western Cape region).

5.5. Conclusions

This thesis presents some of the first work on temporary wetland ecosystems in the context of human transformed landscapes. Using a broad-scale approach, I have shown that transformation of natural vegetation habitat adjacent to temporary isolated depression wetlands in the south-western Cape is associated with changes in physico-chemical conditions (chapter 2) in these wetlands and, to a lesser degree, invertebrate assemblages (chapter 3). These relationships were however weak (albeit mostly significant) due to pronounced natural influences at this scale of analysis, which appeared to mask trends with the anthropogenic variables. From an applied perspective, an invertebrate index for the biological assessment of human disturbances in and around temporary wetlands is not recommended for the south-western Cape region. The environmental data indicate that conservation of relatively narrow (~100 m) buffer strips of indigenous vegetation around temporary wetlands in the region would be an effective step towards maintaining natural physico-chemical conditions in these wetlands.

Using a fine-scale study of a cluster of wetlands within a single landscape (chapter 4), habitat transformation effects (in the form of alien vegetation invasion) on temporary wetlands were considerably more apparent than for the broad-scale studies. The patterns suggested that the replacement of indigenous Sand fynbos habitat around wetlands with alien vegetation lead to highly significant changes in the physico-chemistry and biotope characteristics of these wetlands, which in turn mediated highly significant changes in the composition of invertebrate assemblages inhabiting these wetlands.

The results across chapters 3 and 4 of this thesis indicate that temporary wetland invertebrates are not necessarily resilient habitat generalists, as argued in the theoretical literature, but rather that assemblages are structured by environmental gradients, whether these be natural or human-induced.

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APPENDICES

Appendix 1. List of the candidate covariables for chapter 2 and 3 analyses. Time was incorporated as a quantitative covariable measured as days since first sampling event ("date sampled" is provided for general reference). dd - decimal degrees.

Site code	Date sampled	Latitude (dd)	Longitude (dd)	Time (days)	Altitude (m)	Vegetation group (wetland cluster)
FER01	11/10/2007	-34.6981	19.7202	80	100	Ferricrete Fynbos
FER02	11/10/2007	-34.6970	19.7207	80	100	Ferricrete Fynbos
FER03	11/10/2007	-34.6977	19.7276	80	92	Ferricrete Fynbos
FER04	11/10/2007	-34.7214	19.7566	80	26	Ferricrete Fynbos
FER05	13/10/2007	-34.5954	19.9589	82	30	Ferricrete Fynbos
FER06	14/10/2007	-34.7108	19.9306	83	1	Ferricrete Fynbos
SAN01	23/07/2007	-34.0379	18.7250	0	11	Sand Fynbos
SAN02	23/07/2007	-34.0363	18.7253	0	11	Sand Fynbos
SAN03	08/09/2007	-33.0083	18.3515	47	30	Sand Fynbos
SAN04	08/09/2007	-33.0842	18.3971	47	50	Sand Fynbos
SAN05	20/09/2007	-33.0870	18.3977	59	44	Sand Fynbos
SAN06	20/09/2007	-33.0853	18.3981	59	50	Sand Fynbos
SAN07	20/09/2007	-33.0724	18.3719	59	62	Sand Fynbos
SAN08	07/09/2007	-33.5146	18.6546	46	120	Sand Fynbos
SAN09	07/09/2007	-33.5449	18.6356	46	97	Sand Fynbos
SAN10	07/09/2007	-33.5912	18.6059	46	90	Sand Fynbos
SAN11	11/08/2007	-34.0308	18.7249	19	13	Sand Fynbos
SAN12	25/07/2007	-33.9995	18.4854	2	25	Sand Fynbos
SAN13	25/07/2007	-33.9984	18.4857	2	24	Sand Fynbos
SAN14	25/07/2007	-33.9981	18.4873	2	23	Sand Fynbos
SAN15	25/07/2007	-34.0000	18.4862	2	25	Sand Fynbos
SAN16	25/07/2007	-34.0004	18.4836	2	27	Sand Fynbos
SAN17	31/07/2007	-33.9988	18.4820	8	27	Sand Fynbos
SAN18	31/07/2007	-33.9978	18.4822	8	29	Sand Fynbos
SAN19	31/07/2007	-33.9971	18.4827	8	27	Sand Fynbos
SAN20	31/07/2007	-33.9963	18.4821	8	29	Sand Fynbos
SAN21	31/07/2007	-33.9959	18.4838	8	24	Sand Fynbos
SAN22	31/07/2007	-33.9942	18.4836	8	27	Sand Fynbos
SAN23	31/07/2007	-33.9945	18.4848	8	25	Sand Fynbos
SAN24	01/08/2007	-33.9964	18.4848	9	26	Sand Fynbos
SAN25	01/08/2007	-33.9926	18.4873	9	25	Sand fynbos
SAN26	01/08/2007	-33.9932	18.4838	9	26	Sand Fynbos
SAN27	01/08/2007	-33.9932	18.4832	9	27	Sand Fynbos
SAN28	04/09/2007	-34.0038	18.4875	43	26	Sand Fynbos
SAN29	08/08/2007	-34.0540	18.5053	16	9	Sand Fynbos
SAN30	08/08/2007	-34.0487	18.5104	16	13	Sand Fynbos
SAN31	08/08/2007	-34.0381	18.5356	16	18	Sand Fynbos
SAN32	08/08/2007	-34.0401	18.5340	16	18	Sand Fynbos
SAN33	10/08/2007	-34.0274	18.5397	18	19	Sand Fynbos
SAN34	17/09/2007	-33.7096	18.4544	56	6	Sand Fynbos
SAN35	17/09/2007	-33.6902	18.4547	56	12	Sand Fynbos
SAN36	17/09/2007	-33.7033	18.4687	56	16	Sand Fynbos
SAN37	17/09/2007	-33.7001	18.4683	56	20	Sand Fynbos
SAN38	20/09/2007	-32.8073	18.3598	59	37	Sand Fynbos
SAN39	21/09/2007	-32.7682	18.2391	60	23	Sand Fynbos
SAN40	21/09/2007	-32.7704	18.2308	60	15	Sand Fynbos
SAN41	22/09/2007	-33.3411	18.1848	61	5	Sand Fynbos

(Continued overleaf)

Appendix 1. (continued)

Site code	Date sampled	Latitude (dd)	Longitude (dd)	Time (days)	Altitude (m)	Vegetation group (wetland cluster)
SAN42	22/09/2007	-33.3404	18.1837	61	4	Sand Fynbos
SAN43	22/09/2007	-33.4038	18.2793	61	63	Sand Fynbos
SAN44	22/09/2007	-33.4035	18.2796	62	63	Sand Fynbos
SST01	10/10/2007	-34.7405	19.6794	79	3	Sandstone fynbos
SST02	10/10/2007	-34.7407	19.6783	79	6	Sandstone fynbos
SST03	10/10/2007	-34.7387	19.6407	79	5	Sandstone fynbos
SST04	10/10/2007	-34.7397	19.7325	79	7	Sandstone fynbos
SST05	10/10/2007	-34.7257	19.7334	79	13	Sandstone fynbos
SST06	12/10/2007	-34.7526	19.8017	81	14	Sandstone fynbos
SHA01	05/09/2007	-32.7723	18.8183	44	126	Shale renosterveld
SHA02	05/09/2007	-32.7024	18.8361	44	125	Shale renosterveld
SHA03	06/09/2007	-32.6886	18.9327	45	159	Shale renosterveld
SHA04	06/09/2007	-32.6776	18.9345	45	151	Shale renosterveld
SHA05	06/09/2007	-32.6409	18.8909	45	138	Shale renosterveld
SHA06	07/09/2007	-32.9016	18.7989	46	120	Shale renosterveld
WES01	16/08/2007	-34.0414	18.7241	24	9	Western Strandveld
WES02	17/08/2007	-34.0374	18.7226	25	12	Western Strandveld
WES03	11/08/2007	-34.0346	18.7216	19	11	Western Strandveld
WES04	11/08/2007	-34.0364	18.7214	19	12	Western Strandveld
WES05	04/10/2007	-34.0119	18.6643	73	34	Western Strandveld
WES06	04/10/2007	-34.0129	18.6644	73	32	Western Strandveld
WES07	04/10/2007	-34.0119	18.6675	73	29	Western Strandveld
WES08	05/10/2007	-33.9845	18.6606	74	38	Western Strandveld
WES09	05/10/2007	-33.9889	18.6593	74	33	Western Strandveld
WES10	23/07/2007	-34.0490	18.7170	0	14	Western Strandveld
WES11	23/07/2007	-34.0455	18.7226	0	9	Western Strandveld
WES12	23/07/2007	-34.0435	18.7248	0	10	Western Strandveld
WES13	10/09/2007	-33.6872	18.4356	49	6	Western Strandveld
WES14	10/09/2007	-33.6853	18.4349	49	5	Western Strandveld
WES15	10/09/2007	-33.6842	18.4368	49	8	Western Strandveld
WES16	10/09/2007	-33.6855	18.4374	49	10	Western Strandveld
WES17	10/09/2007	-33.6869	18.4369	49	8	Western Strandveld
WES18	10/09/2007	-33.6922	18.4386	49	7	Western Strandveld
WES19	08/08/2007	-34.0581	18.5046	16	10	Western Strandveld
WES20	08/08/2007	-34.0585	18.5035	16	7	Western Strandveld
WES21	08/08/2007	-34.0581	18.5001	16	7	Western Strandveld
WES22	10/08/2007	-34.0675	18.4950	18	6	Western Strandveld
WES23	10/08/2007	-34.0711	18.4983	18	7	Western Strandveld
WES24	10/08/2007	-34.0696	18.4980	18	8	Western Strandveld
WES25	23/07/2007	-34.0044	18.6435	0	37	Western Strandveld
WES26	01/10/2007	-34.0124	18.6813	70	28	Western Strandveld
WES27	01/10/2007	-34.0090	18.6806	70	28	Western Strandveld
WES28	01/10/2007	-34.0096	18.6785	70	18	Western Strandveld

Appendix 2. Physico-chemical, biotope and hydro-morphometrical variables measured in chapter 2. CV – complex vegetation; SV – simple vegetation; OW – open water; BU – benthic un-vegetated; TSA – total surface area; Max. depth – maximum depth.

