DEVELOPING AND TESTING THE CONGRUENCY OF SELECTED BIOLOGICAL INDICATORS AND AN EXISTING TOOL DESIGNED TO ASSESS WETLAND HEALTH IN AGRICULTURAL SETTINGS IN THE KZN MIDLANDS.

By

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ABSTRACT

Despite the fact that wetlands have been understood to be important for a wide range of ecosystem services, wetlands continue to be degraded globally. There has been a growing need to develop biomonitoring tools that reflect the present ecological state of wetlands, but very few attempts have been made in South Africa to achieve this, and those that have attempted this have generally achieved limited success. This study was conducted to develop and test the congruency of four selected biological indicators (dragonflies, frogs, macroinvertebrates and plants) in relation to the assessment of present ecological state using an existing method in South Africa, "WET-Health". WET-Health assessments rely primarily on transformations to a wetland that result from human impacts in both the catchment and the wetland itself. Using the tool, a health score is obtained that is consistent with the Department of Water Affairs (DWA) current "present ecological state" as applied to river health assessment. The study was conducted in agricultural settings of the Midlands of KwaZulu-Natal based on 13 wetlands. The selected wetlands were scored using WET-Health and grouped in four different ecological condition classes (A, B, C and D). Physical characteristics (wetland area, mean depth), biological characteristics (species cover/abundance, presence and species richness), and chemical characteristics (ammonia, pH, sulphate, nitrogen and phosphate) were also recorded in the selected wetlands.

Nineteen different species of dragonfly were recorded in this study. The study demonstrated that dragonflies are a promising bioindicator of wetland present ecological state as the dragonfly index was found to be closely correlated with WET-Health scores. Open water bodies within the selected wetlands were the focus of dragonfly sampling, as male dragonflies are territorial and they will patrol or be found around this habitat. Emergent vegetation dominated by sedges formed the focus of macroinvertebrate sampling in this study because greater numbers of macroinvertebrate families were found in this biotope in comparison to open water areas with no emergent vegetation. A total of 47 macroinvertebrate families were recorded in this study, but SASS5 scores based on macroinvertebrates showed no correlation with WET-Health scores. A total of 10 different frog species were recorded
in this study. All the species were common frog species found in most parts of the country. Frog species richness and occurrence showed no correlation with WET-Health scores. A total of twenty samples of two meter radius were measured per wetland and sampled for plant species and estimation of cover-abundance of each species per sample. Over 50 different plant species were recorded in this study, and both species accumulation and species richness showed a degree of correlation with WET-Health scores. All the wetlands in class A had generally higher species accumulation rate and species richness compared to the other wetland classes.

In addition to testing the congruency of four selected biological indicators with WET-Health, water quality was measured in all the wetlands. Wetlands in class A were associated with improved water quality as the water passes through the wetland. However, wetlands in class C and D did not show consistently improved water quality between the apex and the toe of these wetlands. In some cases the water quality deteriorated as it passed through wetlands in these two classes.
PREFACE

The experimental work described in this dissertation was carried out in the Department of Environmental Science at Rhodes University, from April 2012 to November 2016, under the supervision of Professor W.N. Ellery. Dr Donovan Kotze was a co-supervisor of this work.

This study represents original work done by the author and has not otherwise been submitted in any form or for any other degree or diploma to any tertiary institution. Where use has been made of the work of others, it has been duly acknowledged in the text.

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Date
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This degree is a challenge to all of my children, I would like them to know that your past should never determine your future. I have come this far with my background and have laid a solid foundation for all of them, sky should not be a limit. Education will not make you rich, however it will open your mind, will change your life and it’s a basis of any career.

I dedicate this degree to my late grandfather (Samuel Mpati Kubheka). He is the greatest man I have ever known to walk this planet. To me he was and still is my Mandela, my Martin Luther King and my Amaru Shakur.
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Chapter 1: INTRODUCTION

1.1 Wetland degradation and assessment of wetland integrity.

Ecosystem degradation is happening across the globe, from marine fisheries and open ocean ecosystems to those on high mountain ranges in remote settings (Qiuying et al., 2006). Humans need to understand the nature, extent and causes of ecosystem change because human well-being is dependent upon the ecosystem services provided by intact, functioning ecosystems (Millennium Assessment 2005).

Because of the massive data requirements involved it is currently extremely difficult to determine ecosystem change using direct comparisons of transformed and natural ecosystems, and impossible to do this for a wide range and number of ecosystems. This has meant that rapid assessments of ecosystem health using bioindicators is necessary. Indicators, including those based on human activities that degrade ecosystems, provide very useful approaches to determine ecosystem health. Such an approach is referred to as “impact-based” or “stressor-based” (Macfarlane et al. 2007; Kotze et al. 2012), and it provides summative indices of present ecological condition but it does not provide explicit indicators of the nature of the alteration of ecological interactions impacts of human activities on ecosystem structure and function or biodiversity.

Another approach is to use biological taxa that are sensitive to human activities and impacts that degrade ecosystems, in order to determine the degree of transformation of ecosystems. This is a “bioindicator” approach and it offers better insights into the impacts of human activities on components of biodiversity. Due to the explicit focus on biological information, conservation agencies, whose mandate is to monitor and protect biodiversity, favour a bioindicator approach.

Wetlands are a major component of South Africa's water resources (Act No. 36 of 1998) and are crucial to life-support functions, human well-being and the natural environment (Palmer et al. 2002 and Birol et al. 2008). Wetlands are valuable ecosystems that occupy about 6% of the world's land surface (Turner 1991, Schuyt & Brander 2004), but they contribute up to 40% of the globe's ecosystem services and are the most biologically productive natural ecosystems on earth (Constaza et al. 2001).
1997, Rolon & Maltchik 2006). Wetlands support a disproportionate number of rare and endangered species and provide a range of goods and services that support human well-being (Begg 1990, Kotze et al. 2005, Junk et al. 2013), but are at the same time ecologically sensitive and adaptive systems (Turner et al. 2000). Regardless of the international acknowledgment that wetlands are extremely important, they continue to be degraded and destroyed faster than any other terrestrial ecosystem (Dahl 2000).

The World Resources Institute (2005) states that about half of the world’s wetlands have been lost and converted or degraded in the twentieth century. Vegetation clearing and drainage for agriculture, infrastructure expansion, invasive species, pollution and global climate change, are the main factors associated with wetland loss and degradation (Laurance et al. 2012).

Although conversion of wetlands to agricultural land is a major problem in many parts of Africa, Asia, and South America, water projects are another major threat (van der Valk 2006). With growing human populations and economies, additional water is needed, which puts pressure on the world’s wetlands. In South Africa the extent of wetland loss has not been quantified, but it has been estimated that in eastern South Africa about 50% of wetlands have been lost or degraded (Kotze et al. 1995). Dini (2004) states that although no official national survey of wetland loss has been conducted in South Africa, studies that have been undertaken in several catchments have indicated that between 35% and 50% of the wetlands have been lost or severely degraded. In the Mfolozi catchment in northern KwaZulu-Natal 58% of the wetland area has been lost or degraded (Begg 1988). Begg (1986 and 1990) argues that more than half of all wetlands in South Africa have been modified resulting in their functioning being impaired. One of the main causes of wetland modification is agricultural development, such as drainage and poor land use practice (Kotze et al. 1995). Degraded wetlands cannot provide goods and services to the same extent as pristine ones as their ecological integrity is compromised (Kotze et al. 1995).