Site code	pH	Conductivity (µS.cm^{-1})	Ave Temp. (°C)	Turbidity (NTU)	Oxygen (mg.L^{-1})	NO_3^-+NO_2^- (µg.L^{-1})	PO_4^{3+} (µg.L^{-1})	NH_4^+ (µg.L^{-1})	%CV	%SV	%OW	%BU	TSA (m^2)	Max. depth (cm)
FER01	8.10	826	18.75	9.5	9.60	1.86	11.20	23.40	0	30	0	70	1963	22
FER02	7.22	825	18.77	3.0	7.87	0.00	3.39	16.41	5	60	0	35	589	42
FER03	7.05	1832	21.83	2.0	6.77	6.73	3.69	21.66	60	40	0	0	48381	70
FER04	8.80	1786	22.42	4.0	10.33	1.99	4.11	8.57	60	20	20	0	14019	80
FER05	10.12	17880	28.58	3.5	12.53	0.00	18.80	19.97	90	10	0	0	7540	15
FER06	8.52	3143	20.97	12.0	10.10	0.00	1.58	17.21	70	0	0	30	29452	28
SAN01	7.86	429	15.07	18.6	5.07	2.57	13.67	33.44	70	20	10	0	1257	45
SAN02	8.57	258	19.40	2.1	10.03	0.88	9.38	26.00	100	0	0	0	471	25
SAN03	7.04	570	17.32	4.4	1.20	28.95	2827.36	2314.41	10	5	85	0	1665	68
SAN04	8.21	3697	18.73	14.7	8.50	0.87	510.89	105.21	15	15	70	0	19242	50
SAN05	7.27	2733	17.57	4.0	1.38	10.55	615.00	1524.48	0	60	40	0	1885	25
SAN06	7.57	3313	24.25	18.5	3.13	17.95	816.31	4231.53	40	10	50	0	1414	29
SAN07	8.08	583	26.33	7.0	6.27	10.26	655.65	65.20	60	0	40	0	7461	39
SAN08	6.90	306	18.20	7.4	1.73	11.62	213.88	2283.93	30	10	60	0	451	80
SAN09	7.38	585	22.50	1.8	1.93	9.66	87.39	56.77	35	5	60	0	2827	44
SAN10	7.25	261	20.45	4.5	4.60	11.24	37.11	47.04	60	0	40	0	1571	70
SAN11	7.40	222	13.90	4.1	7.70	3.95	4.04	19.63	0	100	0	0	62832	45
SAN12	6.68	239	11.88	5.3	3.70	44.19	8.57	33.23	70	0	30	0	611	28
SAN13	6.78	231	11.41	3.8	3.15	5.30	45.79	39.47	20	70	10	0	298	50
SAN14	6.55	169	12.33	1.4	5.00	2.12	5.42	19.46	90	0	10	0	5107	30
SAN15	6.87	345	11.25	3.7	3.97	0.43	20.97	23.64	30	60	10	0	543	52
SAN16	6.70	281	11.80	3.4	5.03	4.55	34.52	42.10	45	15	40	0	651	120
SAN17	6.61	463	12.87	1.7	5.80	0.08	1.55	7.09	100	0	0	0	436	18
SAN18	4.33	229	13.63	1.1	4.63	6.82	1.55	12.72	100	0	0	0	214	30
SAN19	4.07	141	15.12	0.9	6.83	3.28	6.57	11.64	100	0	0	0	738	27
SAN20	6.82	450	15.25	1.6	6.87	2.17	3.10	10.23	60	30	10	0	589	50
SAN21	4.37	234	16.45	2.0	5.73	13.07	1.66	23.04	60	30	10	0	1542	87
SAN22	6.44	184	15.10	1.0	6.17	0.67	2.90	15.26	35	45	20	0	5631	100
SAN23	4.56	216	16.35	1.0	8.30	10.18	2.96	21.27	80	10	10	0	426	39
SAN24	4.48	192	12.08	1.4	5.20	37.48	1.50	23.57	80	10	10	0	233	62
SAN25	6.74	579	16.15	2.7	5.83	6.10	4.51	62.37	95	0	5	0	486	49
SAN26	7.45	764	17.38	1.0	6.77	1.01	11.58	34.55	100	0	0	0	800	30
SAN27	6.98	294	15.45	0.7	2.53	1.47	86.17	22.24	100	0	0	0	1081	40
SAN28	8.31	114	19.20	1.5	11.07	1.33	7.14	11.70	50	0	20	30	721	40
SAN29	7.66	958	15.52	2.8	4.00	5.87	121.89	14.58	30	20	50	0	8652	48
SAN30	7.72	911	16.18	2.0	3.97	2.08	73.89	33.48	40	50	10	0	8234	120
SAN31	7.79	1097	14.72	3.4	9.30	8241.59	1276.73	1087.33	50	10	40	0	18153	70
SAN32	7.95	1048	15.88	2.6	7.47	801.02	1407.49	40.53	20	30	50	0	18153	150
SAN33	8.00	986	13.68	2.0	8.60	602.28	440.30	24.68	20	10	70	0	2151	83
SAN34	9.16	15617	18.70	2.3	14.33	0.01	119.92	6.70	95	0	0	5	716	38
SAN35	8.41	12467	22.83	15.7	11.23	0.06	2.67	24.14	10	30	0	60	1395	27

(Continued overleaf)

Appendix 2. (continued)

Site code	pH	Conductivity (μS.cm^{-1})	Ave Temp. (°C)	Turbidity (NTU)	Oxygen (mg.L^{-1})	NO_3^-+NO_2^- (μg.L^{-1})	PO_4^{3+} (μg.L^{-1})	NH_4^+ (μg.L^{-1})	%CV	%SV	%OW	%BU	TSA (m^2)	Max. depth (cm)
SAN36	8.22	20833	22.18	2.5	16.70	0.02	14.31	8.22	0	60	40	0	1374	150
SAN37	7.25	12983	23.13	17.5	7.13	1.36	23.90	28.38	20	60	0	20	707	21
SAN38	8.27	7653	22.30	10.5	2.80	5.91	140.79	303.03	100	0	0	0	1414	30
SAN39	8.71	2697	18.47	4.0	5.50	0.79	444.40	66.16	10	40	50	0	587	78
SAN40	8.68	10773	21.65	9.0	9.10	4.39	594.89	73.38	0	0	0	100	3181	20
SAN41	9.69	9190	22.12	4.0	15.47	0.12	1.67	8.21	60	0	40	0	518	150
SAN42	8.42	8100	23.25	34.0	6.50	0.18	256.12	73.27	70	0	30	0	1257	30
SAN43	7.93	7847	21.50	5.0	4.97	4.39	175.36	76.22	70	10	20	0	1963	34
SAN44	8.27	876	20.93	6.7	6.77	1.71	68.31	58.76	80	10	10	0	1071	28
SST01	6.71	883	14.97	2.5	6.53	18.50	9.62	73.75	70	30	0	0	1649	63
SST02	7.74	2560	15.95	1.5	10.23	18.54	2.94	41.63	55	40	5	0	393	70
SST03	7.78	2787	24.87	2.5	9.37	5.53	6.34	35.64	100	0	0	0	14137	14
SST04	8.33	13460	23.67	12.5	8.80	8.91	9.42	40.99	100	0	0	0	17593	17
SST05	9.74	3093	20.47	5.0	8.63	0.00	11.16	38.35	0	0	0	100	1571	10
SST06	8.06	488	24.72	1.0	8.90	0.00	6.36	0.23	30	30	40	0	3004	56
SHA01	7.27	353	17.03	9.8	5.87	14.58	66.27	98.39	40	40	20	0	15708	90
SHA02	6.95	354	17.72	157.5	3.33	2.56	15.86	2803.87	0	60	40	0	11781	40
SHA03	7.21	436	16.94	53.9	3.93	5.63	137.35	164.10	30	0	70	0	2513	50
SHA04	7.35	260	19.72	38.1	4.17	5.92	999.41	1534.37	40	10	0	50	3711	38
SHA05	7.72	265	18.25	713.0	6.57	47.65	579.56	130.81	40	10	50	0	1571	40
SHA06	7.31	2730	12.31	439.5	3.83	0.06	12.03	61.72	0	70	30	0	3142	40
WES01	7.94	3110	17.48	1.1	8.27	57.00	26.06	50.33	100	0	0	0	1257	35
WES02	7.81	840	15.67	2.1	8.30	0.12	3.96	15.85	45	40	15	0	1885	45
WES03	7.60	1158	14.80	44.2	7.07	0.71	4.46	2.24	30	20	50	0	942	80
WES04	7.93	1740	15.52	1.8	6.40	2.39	166.28	68.35	40	40	20	0	589	80
WES05	8.11	5093	18.40	2.0	5.53	3.16	14.10	34.33	5	95	0	0	3927	50
WES06	9.01	3553	22.52	1.5	10.43	0.56	2.81	20.06	15	45	40	0	471	150
WES07	8.19	3803	23.02	5.5	5.93	2.33	13.71	96.93	5	95	0	0	7854	20
WES08	8.34	2373	18.93	1.0	2.37	1.82	7.91	15.30	34	33	33	0	9425	75
WES09	8.06	1100	21.10	5.5	2.00	2.28	6.97	27.18	30	60	10	0	1257	80
WES10	8.17	863	18.12	1.4	9.63	3.27	7.76	14.96	60	30	10	0	3927	45
WES11	8.37	1026	16.38	4.6	11.33	2.77	37.06	38.51	30	45	25	0	1731	45
WES12	7.63	8193	15.90	6.1	8.57	0.94	76.59	31.35	20	40	40	0	3310	80
WES13	8.29	6453	18.47	1.9	9.47	1.56	9.29	34.97	60	0	40	0	3793	55
WES14	8.20	9253	19.50	2.7	7.60	0.37	43.87	64.42	60	0	40	0	1885	35
WES15	8.23	10640	22.38	1.7	5.63	1.26	6.33	54.11	70	0	30	0	707	35
WES16	8.18	7723	22.55	1.2	4.97	1.47	14.51	59.75	70	0	30	0	4712	35
WES17	8.64	3473	22.57	0.9	13.33	0.99	2.25	34.44	50	10	40	0	2199	45
WES18	8.12	1178	23.73	8.8	3.33	0.91	4.28	38.75	30	70	0	0	3010	45
WES19	7.43	1362	11.22	1.5	4.90	13.74	5.46	40.80	5	95	0	0	13435	48
WES20	7.63	1311	12.85	1.6	6.97	12.14	9.45	19.32	50	30	20	0	4519	150
WES21	7.33	4023	16.00	12.0	5.97	60.67	34.39	109.57	30	30	40	0	8488	150
WES22	8.12	20833	13.53	0.9	8.40	1.64	6.36	17.64	20	40	40	0	1374	180

(Continued overleaf)

Appendix 2. (continued)

Site code	pH	Conductivity (μS.cm^{-1})	Ave Temp. (°C)	Turbidity (NTU)	Oxygen (mg.L^{-1})	NO_3^-+NO_2^- (μg.L^{-1})	PO_4^{3+} (μg.L^{-1})	NH_4^+ (μg.L^{-1})	%CV	%SV	%OW	%BU	TSA (m^2)	Max. depth (cm)
WES23	7.62	890	13.42	0.4	8.13	4.47	2.72	1.47	25	45	30	0	3000	150
WES24	8.31	948	14.17	1.0	8.60	1.72	15.59	21.14	10	30	60	0	2356	150
WES25	8.02	1146	17.17	1.3	6.90	0.13	2.10	15.52	25	60	15	0	3142	45
WES26	7.51	869	19.95	1.0	3.07	0.04	10.75	42.98	20	70	10	0	1257	55
WES27	8.19	1437	23.02	2.0	7.33	0.04	1.69	5.61	60	40	0	0	17868	45
WES28	8.23	890	22.00	2.0	7.33	0.05	0.00	10.64	30	50	20	0	3000	64

Appendix 3. Ordinal scores for each type of land cover around wetlands used to proxy habitat transformation gradients in chapters 2 and 3. Natural – indigenous vegetation; Invaded – alien invasive vegetation; Agriculture – land converted to agriculture; Urban – land converted to urban surfaces. The areal cover of these categories was scored within 100 m and 500 m radii of each wetland edge using an ordinal scale: 0 - none; 1 – sparse cover (< 33%); 2 – moderate cover (33-66%); 3 – extensive cover (> 66%).

Site code	Natural 100m	Invaded 100m	Agriculture 100m	Urban 100m	Natural 500m	Invaded 500m	Agricultural 500m	Urban 500m
FER01	3	0	1	0	2	0	2	0
FER02	3	0	1	0	2	0	2	0
FER03	2	0	2	0	2	0	2	0
FER04	3	0	0	0	3	1	0	0
FER05	2	1	2	0	2	1	2	0
FER06	2	0	2	0	2	1	2	0
SAN01	1	3	0	0	1	3	0	1
SAN02	0	2	0	2	1	2	0	2
SAN03	2	2	0	0	3	1	0	0
SAN04	3	1	0	0	3	1	0	0
SAN05	2	2	0	0	3	1	0	0
SAN06	2	2	0	0	3	1	0	0
SAN07	1	0	3	0	1	1	3	0
SAN08	0	3	1	0	0	3	1	0
SAN09	1	1	3	0	1	1	3	0
SAN10	1	3	0	0	1	3	0	0
SAN11	0	3	0	0	0	3	0	0
SAN12	2	2	0	1	2	2	0	1
SAN13	1	3	0	1	2	2	0	1
SAN14	3	1	0	0	2	2	0	1
SAN15	1	3	0	0	2	2	0	1
SAN16	2	2	0	0	2	2	0	1
SAN17	2	2	0	0	2	2	0	1
SAN18	3	1	0	0	2	2	0	1
SAN19	3	1	0	0	2	2	0	1
SAN20	2	2	0	0	2	2	0	1
SAN21	3	0	0	0	2	2	0	1
SAN22	2	2	0	0	2	2	0	1

(Continued overleaf)

Appendix 3. (Continued)

Site code	Natural 100m	Invaded 100m	Agriculture 100m	Urban 100m	Natural 500m	Invaded 500m	Agricultural 500m	Urban 500m
SAN23	3	1	0	0	2	2	0	1
SAN24	3	1	0	0	2	2	0	1
SAN25	0	3	0	1	1	2	0	2
SAN26	2	2	0	0	2	2	0	1
SAN27	2	2	0	0	2	2	0	1
SAN28	1	3	0	0	1	3	0	1
SAN29	0	2	0	2	1	1	0	3
SAN30	0	3	0	1	0	1	0	3
SAN31	0	2	1	2	0	2	1	2
SAN32	0	3	1	1	0	2	1	2
SAN33	0	0	3	0	0	1	3	1
SAN34	1	3	0	0	1	3	0	0
SAN35	0	3	0	0	0	2	0	2
SAN36	0	3	0	1	0	2	0	2
SAN37	0	2	0	2	0	2	0	2
SAN38	1	2	2	0	1	2	2	0
SAN39	0	1	3	0	0	1	3	0
SAN40	0	1	3	0	1	1	3	0
SAN41	1	3	0	0	2	2	0	0
SAN42	2	2	0	0	2	2	0	0
SAN43	1	3	0	0	1	3	0	1
SAN44	1	3	0	0	1	3	0	1
SST01	3	1	0	0	3	1	0	0
SST02	2	2	0	0	2	2	0	0
SST03	0	3	0	0	2	2	0	0
SST04	3	1	0	0	3	1	0	0
SST05	2	2	0	0	2	2	0	0
SST06	0	3	0	0	1	3	0	0
SHA01	3	1	0	0	2	1	2	0
SHA02	1	0	3	0	1	0	3	0
SHA03	0	0	3	0	0	0	3	0
SHA04	2	0	2	0	1	0	3	0
SHA05	0	0	3	0	1	0	3	0
SHA06	0	0	3	0	0	0	3	0
WES01	1	3	0	0	1	3	0	0
WES02	0	3	0	1	1	3	0	1
WES03	0	3	0	0	0	3	0	1
WES04	0	3	0	1	0	3	0	1
WES05	1	3	0	0	2	2	0	1
WES06	1	3	0	0	2	2	0	1
WES07	3	1	0	0	2	2	0	0
WES08	3	0	0	0	2	2	0	0
WES09	3	0	0	0	3	1	0	0
WES10	2	2	0	1	1	2	0	2

(Continued overleaf)

Appendix 3. (Continued)

Site code	Natural 100m	Invaded 100m	Agriculture 100m	Urban 100m	Natural 500m	Invaded 500m	Agricultural 500m	Urban 500m
WES11	0	3	0	0	1	3	0	0
WES12	1	3	0	0	1	3	0	0
WES13	2	2	0	0	3	1	0	0
WES14	2	2	0	0	3	1	0	0
WES15	3	1	0	0	3	1	0	0
WES16	3	0	0	0	3	1	0	0
WES17	3	0	0	0	3	1	0	0
WES18	3	0	0	1	2	1	0	2
WES19	1	3	0	1	2	1	0	2
WES20	2	2	0	1	2	1	0	2
WES21	2	0	0	2	2	1	0	2
WES22	3	1	0	0	3	1	0	1
WES23	3	1	0	0	3	1	0	1
WES24	3	1	0	0	3	1	0	1
WES25	0	2	0	2	1	1	0	3
WES26	3	1	0	1	2	1	0	2
WES27	2	1	0	2	2	1	0	2
WES28	2	1	0	2	2	1	0	2

Appendix 4. Summary statistics of the hydro-morphometrical, physico-chemical and biotope variables (untransformed data) collected in chapter 2, reported per wetland cluster (defined by terrestrial vegetation group). TSA – total surface area; EC – electrical conductivity; CV – complex vegetation; SV – simple vegetation; OW – open water; BU – Benthic un-vegetated.