As a contracting party to the Ramsar Convention of 1971, South Africa has an obligation to conserve wetlands and water resources and use them wisely. The broad mandate of conservation authorities in South Africa is the conservation of biodiversity. To gauge whether these organizations are succeeding in this mandate, specific tools
have been developed and implemented for some ecosystems and taxa, but assessments for other ecosystems and taxa have not been undertaken. The rate at which wetlands have been and are being degraded, and the lack of scientific knowledge on patterns of species richness and loss in such systems, highlight the urgent need for ecological studies to provide scientific support to management and conservation programs for wetland biodiversity (Maltchik et al. 2010).

In South Africa there are a large number of wetlands that have been or are being degraded, which has created a need for tools to assess the ecological conditions of wetlands (Kotze et al. 2012). One of the tools that is currently used in South Africa to assess the integrity of individual wetlands is titled ‘WET-Health: A technique for rapidly assessing wetland health’ (Macfarlane et al. 2007). Given the lack of baseline reference-wetland studies in South Africa, WET-Health focuses on both stressor and response indicators, being based on expert knowledge and information gleaned from scientific literature regarding stressors and the nature of their impact on wetland hydrology, geomorphology and vegetation composition. It requires users to determine the extent of an activity that may impact on hydrology, geomorphology and vegetation, and guides users to score the intensity of impact associated with this activity. The magnitude of impact is the product of the extent and intensity of impact for each activity. The magnitude of impacts for all activities in the catchment and the wetland are combined in a structured way to give an overall magnitude of impact score for hydrology, geomorphology and vegetation, which can be combined to produce an overall magnitude of impact score. "Wetland health", which is synonymous with "wetland integrity" and "ecological condition", is viewed as being inversely related to the overall magnitude of impacts as conceptualised in WET-Health (Figure 1.1). The WET-Health scoring system views habitat impact as increasing from 0 to 10 along an imaginary human disturbance gradient. Conversely, habitat health decreases in an inverse manner as habitat impact increases along the human disturbance gradient (Macfarlane et al. 2007).
A major problem identified by conservation agencies is that WET-Health does not include an explicit assessment of the impact of human activities on the loss of biodiversity. However, biomonitoring is one of the most important and widely used tools for monitoring aquatic ecosystem health internationally (US EPA 2002a, Dickens & Graham 2002, Fore 2003).

Diatoms, invertebrates, vascular plants, fish, birds and reptiles have all been used globally with varying degrees of success for the bioassessment of wetland health (US EPA 2002a, Fore 2003 and Butcher 2003). In South Africa, the bioassessment of wetlands using macroinvertebrates has recently received some attention, but it is in its infancy compared with the use of macroinvertebrates for the assessment of river health (Bird et al., 2013). The river health assessment program uses a South African biomonitoring assessment system based on invertebrate taxa collected in a range of habitats in the stream, which is known as the South African Scoring System (SASS). There have been several versions and the current system is SASS5 (Dickens & Graham 2002).
In South Africa there has been very limited work done on biomonitoring of wetlands and the most recent studies on wetland assessments using macroinvertebrates yielded limited success (Bowd *et al.* 2006, Mlambo *et al.* 2011, Bird *et al.* 2013). This was suggested to reflect the fact that wetlands are very inhospitable to aquatic invertebrates that have one or more phases of their life cycle below the soil surface. Due to prolonged anoxic conditions in wetland soils, wetlands are extremely stressful environments for organisms like macroinvertebrates, such that there is a limited selection of macroinvertebrates that inhabit wetlands (Gernes & Helgen 2000, Helgen 2002).

The relationship between the health of a wetland and biological diversity is poorly quantified and largely unknown (Ewart-Smith *et al.* 2006). Conservation agencies need to be able to make statements about the status and trend of wetland biodiversity. WET-Health is a good tool available in South Africa currently, but from experience and informal discussions it has been established that a limited number of people can use the tool comfortably and there has been variation in results using the same tool (Bodman 2011, Ollis and Malan 2014). This study is intended to determine if there is a relationship between the health of individual wetlands as determined using WET-Health, and the diversity and abundance of selected biological taxa. The results will be used to develop guidelines to indicate which taxa are best suited for further development of bioindicators that might be successfully used in wetland health assessment. Four different taxa were selected for this study (macroinvertebrates, dragonflies, frogs and vegetation). These taxa were selected because they are well studied and they are relatively easily identifiable in the field.

### 1.2 Aim and objectives

Given this background, the aim of this study is to develop and test, in agricultural settings in the Midlands of KwaZulu-Natal (KZN), the congruency of selected biological indicators and an existing tool designed to assess wetland health. In order to achieve this aim, the following objectives were identified:

1. Identify a number of wetlands subjected to agricultural impacts from very slightly modified to highly transformed.

2. Obtain the health scores of each wetland based on WET-Health assessments, as determined by independent experts.
3. Determine species composition and diversity of plants and frogs in the selected wetlands

4. Determine the Dragonfly Biotic Index (DBI) and SASS5 scores in the selected wetlands

5. Determine the relationships between bioindicator scores and wetland health scores as determined using WET-Health.

6. Use these relationships to make suggestions about the development of an approach to assess wetland integrity using bioindicators
Chapter 2: LITERATURE REVIEW

2.1. Wetland description

Wetlands are very diverse due to differences in soil texture, climate, landscape setting, hydrology, water quality, flora and fauna (Mitsch & Gosselink 2000, Ewart-Smith et al. 2006, Sims et al. 2013), hence the difficulty in defining them (Roggeri 1995, Keddy 2000). Despite this heterogeneity there is consensus that wetlands are systems that are flooded to a shallow depth or saturated to within 0.5 m of the Earth’s surface for a sufficiently lengthy time to lead to anaerobic conditions in the upper soil horizons (shallower than 0.5 m soil depth) (Mitsch & Gosselink 2000). Anaerobic soil conditions lead to chemical transformations in the soil that cause many metals to become soluble, and it also promotes the accumulation of organic matter as decomposition rates are reduced. These biogeochemical transformations result in a biotic response that allows plant species adapted to having roots in soils starved of oxygen, to establish. Given these factors, wetland hydrology is viewed as the most important determinant of wetland formation, structure and function (Keddy 2000, Mitsch & Gosselink 2000).

In South Africa, according to Section 1 of the National Water Act (Act No. 36 of 1998), wetlands are defined as “land which is transitional between terrestrial and aquatic systems where the water table is usually at or near the surface, or the land is periodically covered with shallow water, and which land in normal circumstances supports or would support vegetation typically adapted to life in saturated soil”. Consequently, any part of the landscape where water accumulates for a long enough time to deplete soils of oxygen, will influence soils, plants and animals occurring in that area, such that it is a wetland (DWAF, 2005).

From the perspective of this study, it should be clear that the absence of oxygen in the soil makes it very stressful for plants and animals. Organisms living in the soil need to have adaptations to ensure that they have access to oxygen. Plants are able to do this by ensuring that oxygen from above-ground organs is translocated to the root zone in some way, but very few animals are adapted to living in anaerobic conditions. Given this, soil-dwelling invertebrates are unlikely to live in wetland soils, and fauna living in wetlands will likely dwell in the water column or above it (Keddy 2000).
2.2 South African wetland classification system

Classification is the process through which similar objects are grouped together and dissimilar objects are separated (Morant 1983). Classification enables scientists and practitioners to organise and begin to comprehend complex and variable objects, systems or ideas, so that they can be more easily examined and managed (O’Keeffe et al. 1994). Ecosystem classification involves the grouping of habitats or natural features into categories with similar characteristics, properties, or functions (Tiner 1999). Morant (1983) states that the three main objectives of the wetland classification system he developed for South Africa are: 1) to group ecologically similar ecosystems so that value judgements can be made; 2) to delineate ecosystem boundaries for the compilation of inventories and for mapping; and 3) to provide a uniform base for concepts and terminology throughout the region in which the classification system is to be applied. Wetland classifications are thus attempts to group wetlands with shared characteristics or to identify the types of environments and biota they contain (Pressey & Adam 1995). The classification of wetland ecosystems goes back as far as they have been recognised as important natural resources worthy of conservation (Tiner 1999). To date, one of the most widely used and influential wetland classification systems worldwide is that of the United States Fish and Wildlife Service (USFWS) developed by Cowardin et al. (1979).