	HYDRO-MORPHOMETRY		PHYSICO-CHEMISTRY								BIOTOPE CHARACTERISTICS			
Ferricrete fynbos (n = 6)	TSA (m^2)	Max. depth (cm)	pH	EC (µS.cm^{-1})	Ave temp. (°C)	Turbidity (NTU)	Dissolved oxygen (mg.L^{-1})	NO_3^- + NO_2^- (µg.L^{-1})	PO_4^{3+} (µg.L^{-1})	NH_4^+ (µg.L^{-1})	%CV	%SV	%OW	%BU
Mean	16990	43	8.30	4382	21.9	5.7	9.53	1.76	7.13	17.87	48	27	3	23
Standard deviation	18620	27	1.13	6667	3.6	4.1	2.02	2.61	6.61	5.26	37	22	8	28
Median	10779	35	8.31	1809	21.4	3.8	9.85	0.93	3.90	18.59	60	25	0	15
25 percentile	3357	24	7.44	1066	19.3	3.1	8.30	0.00	3.47	16.61	19	13	0	0
75 percentile	25594	63	8.73	2816	22.3	8.1	10.28	1.96	9.43	21.24	68	38	0	34
Minimum	589	15	7.05	825	18.8	2.0	6.77	0.00	1.58	8.57	0	0	0	0
Maximum	48380	80	10.12	17880	28.6	12.0	12.53	6.73	18.80	23.40	90	60	20	70
Sand fynbos (n = 44)														
Mean	4320	56	7.28	3102	17.5	5.6	6.41	225.47	254.54	296.55	53	19	24	5
Standard deviation	10193	36	1.29	4918	4.0	6.6	3.50	1245.43	516.20	810.87	34	24	24	18
Median	1256	44	7.43	581	16.9	3.4	5.82	4.17	35.82	33.34	55	10	10	0
25 percentile	576	30	6.77	253	15.0	1.7	3.99	0.98	5.19	19.59	20	0	0	0
75 percentile	2320	70	8.21	3409	21.1	5.6	7.85	10.34	224.44	65.44	80	30	40	0
Minimum	214	18	4.07	114	11.3	0.7	1.20	0.01	1.50	6.70	0	0	0	0
Maximum	62831	150	9.69	20833	26.3	34.0	16.70	8241.59	2827.36	4231.53	100	100	85	100
Sandstone fynbos (n = 6)														
Mean	6069	38	8.06	3943	20.8	4.2	8.74	8.58	7.64	38.43	59	17	8	17
Standard deviation	7679	27	0.99	4760	4.4	4.3	1.23	8.42	3.00	23.37	40	19	16	41
Median	1610	37	7.92	2673	22.1	2.5	8.85	7.22	7.89	39.67	63	15	0	0
25 percentile	1196	15	7.75	1303	17.1	1.8	8.68	1.38	6.34	36.32	36	0	0	0
75 percentile	11015	61	8.26	3017	24.5	4.4	9.25	16.10	9.57	41.47	93	30	4	0
Minimum	392	10	6.71	876	15.0	1.0	6.53	0.00	2.94	0.23	0	0	0	0
Maximum	17592	70	9.74	13460	24.9	12.5	10.23	18.54	11.16	73.75	100	40	40	100

Appendix 4. (Continued)

	HYDRO-MORPHOMETRY		PHYSICO-CHEMISTRY								BIOTOPE CHARACTERISTICS			
Shale renosterveld (n = 6)	TSA (m^2)	Max. depth (cm)	pH	EC (mS.cm^{-1})	Ave temp. (°C)	Turbidity (NTU)	Dissolved oxygen (mg.L^{-1})	NO_3^- + NO_2^- (µg.L^{-1})	PO_4^{3+} (µg.L^{-1})	NH_4^+ (µg.L^{-1})	%CV	%SV	%OW	%BU
Mean	6381	50	7.30	359	17.0	235.3	4.62	12.73	301.75	798.87	25	32	35	8
Standard deviation	5878	20	0.25	91	2.5	282.4	1.29	17.80	403.01	1135.28	20	29	24	20
Median	3357	40	7.29	354	17.4	105.7	4.05	5.78	101.81	147.45	35	25	35	0
25 percentile	2636	40	7.23	287	17.0	42.0	3.86	3.33	28.46	106.49	8	10	23	0
75 percentile	9763	48	7.34	415	18.1	369.0	5.44	12.41	469.01	1191.80	40	55	48	0
Minimum	1570	38	6.95	260	12.3	9.8	3.33	0.06	12.03	61.72	0	0	0	0
Maximum	15708	90	7.72	488	19.7	713.0	6.57	47.65	999.41	2803.87	40	70	70	50
Western strandveld (n = 28)														
Mean	4126	74	8.04	3178	18.2	4.2	7.06	6.38	19.17	35.23	37	40	24	0
Standard deviation	4032	46	0.37	2819	3.6	8.3	2.63	15.17	33.27	26.48	23	28	17	0
Median	3075	53	8.12	2057	18.3	1.7	7.20	1.60	7.84	32.84	30	40	23	0
25 percentile	1612	45	7.77	1134	15.6	1.1	5.61	0.67	4.20	15.77	20	28	10	0
75 percentile	4567	80	8.23	3858	22.1	3.2	8.44	2.86	14.78	44.82	53	53	40	0
Minimum	471	20	7.33	840	11.2	0.4	2.00	0.04	0.00	1.47	5	0	0	0
Maximum	17867	180	9.01	10640	23.7	44.2	13.33	60.67	166.28	109.57	100	95	60	0

*** General comments:** These descriptive characteristics are not a focus in this study, but are included here for general reference. Sites were shallow as expected (maximum depth was 180 cm), although they had highly variable total surface areas. Total surface area displayed the most variability of all the measured constituents in this study, with standard deviations being higher than the means for three of the five wetland clusters. Wetlands generally had a neutral pH or were slightly alkaline, although several highly acidic sites were encountered in the Sand fynbos cluster and several highly alkaline sites were spread among the clusters (as reflected by minimum and maximum values for pH). Conductivity levels (as a proxy for salinity) were generally low with mean and median values all below 5 mS.cm^{-1}. Turbidity levels were low on the whole and had mean and median values across all clusters being < 10 NTU, except for the Shale renosterveld cluster, which stood out for having high mean and median turbidity values. These higher levels of turbidity in Shale renosterveld wetlands are probably to some extent a reflection of the naturally high quantity of clay particles in these shale-derived soils. Dissolved oxygen concentrations varied between moderate and high levels in terms of mean and median values among the wetland clusters. Mean and median nutrient concentrations were low, except for the high values reported for phosphates and ammonium in Shale renosterveld wetlands. Several extremely nutrient-enriched sites were found in the Sand fynbos cluster, as reflected by the very high maximum values for all three nutrient variables in this cluster. Wetlands were generally extensively vegetated, as reflected by the higher mean and median cover values for vegetated biotopes (complex and simple vegetation) relative to un-vegetated biotopes (open water and benthic un-vegetated) for all wetland clusters.

Appendix 5. List of macroinvertebrate taxa collected from the 90 temporary wetlands in chapter 3. Certain taxa could only be identified to family level. Chironomidae were identified to sub-family level.

Order	Family	Genus	Species
Acarina	Arrenuridae	*Arrenurus*	*Arrenurus* sp. 1
			Arrenurus sp. 2
	Erythraeidae		
	Eylaidae	*Eylais*	*Eylais* sp. 1
	Hydrachnidae	*Hydrachna*	*Hydrachna fissigera*
		Hydryphantes	*Hydryphantes parmalatus*
			Hydryphantes sp.
		Mamersa	*Mamersa testudinata*
	Limnocharidae	*Limnochares*	*Limnochares crinita*
	Macrochelidae	*Macrocheles*	*Macrocheles sp.*
	Oribatidae		
	Pionidae	*Piona*	*Piona* sp.
	Trombidiidae		
	Unionicolidae	*Neumania*	*Neumania* sp. 1
			Neumania sp. 2
Amphipoda	Paramelitidae	*Paramelita*	*Paramelita capensis*
			Paramelita pinnicornis
			Paramelita sp.
Anostraca	Streptocephalidae	*Streptocephalus*	*Streptocephalus dendyi*
			Streptocephalus purcelli
			Streptocephalus sp.
Coleoptera	Dytiscidae	*Canthyporus*	*Canthyporus canthydroides*
			Canthyporus hottentottus
			Canthyporus sp. 1
			Canthyporus sp. 2
			Canthyporus sp. 3
			Canthyporus sp. 4
		Cybister	*Cybister* sp.
		Darwinhydrus	*Darwinhydrus solidus*
		Herophydrus	*Herophydrus capensis*
		Hydaticus	*Hydaticus* sp.
		Hydropeplus	*Hydropeplus* sp. 1
			Hydropeplus sp. 2
			Hydropeplus sp. 3
			Hydropeplus trimaculatus
		Hyphydrus	*Hyphydrus soni*
		Laccophilus	*Laccophilus cyclopis*
			Laccophilus sp.
		Primospes	*Primospes* sp.
			Primospes suturalis
		Rhantus	*Rhantus cicurius*
	Georissidae		
	Gyrinidae	*Aulonogyrus*	*Aulonogyrus capensis*

(Continued overleaf)

Appendix 5. (Continued)

Order	Family	Genus	Species
Coleoptera (continued)	Haliplidae	*Haliplus*	*Haliplus rufescens*
			Haliplus sp.
	Hydraenidae	*Ochthebius*	*Ochthebius extremus*
			Ochthebius spatulus
		Parasthetops	*Parasthetops nigritus*
		Parhydraena	*Parhydraena* sp. 1
			Parhydraena sp. 2
	Hydrophilidae	*Amphiops*	*Amphiops senegalensis*
		Anacaena	*Anacaena* sp.
		Berosus	*Berosus* sp. 1
			Berosus sp. 2
		Crenitis	*Crenitis* sp. 1
			Crenitis sp. 2
			Crenitis sp. 3
		Enochrus	*Enochrus continentalis*
			Enochrus picinus
			Enochrus sp. 1
			Enochrus sp. 2
		Helochares	*Helochares* sp. 1
			Helochares sp. 2
			Helochares sp. 3
		Laccobius	*Laccobius* sp.
		Paracymus	*Paracymus* sp. 1
			Paracymus sp. 2
			Paracymus sp. 3
		Regimbartia	*Regimbartia compressa*
	Scirtidae		
	Spercheidae	*Spercheus*	*Spercheus* spp.
Conchostraca	Leptestheriidae	*Leptestheria*	*Leptestheria rubidgei*
Diptera	Ceratopogonidae		
	Chaoboridae	*Chaoborus*	*Chaoborus microstictus*
	Chironomidae:		
	Subfamily Chironominae		
	Subfamily Orthocladinae		
	Subfamily Tanypodinae		
	Culicidae	*Aedes*	*Aedes* spp.
		Culex	*Culex* spp.
		Culiseta	*Culiseta* spp.
	Dixidae		
	Stratiomyidae		
	Tipulidae		
Ephemeroptera	Baetidae	*Cloeon*	*Cloeon* spp.
Hemiptera	Belostomatidae	*Appasus*	*Appasus capensis*
	Corixidae	*Micronecta*	*Micronecta citharista*
		Sigara	*Sigara meridionalis*
			Sigara pectoralis
			Sigara wahlbergi
	Gerridae	*Gerris*	*Gerris swakopensis*
		Limnogonus	*Limnogonus capensis*