Hydrogeomorphic classification describes wetlands in terms of the shape of the basin where water is stored, and the main inputs and outputs of water in that basin. It focuses particularly on how water flows through the wetland, for example as channelled versus diffuse flow (Ollis et al. 2013). The hydrogeomorphic Unit is the main unit by which a single wetland or part of a wetland can be recognized. The South African Wetland Classification System (SAWCS) has been developed based on the principles of the hydrogeomorphic classifications developed elsewhere in the world (Ollis et al. 2013). The system is hierarchical such that seven hydrogeomorphic (HGM) types as defined at level 4 of the SAWCS, are used to classify wetland ecosystem types on the basis of hydrology and geomorphology (Ollis et al. 2013).

1. **Slope seepage**: This is a wetland area located on gentle to steep slopes, driven by discharge of groundwater or by water percolating through the upper layers
of the soil. Slope seepages generally feed into drainage basins via headwater streams.

2. **Valleyhead seepage**: This is a typical concave wetland area located on gently sloping land on a valley floor at the head of a drainage line. Water input is mainly from subsurface flow.

3. **Unchannelled valley bottom wetland**: This is a wetland area on a valley floor that is connected to a drainage network, but without a major channel running through it. It is characterized by the prevalence of diffuse flow, which is at or near the surface. Water mainly enters the wetland through an upstream channel, but sometimes also from adjacent slopes.

4. **Channelled valley bottom wetland**: This is a wetland area on the valley floor that is divided by and typically elevated above a stream channel, which means that this wetland generally drains more rapidly than an unchannelled valley bottom wetland. Water inputs to these areas are from adjacent valley side slopes and occasionally from overtopping of the channel during floods.

5. **Floodplain**: This is a very gently sloping wetland area adjacent to a river channel in its lower reaches that is subject to periodic inundation due to flood events in the wet season. These flood events can be quite turbulent and leave many features in the landscape such as levees, oxbow lakes and depressions. Floodplains are areas where sediment is typically deposited, often for very long periods of time.

6. **Flat**: These represent areas where the groundwater is near the surface and are mostly found on coastal plains. Their main input of water is from rainfall and groundwater, and are features where the flow is imperceptible.

7. **Depression**: This is a closed basin where water accumulates such that they are disconnected (or very weakly connected) with the drainage network. They include pans, which are shallow depressions that receive their water supply via runoff from a local catchment, although they also include shallow depressions that develop where tributary valleys are blocked by sedimentation on trunk stream floodplains (Grenfell et al. 2008). In the latter case they typically receive
their water supply via groundwater inputs, with occasional inputs from the floodplain during floods.

In order to standardize assessment of the impacts of human activities on biota in wetlands and their catchments, only unchannelled valley-bottom wetlands were sampled in this study. This reduced the variability of biotic response due to differences related to hydrological variability associated with the way that water flows through the wetland.

2.3 Wetland values

Wetlands contribute disproportionately to providing ecosystem goods and services that are of benefit to human well-being. Ecosystem services may be of direct benefit to humans through provisioning services such as water provision, fibre for craft and shelter construction, livestock production during critical seasons when forage elsewhere in the landscape is limiting. They may also provide indirect benefits through regulating and supporting ecosystem amenities such as flood and flow control, groundwater recharge and discharge, water quality maintenance, biodiversity and carbon sequestration (Costanza et al. 1997, Hartig et al. 1997, Galbraith et al. 2005, Birol et al. 2006, Tiril 2006 and Holland et al. 2012). Moreover, wetlands are increasingly being recognised for their crucial role in the conservation of biological diversity (Gibbs 2000). Costanza et al. (1997) estimated the global economic value of goods and services provided by wetlands to be US$ 4.9 trillion per year.

The provision of ecosystem services by wetlands is the major motivation for their conservation, including their role in the conservation of biodiversity. Wetlands play a particularly important role in supporting high concentrations of wetland-dependent wild species, including waterfowl and other birds, several species of antelope, hippopotamus, and other freshwater dependent animal species (Michaud 2001, Mitsch and Gosselink 2000). Wetland plants provide food and habitat for these animals, some of which are not able to survive without the habitat that wetlands provide. A surprising number of threatened animal taxa depend on wetlands for a part (or all) of their life cycles. Habitat degradation and loss of wetlands are the main reasons for many of these species being threatened (Breen and Begg 1989).
2.4 Wetland biodiversity

Biodiversity is a characteristic of nature and a property of living Ecosystems. The most common definition of biodiversity is that adopted by the Convention on Biological Diversity that defines biodiversity as the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and ecological complexes of which they are part. Biodiversity includes diversity within species, between species and within and between ecosystems (Maltby and Barker 2009). Globally the term biodiversity has been used loosely by different interest groups due to global interest in the social, economic, political, legal and other dimensions of biodiversity and its loss (Maltby and Barker 2009). Biodiversity in inland aquatic environments has been given attention recently, and wetlands have been extensively investigated for their ecology, management, conservation and restoration (Mitsch and Goselink 2000). Despite growing interest in wetlands, their biodiversity related issues have received fairly limited attention until recently (Maltby and Barker 2009).

The biodiversity of wetland ecosystems is variable and encompasses the range of living things, the degree of genetic variation, and the wealth of different habitats within an ecosystem (Alam 2014). Wetlands are a great source of global biodiversity within the major climatic belts due to the evolved collection of animals and plants (Alam 2014). Animals that utilize wetlands can be grouped in to six categories, (1) resident in wetlands, (2) regular migrants from deep-water habitats, (3) regular migrants from terrestrial uplands, (4) regular migrants from other wetlands (e.g. water birds), (5) occasional visitors and (6) those taxa that are indirectly dependant on wetland biota (e.g. canopy insects; Maltby and Barker 2009). Most wetland dependant species utilise wetlands for feeding, breeding, and nesting, or for habitat at one or more stages in their life cycle. Species may be resident in wetlands or may migrate periodically or seasonally into wetlands from other environments. Despite being dependant on wetlands, most animals use wetlands directly for key activities but do not necessarily reside in them. This shows that assessment of biodiversity in wetlands should take into consideration all those species that depend on wetlands directly or indirectly at some stage in their life cycle, because their conservation requires conservation and management of wetland habitats.
Biodiversity has been indicated as a key factor maintaining ecosystem function (Chapin et al. 2000, Naeem et al. 2009) and the loss of biodiversity globally is a serious global concern. Land use change has been considered to be one of the greatest threats to biological diversity (Castro et al. 2010, Hawksworth and Bull 2010, Laliberté et al. 2010), as has the alteration of abiotic conditions in natural areas that alters environmental conditions to a point that can lead to local extinctions (Breen and Begg 1989, Eppink et al. 2008). The reduction of species richness through local and global extinctions is common in wetland ecosystems (Breen and Begg 1989).