(Continued overleaf)

Appendix 5. (Continued)

Order	Family	Genus	Species
Hemiptera (continued)	Notonectidae	*Anisops*	*Anisops sardea*
			Anisops sp.
		Notonecta	*Notonecta lactitans*
			Notonecta sp.
	Pleidae	*Plea*	*Plea piccanina*
			Plea pullula
	Veliidae	*Mesovelia*	*Mesovelia vittigera*
Isopoda	Amphisopodidae	*Mesamphisopus*	*Mesamphisopus* spp.
Odonata	Aeshnidae	*Anax*	*Anax* spp.
	Coenagrionidae	*Enallagma*	*Enallagma* spp.
		Ischnura	*Ischnura* spp.
	Libellulidae	*Trithemis*	*Trithemis* spp.
		Palpopleura	*Palpopleura* spp.
Pulmonata	Ancylidae	*Ferrissia*	*Ferrissia* sp.
	Helicidae	*Cochlicella*	*Cochlicella* spp.
	Lymnaeidae	*Lymnaea*	*Lymnaea columella*
	Physidae	*Aplexa*	*Aplexa marmorata*
		Physa	*Physa acuta*
	Planorbidae	*Bulinus*	*Bulinus tropicus*
		Ceratophallus	*Ceratophallus natalensis*
Littorinimorpha	Pomatiopsidae	*Tomichia*	*Tomichia* spp.
Trichoptera	Leptoceridae	*Athripsodes*	*Athripsodes* sp.

Appendix 6. List of microcrustacean taxa collected from the subset of 41 temporary wetlands sampled in chapter 3.

Class/Sub-class	**Order**	**Family**	**Genus**	**Species**
Branchiopoda	Cladocera	Chydoridae	*Chydorus*	*Chydorus* sp.
			Leydigia	*Leydigia* sp.
		Daphniidae	*Ceriodaphnia*	*Ceriodaphnia producta*
			Daphnia	*Daphnia barbata*
				Daphnia dolichocephala
				Daphnia pulex/obtusa
				Daphnia (Ctenodaphnia) sp. 1
				Daphnia (Ctenodaphnia) sp. 2
			Megafenestra	*Megafenestra aurita*
			Simocephalus	*Simocephalus* spp.
		Macrothricidae	*Macrothrix*	*Macrothrix propinqua*
		Moinidae	*Moina*	*Moina brachiata*
				Moina sp.
Copepoda	Calanoida	Diaptomidae	*Lovenula*	*Lovenula simplex*
			Metadiaptomus	*Metadiaptomus capensis*
				Metadiaptomus purcelli
			Paradiaptomus	*Paradiaptomus lamellatus*
				Paradiaptomus sp.
	Cyclopoida	Cyclopidae	*Acanthocyclops*	*Acanthocyclops* sp.
				Acanthocyclops vernalis
			Mesocyclops	*Mesocyclops major*
			Microcyclops	*Microcyclops crassipes*
	Harpacticoida	Ameiridae	*Nitocra*	*Nitocra dubia*
Ostracoda	Podocopida	Cyprididae	*Bradycypris*	*Bradycypris intumescens*
			Chrissia	*Chrissia* sp. 1
				Chrissia sp. 2
			Cypretta	*Cypretta* sp.
			Cypricercus	*Cypricercus episphaena*
				Cypricercus maculatus
			Heterocypris	*Heterocypris* sp.
			Paracypretta	*Paracypretta acanthifera*
				Paracypretta sp.
			Physocypria	*Physocypria capensis*
			Pseudocypris	*Pseudocypris acuta*
			Ramotha	*Ramotha capensis*
				Ramotha producta
				Ramotha trichota
			Zonocypris	*Zonocypris cordata*
		Cypridopsidae	*Cypridopsis*	*Cypridopsis* sp.
			Sarscypridopsis	*Sarscypridopsis* sp. 1
				Sarscypridopsis sp. 2
				Sarscypridopsis sp. 3
		Limnocytheridae	*Gomphocythere*	*Gomphocythere* sp.

Appendix 7. Template score sheet for calculating the human disturbance score ("HDS") at each site in chapter 3. The score sheet consists of two main components, firstly, the land use characterization table used for scoring the expected effects of immediate and surrounding land use on wetland water quality, hydrology and physical structure. WQ - water quality; Hydrol – hydrology; Phys struc - physical structure. Secondly, the table is used for scoring plant community indicators.

Rate areal extent: 0 = none, 1 = (<33%), 2 = (33-66%), 3 = (66 – 100%) If present, score as per below SCORE table												
Present Landuse / Activity	In wetland				Within 100m				Within 500m			
	Extent	Score Impact on:			Extent	Score Impact on:			Extent	Score Impact on:		
		WQ	Hydrol	Phys struc		WQ	Hydrol	Phys struc		WQ	Hydrol	Phys struc
Commercial afforestation												
Agriculture – crops												
Agriculture – livestock												
Abandoned lands												
Rural development												
Urban development												
Suburban gardens												
Deep flooding (too deep for emergent vegetation)												
Shallow flooding												
Dead brush piles of alien vegetation												
Dead/dying plants												
Drowned vegetation												
Stranded aquatic vegetation												
Old high water marks												
Industrial												
Informal settlement												
Mining / excavation												
Recreational (sports field, golf estate etc.) specify												
Infilling												
Stormwater outlets												
Sewage disposal												
WWTW outlets												
Solid waste disposal (including dumping and litter)												
Weirs												
Berms												
Dams												
Water abstraction												
Drainage channels												
Roads / Railway												

(Continued overleaf)

Appendix 7 (continued)

Rate areal extent: 0 = none, 1 = (<33%), 2 = (33-66%), 3 = (66 – 100%) If present, score as per below SCORE table												
Present Landuse / Activity	In wetland				Within 100m				Within 500m			
	Extent	Score Impact on:			Extent	Score Impact on:			Extent	Score Impact on:		
		WQ	Hydrol	Phys struc		WQ	Hydrol	Phys struc		WQ	Hydrol	Phys struc
Culverts												
Dredging												
Pedestrian paths												
Off road vehicle use												
Habitat modifiers fish stocking												
Dense woody alien vegetation patches												
Dense aquatic alien vegetation patches												
Erosion e.g. gullies / headcuts												
Deposition / sediment												
Other												

SCORE TABLE: total impact on scale of 0 to 5:
5 = Highly extensive: currently active and major disturbance to wetland
4 = Extensive: less intense than “highly extensive”, but current or active alteration of wetland
3 = Immoderate: active alterations that have changed wetland
2 = Moderate: low intensity alteration that has minor impact on wetland
1 = Minimal: low intensity alteration or past alteration that is not currently affecting wetland
0 = Least: as expected for reference, no evidence of disturbance to wetland

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

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

Appendix 8A. Example score sheet for calculating the human disturbance score ("HDS") at SAN13, a relatively impacted site (see Appendices 1-3 for further site details).

Only the land use categories which received a score at this site are presented. Appendix 7 provides details of the scoring criteria. WQ - water quality; Hydrol – hydrology; Phys struc - physical structure. For each column scored for human impacts (and in turn within each of the distance bands), the maximum score of impact across all land use activities (see "max. impact scores") was used in the next step, which was to sum the maximum scores of impact across all impact categories (namely WQ, Hydrol, Phys struc) and distance bands (see "sum of max. impact scores"). The plant community indicator scores were summed and this score was added to the "sum of max. impact scores" to produce a final impact score for the site. This was divided by the maximum possible score (70) to obtain the final HDS (%) for each wetland.

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

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

Appendix 8B. Example score sheet for calculating the human disturbance score ("HDS") at SAN23, a minimally impacted site (see Appendices 1-3 for further site details).

Only the land use categories which received a score at this site are presented. Appendix 7 provides details of the scoring criteria. WQ - water quality; Hydrol – hydrology; Phys struc - physical structure. For each column scored for human impacts (and in turn within each of the distance bands), the maximum score of impact across all land use activities (see "max. impact scores") was used in the next step, which was to sum the maximum scores of impact across all impact categories (namely WQ, Hydrol, Phys struc) and distance bands (see "sum of max. impact scores"). The plant community indicator scores were summed and this score was added to the "sum of max. impact scores" to produce a final impact score for the site. This was divided by the maximum possible score (70) to obtain the final HDS (%) for each wetland.

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

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

Appendix 9. Human disturbance scores ("HDS") for each wetland in chapter 3, reported per vegetation group (wetland cluster). Scores are expressed as percentages and were calculated using the rapid-assessment index of human impacts in and around wetlands (see Appendices 7 and 8 and section 3.2.3 of chapter 3). Low scores indicate minimal impacts, whilst high scores indicate extensive impacts.

Ferricrete fynbos		Sand fynbos		Sandstone fynbos		Shale renosterveld		Western strandveld	
Site code	HDS (%)	Site code	HDS (%)	Site code	HDS (%)	Site code	HDS (%)	Site code	HDS (%)
FER01	53	SAN01	66	SST01	36	SHA01	44	WES01	66
FER02	50	SAN02	74	SST02	39	SHA02	76	WES02	69
FER03	44	SAN03	27	SST03	43	SHA03	74	WES03	61
FER04	23	SAN04	46	SST04	40	SHA04	69	WES04	50
FER05	51	SAN05	31	SST05	36	SHA05	74	WES05	63
FER06	47	SAN06	33	SST06	60	SHA06	73	WES06	57
		SAN07	47					WES07	47
		SAN08	53					WES08	43
		SAN09	59					WES09	41
		SAN10	69					WES10	70
		SAN11	60					WES11	67
		SAN12	50					WES12	70
		SAN13	66					WES13	14
		SAN14	40					WES14	10
		SAN15	50					WES15	17
		SAN16	46					WES16	13
		SAN17	36					WES17	10
		SAN18	31					WES18	30
		SAN19	36					WES19	63
		SAN20	34					WES20	54
		SAN21	33					WES21	59
		SAN22	36					WES22	36
		SAN23	33					WES23	21
		SAN24	37					WES24	24
		SAN25	63					WES25	74
		SAN26	47					WES26	66
		SAN27	51					WES27	69
		SAN28	60					WES28	69
		SAN29	74						
		SAN30	77						
		SAN31	67						
		SAN32	67						
		SAN33	69						
		SAN34	50						
		SAN35	59						
		SAN36	64						
		SAN37	51						
		SAN38	59						
		SAN39	69						
		SAN40	74						
		SAN41	51						
		SAN42	44						
		SAN43	56						
		SAN44	63						

Appendix 10. Macroinvertebrate and microcrustacean metrics tested in chapters 3 and 4. All families (excluding those present in < 5% of samples) were also tested as indicator taxa (see section 3.2.4). Blank sources (i.e. "–") imply that metrics were developed during this study. SASS: South African Scoring System for streams (Dickens and Graham 2002); FBI: Family Biotic Index for wetlands (Hicks and Nedeau 2000). The terms "tolerant" and "intolerant" used for certain macroinvertebrate metrics in this study are derived from Hicks and Nedeau (2000) for New England (USA) and are not based on levels of known tolerance for taxa in the south-western Cape study region.

METRIC	SOURCE
Macroinvertebrates	
Total number of individuals	Gernes & Helgen (2002)
Total number of water mite individuals (Acarina)	-
Total number of "intolerant" individuals ("AAA" - Acarina+Aeshnidae+Amphipoda)	Hicks & Nedeau (2000)
% "Intolerant" individuals (AAA) of total sample count	Hicks & Nedeau (2000)
Total number of "intolerant" individuals (all "intolerant" taxa)	Intolerant taxa defined by Hicks & Nedeau (2000)
% "Intolerant individuals" of total sample count (all "intolerant" taxa)	Intolerant taxa defined by Hicks & Nedeau (2000)
Total number of "tolerant" Coleopteran individuals	Tolerant taxa defined by Hicks & Nedeau (2000)
Total number of Coleopteran individuals	-
% Coleopteran individuals (of total sample count)	-
Corixidae (as % of Coleoptera and Hemiptera)	Gernes &Helgen (2002)
Total number of individuals in dominant taxon	Gernes &Helgen (2002)
% Dominant taxon (of total sample count)	Gernes &Helgen (2002)
Total number of Gastropod individuals	-
% Gastropod individuals (of total sample count)	-
Total number of Hemipteran individuals	-
% Hemipteran individuals (of total sample count)	-
Sum (Corixidae + Notonectidae individuals)	-
Sum (Physidae + Planorbidae individuals)	-
Total number of individuals in dominant three taxa	-
% Dominant three taxa (of total sample count)	Gernes &Helgen (2002)
Total number of families	Gernes & Helgen (2002)
Family Biotic Index (FBI)	Hicks & Nedeau (2000)
Average score per taxon (FBI)	Hicks & Nedeau (2000)
% Predators	Functional Feeding Guilds of Hicks & Nedeau (2000)
% Scrapers	Functional Feeding Guilds of Hicks & Nedeau (2000)
% Grazer-collectors	Functional Feeding Guilds of Hicks & Nedeau (2000)
% Omnivores	Functional Feeding Guilds of Hicks & Nedeau (2000)
% Shredders	Functional Feeding Guilds of Hicks & Nedeau (2000)
SASS score	Dickens & Graham (2002)
Average score per taxon (SASS)	Dickens & Graham (2002)

(Continued overleaf)

Appendix 10. (Continued)

METRIC	SOURCE
Microcrustaceans	*All microcrustacean metrics developed for this study*
Total number of individuals	-
Total number of taxa	-
Total number of families	-
Total number of Copepod individuals	-
Total number of Ostracod individuals	-
Total number of Cladoceran individuals	-
% Copepods (of total sample count)	-
% Ostracods (of total sample count)	-
% Cladocerans (of total sample count)	-
Number of Copepod taxa	-
Number of Ostracod taxa	-
Number of Cladoceran taxa	-
% Copepod taxa (of total taxa)	-
% Ostracod taxa (of total taxa)	-
% Cladoceran taxa (of total taxa)	-

Appendix 11. Sites 1 – 4 inside the core conservation area at Kenilworth. These wetlands are surrounded by extensive Sand fynbos vegetation. The beds of aquatic vegetation (*Isolepis rubicunda*) are clearly visible at sites 3 and 4. The photographs were taken at various times.

Appendix 11 (continued). Sites 5 – 7 surrounded by moderately transformed land on the periphery of the core conservation area at Kenilworth. Site 8 occurs within the extensively transformed area at Youngsfield. Note the conspicuous aquatic vegetation beds (*Isolepis rubicunda*) at sites 5 and 7. Also note the presence of the alga *Cladophora* sp. at site 8. The photographs were taken at various times.

Appendix 11 (continued). Sites 9 – 12 occurring within the extensively transformed area at Youngsfield. The photographs were taken at various times.

Appendix 12. List of the macroinvertebrate taxa collected in chapter 4. Certain taxa could only be identified to family level.

Order	Family	Genus	Species
Acarina	Arrenuridae	*Arrenurus*	*Arrenurus* sp.
	Eylaidae	*Eylais*	*Eylais* sp.
	Hydrachnidae	*Hydrachna*	*Hydrachna* sp.
	Hydryphantidae	*Hydryphantes*	*Hydryphantes* sp.
	Limnocharidae	*Limnochares*	*Limnochares* sp.
	Pionidae	*Piona*	*Piona* sp. 1
			Piona sp. 2
Amphipoda	Paramelitidae	*Paramelita*	*Paramelita capensis*
			Paramelita pinnicornis
Anostraca	Streptocephalidae	*Streptocephalus*	*Streptocephalus dendyi*
Coleoptera	Curculionidae	*Bagous*	*Bagous* sp.
	Dryopidae	*Rapnus*	*Rapnus raffrayi*
			Rapnus sp.
	Dytiscidae	*Canthyporus*	*Canthyporus hottentotus*
			Canthyporus sp.
		Darwinhydrus	*Darwinhydrus solidus*
		Herophydrus	*Herophydrus capensis*
		Hydropeplus	*Hydropeplus trimaculatus*
		Laccophilus	*Laccophilus cyclopis*
			Laccophilus sp. 1
			Laccophilus sp. 2
	Georissidae	*Georissus*	*Georissus* sp.
	Gyrinidae	*Gyrinus*	*Gyrinus vicinus*
	Haliplidae	*Algophilus*	*Algophilus lathridioides*
		Haliplus	*Haliplus rufescens*
			Haliplus sp. 1
			Haliplus sp. 2
	Hydraenidae	*Aulacochthebius*	*Aulacochthebius* sp.
		Hydraena	*Hydraena* sp.
		Ochthebius	*Ochthebius* sp.
	Hydrochidae	*Hydrochus*	*Hydrochus* sp.
	Hydrophilidae	*Berosus*	*Berosus crassipes*
		Enochrus	*Enochrus picinus*
			Enochrus sp.
		Helochares	*Helochares dilutus*
			Helochares sp.
		Laccobius	*Laccobius* sp.
	Scirtidae	*Scirtidae*	*Scirtidae*
	Spercheidae	*Spercheus*	*Spercheus* sp.
	Staphylinidae		
Diptera	Ceratopogonidae		
	Chironomidae	*Acinoretractus*	*Acinoretractus* sp.
		Cardocladius	*Cardocladius hessei*
		Cladotanytarsus	*Cladotanytarsus* sp. 1
			Cladotanytarsus sp. 2
		Corynoneura	*Corynoneura* sp.
		Cricotopus	*Cricotopus* sp.
		Dicrotendipes	*Dicrotendipes* sp.
		Endochironomus	*Endochironomus* sp.
		Polypedilum	*Polypedilum* sp. 1
			Polypedilum sp. 2
			Polypedilum sp. 3
		Psectrocladius	*Psectrocladius* sp. 1
			Psectrocladius sp. 2
			Psectrocladius sp. 3
		Smittia	*Smittia* sp.