The increased in both human population and increased socio-economic development has led to severe pressures being placed on freshwater systems globally and this has resulted in an estimated extinction risk amongst freshwater species being significantly higher than for terrestrial species (Holland et al. 2012, Dudgeon et al. 2006 and Saunders et al., 2002). Wetlands are important for biodiversity because they have diverse habitat types, reflected in many classification schemes which differentiate between wetlands on the basis of hydrology, geomorphology, water chemistry, edaphic characteristics and dominant vegetation (Maltby and Barker 2009).

### 2.5 Threats to wetlands

One of the major threats to wetlands globally is from the direct conversion of land by draining and/or filling (Maltby & Barker 2009). Wetlands have been and continue to be destroyed through conversion to purposes considered to provide greater direct benefits to humans. Direct impacts on wetland occur through conversion to agriculture and overexploitation of wetlands for their plant and animal resources (Mitsch and Gosselink 2000, Barbier 1993).

Several studies in South Africa have demonstrated that agricultural activity causes complex effects on wetland ecosystems (Kotze 2010). The need for irrigation water beyond the wetland boundary directly influences the water balance of wetlands, as water flows are regulated and diverted. Shallow and smaller wetlands in drier climates are more seriously affected as water from the wetland is often used for irrigation (Gopal 2000). Factors contributing to wetland degradation are highly variable, ranging from catchment based activities such as urbanization, abstraction and dam development to site-scale factors such as cultivation, drainage and over-grazing.
The dominant factors impacting on wetland areas are different in different regions of the province. In the KwaZulu-Natal Midlands, agricultural activity forms the basis of the economy. Kotze (2004) states that in agricultural areas such as the upper Mgeni catchment, located in the KwaZulu-Natal Midlands, primary impacts were attributed to cultivation and associated wetland drainage, and the construction of dams (74% and 19% of impacts to wetlands were due to these activities respectively) whereas other impacts were limited.

Based on studies elsewhere in the world, the effects of agriculture on wetlands include (Galbraith et al. 2005, Wiseman 2001): 1) direct loss of wetlands due to draining and conversion to agricultural land, 2) indirect loss of wetland area due to water withdrawal from upstream rivers and streams for irrigation, 3) runoff of fertilizers causing excessive eutrophication leading to fish kills, toxic algal blooms and local extinction of aquatic flora and fauna, 4) loss of wetland area and function due to damming for water storage, abstraction and water diversion upstream of the wetland, 5) loss of seasonal wetlands due to changed hydrological regimes from water storage, 6) loss of wetland functions due to salinization, sediment deposition and erosion 7) pollution from use of pesticides, road works and other toxic chemicals.

Although conversion of wetlands to agricultural land is a major problem in parts of Africa, Asia, and South America, water schemes are another major threat (van der Valk 2006). With the growing human population and pressure for economic growth (mining, increased agricultural lands and dams for human consumption), additional water is needed that puts pressure on the world’s major wetlands. For example, water entering the Okavango Delta in Botswana comes from Angola and passes through Namibia before it reaches the Delta, and all three countries are short of water. Various plans to divert water from the Okavango River and Delta have therefore been proposed (van der Valk 2006). Irrigation projects, including in the Okavango drainage basin, extract large amounts of water, resulting in modification of the hydrological regime of downstream wetlands. Water projects also alter delivery of sediments to deltaic wetlands, which eventually leads to a major loss of freshwater wetland habitats because they subside due to dewatering, which is compounded by sea-level rise (van der Valk 2006).
The most important consequences of wetland loss in South Africa are lower agricultural productivity, poorer water quality, less reliable water supplies, increased incidence and severity of downstream flooding, threatened wildlife resources and the inevitable increased incidence of species extinction (Kotze et al 1995).

2.6 Ecosystem health assessment

In South Africa, as is the case internationally, there is considerable concern regarding the health or ecological condition of aquatic ecosystems. Consequently methods to assess the status and trends of such systems are receiving attention with the aim to identify causes of modification and deterioration, and ultimately to apply measures to improve the condition of these systems (Kleynhans 1995). The need for monitoring and assessment of water quality needs to be an integral part of water resource management in South Africa. This is stated explicitly in the National Water Act (Act No. 36 of 1998), which mandates the Minister of Water Affairs and Forestry to establish national systems that monitor, record, assess and disseminate information on water resources. Information provided by appropriate resource monitoring techniques play a critical role in the on-going process of harmonizing economic development, human welfare and environmental protection.

Traditionally, information gathered to assist the management of water resources was predominantly non-ecological in nature. Monitoring actions focussed largely on chemical and physical water quality variables, and regulatory efforts were aimed at controlling individual physico-chemical stressors (Roux, 1999). It was assumed that improvements in water quality would result in an improvement in ecosystem condition. However, the measurement of only the physico-chemical variables cannot provide an accurate account of the overall condition of an ecosystem as it fails to detect the cumulative and/or synergistic effects of multiple stressors on aquatic systems (Jackson and Davis 1994). Many factors other than chemical water quality may also have an influence on the ecological state of an ecosystem. Examples include habitat and streamflow alteration, water abstraction and introduction of exotic species. Effective management of aquatic ecosystems must therefore adopt a holistic approach that draws hydrological, physio-chemical, toxicological and ecological variables into an integrated assessment of aquatic ecosystem health (Jackson and Davis 1994).
South Africa has developed two approaches to assess aquatic ecosystem health in response to specific information needs (Wepener 2008). The first involves biomonitoring, which was used initially in the South African River Health Programme (RHP), which was developed to assess the ecological status of riverine ecosystems in relation to all the anthropogenic disturbances affecting them. The RHP assessment is based on the concept of biological integrity, and makes use of identified biological indices such as fish, invertebrates and riparian vegetation for evaluating in-stream and riparian habitats (Roux 1999). The biological responses, as indicators of an ecosystem, are recorded in terms of a river health classification scheme that allocates a specific category of health to each river reach (Roux 2004). However, these biological responses deal only with indistinct cause-effect relationships between environmental drivers and consequential biological responses. Should the biological integrity indicate the likelihood of unacceptable conditions, then there should be a process to assess these conditions and responses in a critical manner (Wepener 2008).

The biological indices that are applied in the RHP were expanded upon as part of the Ecological Reserve assessment process, and have given rise to the development of EcoClassification indicators. The "EcoStatus" in this case can be defined as the totality of the features and characteristics of the river and its riparian areas that bear upon its ability to support appropriate natural floral and faunal communities (Kleynhans et al. 2007). Measures such as the macro-invertebrate response assessment index (MIRAI) and fish response assessment index (FRAI) interpret the biophysical components of a river in terms of drivers and biological responses and endpoints in an integrated way, thereby deriving a realistic and reproducible conclusion regarding the EcoStatus of the river (Kleynhans et al. 2007).

The other water quality assessment approach has been to adopt whole effluent toxicity (WET) testing as a tool, in order to evaluate the acceptability of potentially hazardous effluents for discharge into receiving waters (Slabbert et al. 1998). The WET testing approach measures wastewater's effects on specific test organisms' ability to survive, grow and reproduce. The WET testing approach is an integrative tool that measures the toxic effect of an effluent mixture as a whole, and assesses uncharacterized sources of toxicity and toxic interactions such as synergism and antagonism (Smolders et al. 2003). The methods used in WET focus on acute and chronic toxicity.
testing using standardized laboratory-based bioassays involving laboratory-reared organisms (Slabbert et al. 1998). These WET methods have been incorporated into the Direct Estimation of Ecological Effects Potential (DEEEP) toxicity test, the current application of choice within the National Toxicity Monitoring Programme (Slabbert 2004). Smolders et al. (2003) proposed that a more ecologically sound approach would be to use the WET concept in an *in situ* environment to assess in-stream toxicity, rather than conducting laboratory toxicity tests on water samples collected from the environment.