(Continued overleaf)

Appendix 12. (Continued)

Order	Family	Genus	Species
Diptera	Chironomidae	*Virgatanytarsus*	*Virgatanytarsus* sp.
	Culicidae	*Culex*	*Culex* sp.
		Culiseta	*Culiseta* sp.
	Ephydridae		
Ephemeroptera	Baetidae	*Cloeon*	*Cloeon* sp.
Haplotaxida	Naididae	*Pristina*	*Pristina longiseta*
Hemiptera	Belostomatidae	*Appasus*	*Appasus capensis*
	Corixidae	*Sigara*	*Sigara* spp.
	Gerridae	*Gerris*	*Gerris swakopensis*
	Nepidae	*Ranatra*	*Ranatra grandicollis*
	Notonectidae	*Anisops*	*Anisops* spp.
		Enithares	*Enithares sobria*
		Notonecta	*Notonecta lactitans*
	Pleidae	*Plea*	*Plea* spp.
	Veliidae	*Mesovelia*	*Mesovelia vittigera*
Hydroida	Hydridae	*Hydra*	*Hydra* sp.
Isopoda	Amphisopodidae	*Mesamphisopus*	*Mesamphisopus* sp.
Lepidoptera	Crambidae		
Lumbriculida	Lumbriculidae	*Lumbriculus*	*Lumbriculus variegatus*
Neorhabdocoela	Typhloplanidae	*Mesostoma*	*Mesostoma* sp. 1
			Mesostoma sp. 2
Odonata	Aeshnidae	*Anax*	*Anax* sp.
	Coenagrionidae	*Enallagma*	*Enallagma* sp.
	Libellulidae	*Palpopleura*	*Palpopleura* sp.
Pulmonata	Ancylidae	*Ferrissia*	*Ferrissia* sp.
	Lymnaeidae	*Lymnaea*	*Lymnaea columella*
	Physidae	*Aplexa*	*Aplexa marmorata*
	Planorbidae	*Bulinus*	*Bulinus tropicus*
		Ceratophallus	*Ceratophallus natalensis*
Rhynchobdellida	Glossiphoniidae	*Helobdella*	*Helobdella conifera*
			Helobdella stagnalis
Trichoptera	Hydroptilidae		
	Leptoceridae	*Athripsodes*	*Athripsodes* sp.

Appendix 13. List of the microcrustacean taxa collected in chapter 4.

Class/Sub-class	**Order**	**Family**	**Genus**	**Species**
Branchiopoda	Cladocera	Chydoridae	*Chydorus*	*Chydorus* sp.
			Leydigia	*Leydigia* sp.
		Daphniidae	*Ceriodaphnia*	*Ceriodaphnia producta*
			Daphnia	*Daphnia laevis*
				Daphnia obtusa
				Daphnia (*Ctenodaphnia*) sp.
			Megafenestra	*Megafenestra aurita*
			Scapholeberis	*Scapholeberis kingi*
			Simocephalus	*Simocephalus* sp.
		Macrothricidae	*Macrothrix*	*Macrothrix propinqua*
Copepoda	Calanoida	Diaptomidae	*Lovenula*	*Lovenula simplex*
			Metadiaptomus	*Metadiaptomus purcelli*
			Paradiaptomus	*Paradiaptomus lamellatus*
	Cyclopoida	Cyclopidae	*Mesocyclops*	*Mesocyclops major*
			Microcyclops	*Microcyclops crassipes*
Ostracoda	Podocopida	Cyprididae	*Chrissia*	*Chrissia* sp.
			Cypretta	*Cypretta turgida*
			Cypricercus	*Cypricercus episphaena*
			Paracypretta	*Paracypretta acanthifera*
			Physocypria	*Physocypria capensis*
			Ramotha	*Ramotha capensis*
			Zonocypris	*Zonocypris cordata*
		Cypridopsidae	*Cypridopsis*	*Cypridopsis vidua*
			Sarscypridopsis	*Sarscypridopsis* sp.
		Lymnocytheridae	*Gomphocythere*	*Gomphocythere* sp.

Appendix 14. Static environmental variables recorded at each site in chapter 4. Latitude and longitude at the centre point of each wetland is recorded in decimal degrees. The four habitat transformation variables are expressed as percentage cover within 100 m of wetlands.

Sites	Latitude	Longitude	% Indigenous vegetation	% Kikuyu	% *Acacia*	% Road
1	-33.9942	18.4834	85	14	0	1
2	-33.9962	18.4841	93.5	5	0	1.5
3	-33.9964	18.4848	98.5	0	0	0.5
4	-33.9986	18.4833	74.5	20	0	5.5
5	-34.0002	18.4855	33	36	0.5	0
6	-34.0005	18.4836	38	47	0	2.5
7	-34.0012	18.4878	50.5	31.5	0	15.5
8	-34.0033	18.4886	0	74	11.5	7
9	-34.0099	18.4891	0	60	35	1.5
10	-34.0098	18.4892	0	62.5	29	0.5
11	-34.0100	18.4900	0	51	29	2
12	-34.0113	18.4904	0	43	10	30.5

Appendix 15. Labile environmental variables collected at each wetland in chapter 4 (untransformed raw data). EC – electrical conductivity; UVA_{254} – ultraviolet absorbance at 254nm (proxy for the concentration of humic substances in the water column); TSA – total surface area; Veg. – vegetation.

Site code	Date sampled	Temporal category (covariables)	pH	EC ($\mu S.cm^{-1}$)	Turbidity (NTU)	Oxygen ($mg.L^{-1}$)	Ave temp. (°C)	$NO_3^-+NO_2^-$ ($\mu g.L^{-1}$)	NH_4^+ ($\mu g.L^{-1}$)	PO_4^{3+} ($\mu g.L^{-1}$)	Chl *a* ($\mu g.L^{-1}$)	UVA_{254} (cm^{-1})	TSA (m^2)	Maximum depth (cm)	% Macro-algae	% Simple veg. (sedge)	% Simple veg. (reeded)	% Open water	% Complex veg.
1A	12/06/08	June 2008	6.49	878	2.7	7.74	15.0	1.93	2.75	2.40	50.24	1.254	4262	88	0	30	0	50	20
2A	13/06/08	June 2008	4.16	509	3.3	4.58	15.0	10.48	3.29	6.40	71.45	2.349	2421	55	0	5	10	0	85
3A	13/06/08	June 2008	4.20	531	1.3	5.43	16.4	8.84	6.04	1.20	21.50	2.196	142	69	0	20	0	25	55
4A	13/06/08	June 2008	6.24	549	1.3	4.40	15.8	0.92	3.29	1.20	64.49	1.344	1895	120	0	0	20	60	20
5A	26/06/08	June 2008	5.81	669	25.0	1.03	12.7	0.61	44.47	32.80	0.00	1.452	435	88	10	10	75	15	0
6A	26/06/08	June 2008	6.38	485	3.3	1.28	14.1	14.08	1.10	46.80	20.46	1.691	692	110	10	10	5	20	65
7A	27/06/08	June 2008	6.20	320	2.0	2.94	15.2	2.75	0.55	1.20	64.49	1.184	1429	85	0	5	5	35	55
8A	24/06/08	June 2008	7.23	589	1.0	2.03	16.8	24.83	17.02	2.80	22.61	0.928	2441	116	0	15	0	70	15
9A	25/06/08	June 2008	6.35	245	3.3	0.35	15.2	33.10	4.94	88.00	40.92	1.56	199	76	30	5	15	40	40
10A	25/06/08	June 2008	5.93	184	3.0	1.09	15.1	15.34	2.20	9.20	21.50	1.223	546	118	20	0	15	45	40
11A	25/06/08	June 2008	6.34	441	3.3	0.57	13.9	93.51	0.55	14.80	0.00	2.163	1366	94	20	10	0	90	0
12A	25/06/08	June 2008	6.53	438	2.7	0.45	13.3	10.38	18.12	27.20	0.00	1.974	664	89	40	40	0	20	40
1B	04/09/08	August/September 2008	6.68	222	1.1	5.47	16.3	1.33	18.03	0.00	0.00	0.956	5631	100	0	20	0	10	70
2B	04/09/08	August/September 2008	4.83	170	1.3	3.79	14.6	11.65	35.19	8.92	45.22	2.658	1542	87	0	0	0	5	95
3B	04/09/08	August/September 2008	4.22	156	2.8	5.18	15.3	9.07	33.48	7.64	0.00	2.625	233	69	0	0	0	0	100
5B	01/09/08	August/September 2008	6.25	219	7.0	4.80	11.0	1.50	51.66	38.25	19.50	1.349	430	92	10	25	45	30	0
6B	01/09/08	August/September 2008	7.02	211	4.6	5.81	11.8	0.92	19.74	39.49	0.00	0.991	651	120	10	5	5	25	65
7B	01/09/08	August/September 2008	6.64	130	1.7	6.30	12.0	5.09	18.88	16.56	0.00	1.066	3775	94	10	25	0	45	30
9B	29/08/08	August/September 2008	6.74	152	2.8	6.44	15.8	13.94	29.18	310.85	22.61	1.182	327	98	10	5	15	60	20
10B	29/08/08	August/September 2008	6.73	117	1.5	7.78	15.9	2.09	16.31	25.48	42.99	0.866	596	120	10	10	0	55	35
12B	29/08/08	August/September 2008	6.85	323	3.3	4.20	14.1	74.16	21.46	30.58	0.00	1.488	1042	81	40	20	0	65	15
1C	03/09/09	August/September 2009	6.53	205	0.8	5.65	14.4	1.71	160.92	8.52	0.00	1.058	6014	103	0	50	0	15	35
2C	28/08/09	August/September 2009	4.48	188	1.5	2.28	15.0	27.01	204.07	3.65	8.95	3.27	1974	81	0	10	0	10	80
3C	28/08/09	August/September 2009	4.19	187	1.7	4.91	16.0	18.44	210.85	7.15	9.24	2.895	135	67	30	10	0	5	85
4C	03/09/09	August/September 2009	6.20	217	1.5	4.11	14.6	4.68	148.48	2.40	8.67	1.383	4453	111	0	15	5	20	60
5C	27/08/09	August/September 2009	5.59	327	2.9	1.47	13.4	5.79	200.08	92.43	0.00	1.85	1383	93	20	0	60	40	0
6C	27/08/09	August/September 2009	6.36	256	2.7	2.69	14.9	2.96	148.48	39.41	8.40	1.405	198	93	20	0	60	40	0
7C	27/08/09	August/September 2009	6.14	156	2.4	4.45	16.9	0.92	148.17	9.70	8.67	1.399	2658	94	35	30	5	20	45
8C	25/08/09	August/September 2009	8.75	476	1.1	15.08	17.1	1.08	133.46	4.05	8.95	0.54	3041	120	45	10	5	75	10
9C	25/08/09	August/September 2009	7.88	160	3.3	9.17	16.5	2.51	148.31	208.45	0.00	0.862	464	61	80	15	0	60	25
10C	25/08/09	August/September 2009	6.45	142	1.3	5.86	17.0	7.19	181.74	23.13	8.40	0.791	463	109	40	15	10	45	30
11C	26/08/09	August/September 2009	6.64	379	2.4	3.76	16.1	722.24	524.60	12.73	8.40	1.328	3517	87	50	0	30	70	0
12C	26/08/09	August/September 2009	7.12	409	2.7	6.14	16.9	396.83	227.08	22.33	8.95	1.286	3688	72	0	0	35	30	35