The difference between the two approaches is that WET predominantly uses mortality to describe toxicity whereas in-stream toxicity assessment makes use of assessment endpoints that measure biological responses, which in turn can provide a measure of exposure, and sometimes also of toxic effect (Smolders et al. 2003). These biological responses are termed biomarkers and the fundamental assumption upon which they are based centres on some biochemical process (biomarker) being compromised as a result of pollutant exposure (Handy et al. 2003, Slabbert 2004). However, the use of biomarkers in resident organisms is severely constrained by a substantial variability in endpoints, as prolonged pre-exposure to any ambient environmental condition can drastically alter the 'normal' range of a biomarker response (Wepener 2008). The use of caged organisms in streams has been successful in reducing this degree of variability (Wu et al. 2005).

### 2.7 Biomonitoring and bio-indicators

Biomonitoring is generally defined as “the systematic use of living organisms or their responses to determine the condition or changes of the environment” (Rosenberg 1998, Gerhardt 1999, Oertel and Salánki 2003). In this study, the term biomonitoring tends to follows the definition of Markert et al. 1999: “Biomonitoring is a method of observing the impact of external factors on ecosystems and their development over a period, or of ascertaining differences between one location and another.” Compared to the former definition, the second definition is considered to better reflect the ecological content of biomonitoring.

Dickens and Graham (2002) state that biomonitoring tools maybe used to 1) assess the ecological state of aquatic ecosystems, 2) assess the spatial and temporal trends in ecological state, 3) assess emerging problems and set objectives for rivers, 4)
assess the impact of developments and predict changes in the ecosystems due to developments, and 5) contribute to the determination of the Ecological Reserve, which sets the quantity and timing of flows in order to sustain ecological processes in aquatic ecosystems. Aquatic ecosystem health indicators are used to assess the state of the water and aquatic ecosystems, and determine if ecosystem processes are being compromised by natural or human-caused environmental disturbances. Aquatic ecosystem health indicators include water and sediment quality and quantity parameters and flow measurements that look at physical and chemical conditions of the water and sediments, as well as biological indicators that measure the population, health or habitat of aquatic fauna and flora. There are several alternative indicators of biomonitoring in streams and rivers, however periphyton taxa, benthic macroinvertebrate taxa and fish taxa are the most frequently utilized (Dickens & Graham 2002).

Some of the most commonly used biomonitoring techniques in South African aquatic systems are SASS5, MIRAI (Macro-Invertebrate Response Assessment Indices), VEGRAI (riparian Vegetation Response Assessment Index), IHAS (Integrated Habitat Assessment System), FRAI (Fish Response Assessment Index). These monitoring systems form part of the National Biomonitoring Programme for Aquatic Ecosystems (Kleynhans 1999), and are mostly conducted in riverine systems where organisms such as fish and macro-invertebrates can be readily found and used for FRAI and SASS5 respectively. However these biomonitoring techniques are usually not used in aquatic systems such as wetlands, where the environment is not really comparable to riverine systems (Matlaba et al. 2011).

As primary producers, periphyton taxa act as an important foundation of food webs in river ecosystems, which makes them valuable indicators of environmental conditions in streams and rivers (Mayer and Likens 1987, Lamberti 1996). Periphyton generally have rapid reproduction rates and short life cycles and therefore can be expected to reflect short-term impacts and sudden changes in the environment (McCormick and Stevenson 1998, Babour et al. 1999). Because the assemblages usually attach to a substrate, their growing and flourishing can respond directly and sensitively to many physical, chemical and biological variations occurring in the stream reach, including temperature, nutrient levels, current regimes and grazing (McCormick and Stevenson 1998). Periphyton taxa, especially diatoms, have been preferred for river biomonitoring.
purposes by many authors (Whitton 1996). Matlaba et al., 2011, used diatoms to indicate water quality in wetlands and concluded that 1) Diatoms do occur in wetlands and samples may be easily collected from these environments. 2) Diatom community structure seems to be dictated by the prevailing physical, biological and chemical water quality and (most importantly) the structure of these communities can be used to infer water quality in wetlands.

Benthic macroinvertebrates are key components of aquatic food webs that link organic matter and nutrient resources (such as leaf litter, algae and detritus) with higher trophic levels (Wallace and Webster 1996). These organisms have mostly sedentary habits and are therefore representative of site specific ecological conditions (Cook 1976). With the sensitive life stage and relatively long life span, benthic macroinvertebrates have the ability to integrate the effects of short-term environmental variations (Pratt and Coler 1976, Hutchinson et al. 1998). Furthermore, benthic macroinvertebrate assemblages are made up of many species among which there are a wide range of trophic levels and pollution tolerances (Cook 1976), thus providing useful information for interpreting cumulative effects. Community structure of the assemblages frequently changes in predictable ways in response to environmental disturbances, which is the basis for development of biocriteria to evaluate anthropogenic influences (Boyle and Fraleigh 2003). Gray (1989) has summarized these responses into three categories: 1) reduction in diversity; 2) retrogression to dominance by opportunistic (e.g. shorter life-cycle, faster reproducing) species; and 3) reduction in individual size of dominant species.

In South Africa the South African Scoring System version 5 (SASS5) is used to assess the general river condition or health as influenced by a variety of abiotic factors, but principally water quality (Dickens and Graham 2002). This method has become the standard for the rapid bioassessment of rivers in southern and South Africa. SASS 5 is a key tool used in the National River Health Programme and, as required by the South African National Water Act (1998), it is included in the determination of the Ecological Reserve (Dickens and Graham 2002).

Fish are often at the top of the aquatic food web and are consumed by humans, which makes them important for assessing contamination (Babour et al. 1999). Due to their relatively long life cycle and mobility, they can be good indicators of long-term
years) effects and broad habitat conditions (Babour et al. 1999). In addition, given that they occupy a wide range of trophic levels, including the highest level occupied by top predators, community structure of fish assemblages is reflective of integrated aquatic environment health (Karr 1981, Harris 1995). Fish communities respond significantly and predictably to almost all kinds of anthropogenic disturbances, including eutrophication, acidification, chemical pollution, flow regulation, physical habitat alteration and fragmentation, human exploitation and introduced species (Karr et al. 1986, Ormerod 2003). Their sensitivities to the health of surrounding aquatic environments form the basis for using fishes to monitor environmental degradation (Fausch et al. 1990).

2.8 Wetland assessment in South Africa: the WET-Health assessment tool

The broad mandate of conservation authorities is the conservation of biodiversity. To gauge whether the organizations are succeeding in this mandate, specific tools have been developed and implemented for some ecosystems and taxa, but not for others. The rate at which wetlands have been and are being degraded, and the insufficient scientific knowledge on patterns of species associated with such degradation, highlight the urgent need for ecological studies to provide scientific support to biodiversity management and conservation programs in these systems (Maltchik et al. 2010).

One of the tools that is currently used in South Africa to measure the state of wetland integrity is ‘WET-Health’ (Macfarlane et al. 2007). The development of WET-Health is based on expert knowledge and information gathered from scientific literature on how human activities impact wetland integrity. It was developed through an iterative process, starting with a simple and incomplete demonstration prototype, and then refining the system by obtaining feedback from experts and through application in the field to produce, through a series of draft versions, the more complete current system. Such an approach is consistent with the general process of developing an expert system (Kotze et al. 2012).

WET-Health relies primarily on transformations to a wetland that result from human impacts in both the catchment and the wetland itself. Using the tool, a health score is obtained that is consistent with the Department of Water Affairs (DWA) current "present ecological state" as applied to river health assessment. The system used in
WET-Health is described in Table 2.1, showing the relationship between the magnitude of impact score, the present ecological condition category and a description of the present ecological condition category. The intervals on the magnitude of impact score are not equal for all health classes, but they do not overlap. The present ecological condition of a wetland is given a score of “A” if it is unmodified and a score of “F” if it critically modified. Wetland integrity is defined as a measure of deviation of wetlands structure and function from the pristine state (“natural reference condition”) of the wetland.

**Table 2.1:** Health classes used by WET-Health for describing wetland ecological condition (Macfarlane *et al.* 2007 & Kotze *et al.* 2012)

<table>
<thead>
<tr>
<th>Magnitude of impact score</th>
<th>Present ecological condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.9</td>
<td>A</td>
<td>Unmodified, natural.</td>
</tr>
<tr>
<td>1-1.9</td>
<td>B</td>
<td>Largely natural with few modifications. A slight change in ecosystem processes is discernible and a small loss of natural habitats and biota may have taken place.</td>
</tr>
<tr>
<td>2-3.9</td>
<td>C</td>
<td>Moderately modified. A moderate change in ecosystem processes and loss of natural habitats has taken place but the natural habitat remains predominantly intact</td>
</tr>
<tr>
<td>4-5.9</td>
<td>D</td>
<td>Largely modified. A large change in ecosystem processes and loss of natural habitat and biota and has occurred.</td>
</tr>
<tr>
<td>6-7.9</td>
<td>E</td>
<td>The change in ecosystem processes and loss of natural habitat and biota is great but some remaining natural habitat features are still recognizable.</td>
</tr>
<tr>
<td>8-10</td>
<td>F</td>
<td>Modifications have reached a critical level and the ecosystem processes have been modified completely with an almost complete loss of natural habitat and biota.</td>
</tr>
</tbody>
</table>

WET-Health examines the degree of deviation from the natural reference condition for three components of health, which are hydrology, geomorphology and vegetation (Kotze *et al.* 2012). Hydrology is the distribution and movement of both surface and sub-surface water into, through and out of a wetland (Macfarlane *et al.* 2007). To assess hydrology one needs to look at water inputs (from the wetlands catchment) and water distribution and retention (activities within the wetland). The main land-use activities considered to contribute greatly to the reduction of the quantity of water...
flowing into the wetland are (a) abstraction of water for irrigation and human consumption, (b) timber plantations, (c) alien plants and (d) dams. The degree to which human activities have affected the distribution and retention patterns of water within a wetland is also assessed. Activities it considers include (a) canalisation and stream modification, (b) presence of impeding features such as roads across the wetland or impoundments in the wetland, (c) alteration of surface roughness, (d) direct water losses such as by abstraction or the presence of alien vegetation, and (e) infilling or excavation (Macfarlane et al. 2007).

Geomorphology is the distribution and retention pattern of sediment within the wetland, or the loss of sediment through erosion. Geomorphic health focuses on the presence of indicators that result in excessive sediment inputs and/or losses for clastic (minerogenic particles) and organic sediment (Macfarlane et al. 2007). The geomorphic health is assessed by evaluating (a) activities and impacts which are known to commonly influence geomorphic processes such as sediment erosion and/or deposition and (b) direct on-site impacts which provide clear clues of changes to geomorphic processes (Macfarlane et al. 2007).

Vegetation has compositional and structural characteristics that provide specialised habitats for a range of important wetland dependent species such as the red chested flufftail and wattled crane (Macfarlane et al. 2007). WET-Health evaluates changes in vegetation composition and structure as a consequence of current and historic on site transformations and/or disturbances. Vegetation is assessed by evaluating the degree to which the current vegetation composition has deviated from the perceived natural or reference conditions. Assessing this deviation is based on what 'should not be there' (e.g invasive alien species or a high abundance of ruderal (weedy) species) rather than on the composition of indigenous plants that ‘should be there’ (Macfarlane et al. 2007). This creates a problem when trying to relate vegetation biotic indices to WET-Health, as some vegetation indices like Floristic Quality Assessment Index (FQAI) exclude alien species and also plant rating is based on species that have preference for non-degraded natural communities (Miller and Wardrop 2006).

Prior to assessment, a wetland is divided into hydrogeomorphic (HGM) units and their associated catchments. Each hydrogeomorphic unit is analysed separately for hydrological, geomorphological and vegetation health based on assessments of the
extent and intensity of a range of human impacts in the HGM unit’s catchment and in the HGM unit itself. Impacts are assessed as they relate to water supply and timing of inflow, and the residence of water in the wetland (hydrological impacts), and sediment supply and retention in the wetland (geomorphological impacts). The extent of impact is measured as the proportion of a wetland and/or its catchment that is affected by an activity (scaled from 0 to 1), while the intensity of impact is estimated by evaluating the degree of alteration that results from a given activity (scaled from 0 to 10). The results are translated to a magnitude of impact score for each activity, which is calculated as the extent of the impact in the catchment or wetland multiplied by the intensity of the impact, to produce a magnitude of impact score on a scale of 0 to 10.

The magnitudes of impact scores for all activities in the catchment and the wetland are combined in a structured way to produce an overall magnitude of impact score. Magnitude of impact scores for all activities or impacts are translated into a health score for hydrology and geomorphology. For the assessment of vegetation health, the HGM unit is divided into areas where there are indications of particular disturbance activities such as abandoned fields or impoundments. The extent of each disturbance class in the wetland is determined and the intensity of impact associated with each disturbance class is assessed. The magnitude of impact for each disturbance class is calculated as extent multiplied by intensity of impact, and these are summed to give the overall magnitude of impact score for vegetation. The scores for hydrology, geomorphology and vegetation can in turn be combined in a structured way to calculate the overall impact of all activities that affect the integrity of each HGM unit (Macfarlane et al. 2007), which gives an indication of the deviation from the natural reference condition.

2.9 Potential biotic indices in the assessment of wetland health

A shortage of funding, time and skilled personnel are some of the limitations faced by conservation agencies, and they rely on alternate approaches to species inventories for biodiversity assessment (Simaika and Samways 2011). There is a need to develop bioindicators to monitor changes, particularly indices that indicate a general decline in biodiversity. A good bioindicator must readily reflect the state of an environment, it must reflect the impact of environmental change at different scales, or be a suitable
surrogate of other taxa (McGeoch 1998). Bioindicators can be used in measuring any of three indicator categories: biological diversity, environmental change, and ecological processes.

The biotic index approach, as defined by Tolkamp (1985), combines the relative abundance of certain taxonomic groups with their sensitivities or tolerances of environmental change into a single index or score. Biotic indices are increasingly popular because they are 1) responsive to different types of anthropogenic impact (Growns et al. 1995); 2) robust to variations in sample size (Armitage et al. 1983 and Growns et al. 1997); and 3) have low variability both within a site and over time (Hannaford and Resh 1995). They are also subject to continuing evaluation and refinement (Chessman et al. 1997).

The difficulty in constructing a biotic index is that individual taxa may not be equally sensitive to all types of anthropogenic disturbance (Chessman & McEvoy 1998). For example, laboratory studies show that particular species of aquatic macroinvertebrates vary quite widely in their tolerances of specific pollutants (Chapman et al. 1982, Ewell et al. 1986). Another difficulty is that the abundances of particular macroinvertebrate taxa can differ greatly between streams affected by flow alteration and various types of landuse activities and wastewater discharges (Yoder and Rankin 1995). In these circumstances, it is difficult to assign representative sensitivity values to individual taxa. However, given sufficient data and experimental evidence, species-specific pollution indications can be used to infer environmental conditions in a habitat.