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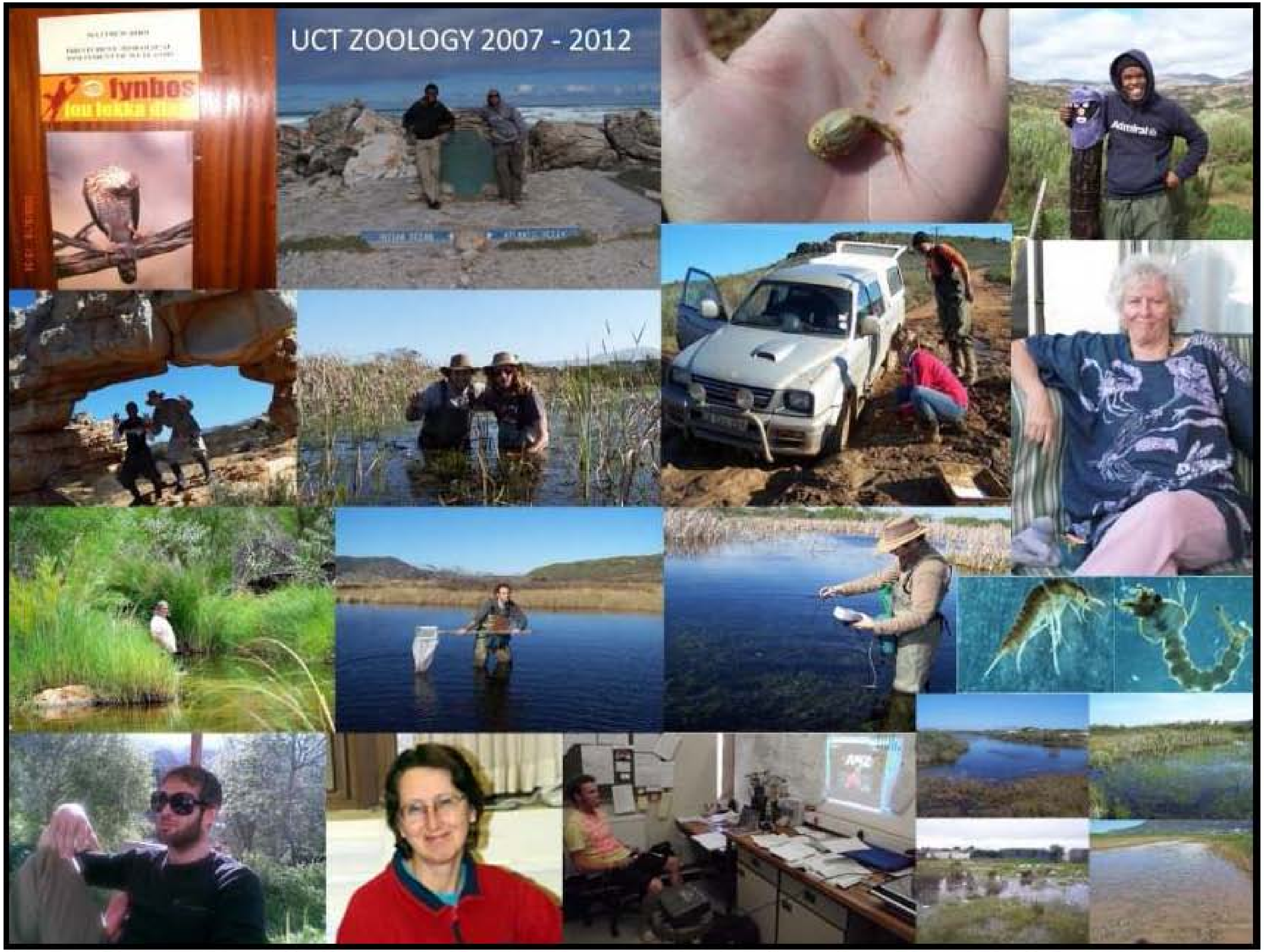
Appendix 15. Labile environmental variables collected at each wetland in chapter 4 (untransformed raw data). EC – electrical conductivity; UVA_{254} – ultraviolet absorbance at 254nm (proxy for the concentration of humic substances in the water column); TSA – total surface area; Veg. – vegetation.

Site code	Date sampled	Temporal category (covariables)	pH	EC ($\mu S.cm^{-1}$)	Turbidity (NTU)	Oxygen ($mg.L^{-1}$)	Ave temp. (^oC)	NO_3^-+NO_2^- ($\mu g.L^{-1}$)	NH_4^+ ($\mu g.L^{-1}$)	PO_4^{3+} ($\mu g.L^{-1}$)	Chl *a* ($\mu g.L^{-1}$)	UVA_{254} (cm^{-1})	TSA (m^2)	Maximum depth (cm)	% Macro-algae	% Simple veg. (sedge)	% Simple veg. (reeded)	% Open water	% Complex veg.
1D	20/10/09	October 2009	6.57	316	1.1	2.87	22.8	7.97	13.73	3.60	29.85	1.423	2393	87	0	15	0	5	80
2D	20/10/09	October 2009	4.60	236	1.8	1.62	22.6	22.15	2.20	3.20	104.49	3.756	2422	86	0	0	0	5	95
3D	20/10/09	October 2009	4.75	242	1.0	3.30	22.4	22.40	4.39	10.00	14.93	3.204	134	67	0	5	0	0	95
4D	20/10/09	October 2009	6.33	298	0.9	2.21	21.3	14.57	4.94	2.80	25.44	1.666	1000	100	0	0	5	25	70
5D	20/10/09	October 2009	6.09	388	2.4	0.87	19.6	16.21	2.75	38.40	13.76	1.9	349	85	45	25	35	40	0
6D	20/10/09	October 2009	6.52	410	1.1	1.31	19.7	4.15	0.55	12.40	44.78	1.532	78	87	0	0	5	10	85
7D	20/10/09	October 2009	6.56	211	2.4	2.17	21.0	14.08	4.94	3.20	26.45	1.5	938	68	25	60	0	10	30
8D	19/10/09	October 2009	7.82	560	1.2	3.87	24.9	8.10	22.51	7.60	0.00	0.551	2544	118	15	10	0	55	35
9D	19/10/09	October 2009	6.73	230	4.9	2.50	24.4	2.98	3.29	167.60	46.71	0.913	87	51	55	15	60	10	15
10D	19/10/09	October 2009	6.65	163	2.7	1.88	23.1	2.94	1.10	24.80	44.78	0.85	273	82	25	10	10	20	60
11D	19/10/09	October 2009	7.10	394	1.8	3.86	21.0	10.65	9.33	26.40	15.57	1.24	1007	77	50	5	10	55	30
12D	19/10/09	October 2009	7.55	606	3.5	5.69	22.2	2.97	18.12	37.20	41.27	1.505	1066	39	90	0	20	15	65
1E	25/11/09	November 2009	6.65	238	0.9	3.51	24.3	8.61	0.00	0.80	10.32	1.155	2248	101	0	25	0	10	65
2E	25/11/09	November 2009	4.63	131	0.8	1.34	21.8	17.10	2.75	0.00	6.02	2.805	1965	48	0	5	0	5	90
3E	25/11/09	November 2009	4.82	199	1.5	2.65	23.5	16.55	7.14	0.00	6.88	3.324	171	54	5	0	0	15	85
4E	25/11/09	November 2009	5.97	206	0.7	1.89	21.9	3.30	2.75	3.20	13.76	1.588	1408	99	0	0	0	15	85
5E	25/11/09	November 2009	5.92	320	3.5	0.57	21.7	6.22	2.20	10.80	477.67	1.705	610	78	25	10	40	50	0
6E	25/11/09	November 2009	6.52	292	1.7	2.20	24.4	1.57	1.65	5.60	57.32	1.529	170	88	0	0	5	10	85
7E	25/11/09	November 2009	6.49	199	3.7	3.70	24.4	3.71	1.65	2.00	64.49	1.381	1783	89	0	40	0	25	35
8E	24/11/09	November 2009	8.03	460	1.2	7.11	28.1	2.13	4.94	5.60	3.44	0.518	2909	119	50	5	0	45	50
9E	24/11/09	November 2009	6.61	209	4.5	4.73	26.4	12.49	1.10	92.40	24.07	0.935	200	57	55	0	25	20	55
10E	24/11/09	November 2009	6.39	154	2.5	2.91	27.1	8.91	1.10	30.40	34.39	0.952	252	94	0	5	10	20	65
11E	24/11/09	November 2009	6.70	409	1.5	1.83	24.2	11.88	3.84	6.00	20.64	1.441	941	78	65	0	20	50	30
12E	24/11/09	November 2009	6.93	495	1.8	2.06	24.4	4.11	14.82	23.20	6.11	1.355	997	60	45	10	15	35	40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