Although some aspects of stream bioassessment may apply to wetland bioassessment, specific information from wetland assemblages is needed to develop indices and metrics for wetlands (Teels and Adamus 2002). Several studies that have developed bioassessment methods for wetlands have adapted bioassessment frameworks that were originally designed for streams (Teels & Adamus 2002). This approach resulted in two views. The first is that rapid wetland assessment methods should be similar to those methods being used in national river health programmes (Butcher 2003). This view has developed because of the belief that rivers and wetlands are ecologically similar and therefore can be monitored using the same methods (Butcher 2003). The second view argues that aquatic macroinvertebrate assemblages
vary too greatly between wetlands and rivers for the same methods to be used (Butcher 2003). The difference in macroinvertebrate distribution between rivers and wetlands has been attributed to the ecological processes and biotope structure of wetlands being distinct from that of rivers (Wissinger 1999). However, Butcher (2003) has noted that there are significant overlaps in taxa between river and wetland macroinvertebrate assemblages, particularly at family level.

There are many biotic indices that have been developed all over the world and in South Africa including SASS5, MIRAI, VEGRAI, IHAS, FRAI, HAI, DBI and GAI. However in this study only two biotic indices will be used, as they are well studied and developed and also are cost effective: the South African Scoring System version 5 (SASS5) and Dragonfly Biotic Index (DBI).

### 2.9.1 Dragonflies and the Dragonfly Biotic Index

A well-known and used method of assessing the integrity of aquatic ecosystems is the Dragonfly Biotic Index (DBI). The DBI was developed in South Africa as it provides a measure of a rivers ecological integrity (Simaika and Samways 2008). The DBI relies on dragonfly species presence/absence data and it comprises three sub-indices (Table 2.2) 1) the relative geographic distribution of a species, 2) the threat status of the species based on IUCN Categories and Criteria and 3) species sensitivity to habitat disturbance (Simaika and Samways 2011). Values for each sub-index range from 0 to 3 and the sum of the values for any one species is the standard DBI score, which can range from 0 to 9 (Simaika and Samways 2011). A common, widespread, not-threatened and highly tolerant (of disturbance) species would score 0 (0 + 0 + 0), while a highly range-restricted, threatened and sensitive species would score 9 (3 + 3 + 3). To determine a DBI score per site, the total of all the species DBIs must be divided by the total number of species. This method thus standardizes the DBI score to give the DBI site value, which ranges between 0 and 9, and can be compared to DBI site values of other sites (Simaika and Samways 2011). During the development and testing of the index, the DBI was found to be very useful for site selection, as well as for measuring ecological integrity at the global and regional scale (Simaika and Samways 2011).

Dragonflies have proven to be an essential tool for assessing integrity of aquatic systems (Simaika and Samways 2011). There are reasons why dragonflies are
frequently identified as bioindicators: 1) they are well known taxonomically; 2) most are readily identifiable in the field; 3) they occupy a spectrum of habitats; 4) they are sensitive to changes in water quality and the ecological conditions of their habitats; and, 5) their species assemblages are large enough for assessments (Simaika and Samways 2011). It is for these reasons that adult dragonflies are valuable candidates for medium to long-term monitoring programs.

Table 2.2: The sub-indices of Dragonfly Biotic Index (DBI) range from 0 to 3 (Simaika and Samways 2008)

<table>
<thead>
<tr>
<th>Score</th>
<th>Sub-indices</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Very common throughout South Africa and Southern Africa</td>
</tr>
<tr>
<td>1</td>
<td>Localised across a wide area in South Africa; localised or common in southern Africa; or very common in 1-3 provinces and localised or common in Southern Africa.</td>
</tr>
<tr>
<td>2</td>
<td>National endemic confined to 3 or more provinces; or widespread in southern Africa but marginal and very rare in South Africa.</td>
</tr>
<tr>
<td>3</td>
<td>Endemic or near-endemic and confined to only 1 or 2 provinces</td>
</tr>
</tbody>
</table>

Abbreviations: IUCN species threat status (IUCN 2001): LC least concern, NT near threatened, VU vulnerable, CE critically endangered, EN endangered, GS global status, and NS national status

2.9.2 South African Scoring System version 5 (SASS5)

Aquatic macroinvertebrates are well represented in most freshwater bodies throughout the world and play a critical role in the structure and function of most aquatic ecosystems, including wetlands (Williams 2006). Macroinvertebrates are
found in all types of wetlands and are abundant in large and small, permanent and seasonal wetlands, and occur in the sediments, in the water column, and on and amongst the submerged and emergent vegetation (DWAF 2004). Karr (1981) suggests that aquatic organisms are affected by external forces imposed on the freshwater environment that alter the physical, chemical and biological processes of freshwater systems. This in turn influences the biotic communities in the freshwater ecosystem.

Macroinvertebrates are the most important component of wetland food webs, making them important in the ecology and functioning of wetlands (Murkin and Wrubleski 1988). One of the most important roles performed by macroinvertebrates is nutrient cycling and energy transfer within wetland ecosystems and between wetlands and other habitats (DWAF 2004). Wetland macroinvertebrates are not as diverse as their river counterparts, but they are more tolerant of low dissolved oxygen concentrations and they dominate wetland faunal communities (Wissinger 1999).

Given that aquatic invertebrates are directly exposed to physical, chemical and biological disturbances within the wetland, they might be useful bioindicators for assessing the integrity of a wetland (Uzarski et al. 2004, Bowd et al. 2006). There are two schools of thought with regard in using macroinvertebrates as indicators of wetland integrity: one is supportive of the development of invertebrate bioassessment methods and the second is that macroinvertebrates are too scarce and variable in a wetland, and that only community composition of macroinvertebrates at the level of wetland category can be useful (DWAF 2004). General opinion indicates that invertebrates have the potential to be used as a measure of broader wetland condition, because they may integrate the spectrum of available aquatic wetland habitats and condition (DWAF 2004). Secondly, their sensitivity to human-induced disturbance such as pollution influences their occurrence in aquatic ecosystems and how they respond to the pollution varies (Vlok et al. 2006), which is a useful trait for their selection as indicators.

2.9.3 Frogs

Frogs have been fairly well studied as indicators of aquatic habitat condition (Cranston et al. 1996). They are sensitive to environmental change and can be considered good indicators of the state or condition of an ecosystem (Fairweather and Napier 1998).
Frogs are good aquatic indicators because they have complex life cycles encompassing both terrestrial and aquatic habitats, and they have a highly permeable skin at all life history stages (Stebbins and Cohen 1995). Aquatic stages of frogs have highly specialised usage of microhabitats for foraging and maintaining cover from predators (Jansen and Healey 2003).

Frog populations are in decline worldwide due to the loss of habitat and changes to habitat quality (Pechmann and Wilbur 1994, Boyer and Grue 1995). Tyler (1997) suggests that some of these declines may be related to loss and degradation of habitat, land-use activities, pollution, predation and changes to hydrology. Mensing et al. (1998) state that stock grazing and alterations to the hydrology of wetlands influence amphibians associated with such wetlands. Grazing livestock alter habitat complexity in terrestrial habitats, destroying foraging and shelter sites occupied by ground dwelling frogs (Jansen and Healey 2003). Agricultural activities can also reduce habitat complexity in wetlands (Brock and Jarman 2000).

2.9.4 Wetland Plants

The most visible aspect of the wetland environment is the vegetation, which also plays an important role in the functioning of wetlands (Cronk and Fennessey 2001). The available literature on plants suggests that plants are one of the best understood components of wetland biota, particularly in terms of their relationship to soil types and hydrology (Reid and Brooks 1998). Wetland plants are those plants that are adapted to growing in a substrate that is, at least for part of the year, deficient in oxygen and affected by the altered soil chemistry in reducing environments (Sieben et al. 2014). Most wetland plants are herbaceous plants, but in some situations woody species are also prominent. Some wetland plants are floating or submerged (e.g. water lilies, pondweeds and algae), but the largest numbers are emergent, which means that a large portion of their shoot biomass emerges above the water surface (Cronk and Fennessy 2001, Cook 2004).

Wetland specialists in South Africa use wetland plants as indicators of wetland conditions when delineating wetlands (DWAF 2008). Currently there is no vegetation-based health assessment tool developed in South Africa, however Corry (2012) developed a (field-based) methods and applied it in order to determine the attributes of wetland plant assemblages that best indicate the environmental condition of
wetlands. Although WET-Health includes a vegetation module that assesses the
degree to which the current vegetation composition has deviated from perceived
natural or reference conditions (Macfarlane et al. 2007), it is rather crude as it is based
on the presence of alien and ruderal taxa only. The location of wetland plant
community zones and their species composition are influenced largely by factors such
as water regime and salinity, anthropogenic disturbance being the next most important
factor (DeKeyser et al. 2003).

Plants are very important to wetland ecosystems as they are autotrophs at the base
of the food chain and a primary pathway for energy flow in the system (Mitsch and
Gosselink 2000, Cronk and Fennessy 2001). Wetland vegetation provides critical
protection and habitat for other taxonomic groups, such as epiphytic bacteria,
phytoplankton, and some species of algae, periphyton, macroinvertebrates,
amphibians, and fish (DWAF 2004). Plants improve water quality by removing
nutrients through uptake and accumulation in tissues, but they also act as a nutrient
pump by moving compounds from the sediment into the water column (DWAF 2004).
Some plant species remove toxic substances by sequestering them in their tissues
and generally they trap sediments in an anoxic environment where anaerobic bacteria
reduce some nutrients to a gaseous form (Cronk and Fennessy 2001). Vegetation
influences the hydrology and sediment regime through processes such as sediment
and shoreline stabilisation, or by modifying currents (Mitsch & Gosselink 2000).

Wetland plants are considered one of the best indicators of the factors that shape
wetlands within a landscape (DWAF 2004). Plants are regarded as good indicators of
wetland condition because they may have a high species richness, rapid growth rates,
and they respond relatively quickly to environmental changes (US EPA 2002b, DWAF
2008). Globally wetland plants are considered to be useful indicators of wetland
conditions as they represent both structural and functional elements of ecological
character (Butcher 2003). Vegetation has also been shown to provide a sensitive
measure of human-induced changes in wetland ecosystems (DWAF 2004). Changes
in the environment, whether they are due to natural causes or human actions, result
in shifts in plant community composition. Individual species of plants may be used as
indicators because each species shows a differential tolerance to environmental
conditions (van der Maarel 2005).
Chapter 3: STUDY AREA

3.1. Location

The study was conducted in the KwaZulu-Natal Midlands (which includes Pietermaritzburg all the way to the foothills of the berg around Nottingham and Mooi River town) of South Africa, which is a gently undulating area at an altitude between approximately 1,000 and 1,800 m asl and is home to a wide range of agricultural activities (Figure 3.1). It is an area with a high concentration of wetlands because of the relatively high rainfall, but most of the wetlands have been impacted to varying degrees by human disturbance, particularly as a consequence of agricultural activities. The extent of the KwaZulu-Natal Midlands as indicated in Figure 3.1 is a generalisation as there is no clear boundary of this region.
Figure 3.1: The distribution of wetlands examined in this study in relation to the approximate extent of the KwaZulu-Natal Midlands.
3.2. Vegetation

The Midlands is in South Africa’s grassland biome (Rutherford and Westfall 1994, Mucina & Rutherford 2006). The most common vegetation types in the Midlands of KwaZulu-Natal are Moist Midlands Mistbelt, Dry Midland Mistbelt, Dry Midland Sourveld and Dry Tall Grassland (Natural Resources Section 2012). The Moist Midlands Mistbelt Grassland is the most frequently fragmented and altered grassland type in South Africa, and faces imminent danger of disappearing completely (Mucina & Rutherford 2006).

The Grassland Biome is divided into four bioregions, the Drankensberg Grassland, Dry Highveld Grassland, Mesic Highveld Grassland and Sub-Escarpment Grassland. The Sub-Escarpment grassland bioregion is divided into 18 vegetation types (Mucina and Rutherford 2006), and in this study the focus is on 4 of the 18 classes (Midlands Mistbelt Grassland, Drakensberg Foothill Moist Grassland, Mooi River Highland Grassland and Southern KwaZulu-Natal Moist Grassland; Figure 3.2)

The Midlands Mistbelt Grassland covers an area of about 6580 km², with only 0.4 % being formally protected (Mucina and Rutherford 2006). In KwaZulu-Natal it occurs in the Midlands and is scattered in a broad band as several major patches, extending from Dundee in the north to Ixopo in the south. The Midlands Mistbelt Grassland is endangered, and occurs at an altitude ranging between 760-1 400 m asl, with mean annual precipitation of 915 mm summer rainfall and mean annual temperature of 15.8°C, with are only a few patches of the original species-rich grasslands remaining (Mucina and Rutherford 2006). This vegetation type is dominated by forb-rich, tall, sour, Themeda triandra grasslands, which have been transformed by the invasion of native "Ngongoni grass" (Aristida junciformis subsp. Junciformis; (Mucina & Rutherford 2006).

The Drakensberg Foothill Moist Grassland occurs in KwaZulu-Natal and the Eastern Cape Province. In KwaZulu-Natal it extends from the Drakensberg foothills west of Ladysmith in the north to Kokstad in the south (Mucina and Rutherford 2006). The Drakensberg Foothill Moist Grassland occurs at an altitude of 880-1 860 m asl, with summer rainfall and mean annual temperature of 14.6°C. This vegetation type is in the conservation status class of "least threatened", with a conservation target of 23% and
with about 3% already within statutory conservation areas (Mucina and Rutherford 2006)

The Mooi River Highland Grassland is only found in KwaZulu-Natal, with its centre of occurrence in the Mooi River drainage basin and a number of large patches further to the west near Underberg and to the north near Dundee (Mucina and Rutherford 2006). This vegetation class has a mean annual rainfall of 785 mm falling mostly in summer, and it occurs at an altitude of 1 340-1 620 m asl and mean annual temperature of 14°C. Mooi River Highland Grassland is classed as vulnerable, with a conservation target of 23% and only a small portion conserved in a single Nature Reserve, the Swamp Nature Reserve. One quarter of this vegetation type has been transformed for agriculture, including mainly maize cultivation, beef and dairy farming, or commercial forestry plantations of pine (Mucina and Rutherford 2006). The Mooi River Highland Grassland is found in rolling and partly dissected landscapes covered in grassland dominated by short bunch grasses, with *Themeda triandra* and *Tristachya leucothrix* dominant in well-managed veld (Mucina and Rutherford 2006).

Southern KwaZulu-Natal Moist Grassland is found in KwaZulu-Natal and the Eastern Cape Provinces, at an altitude range of 880-1 480 m asl, with mean annual precipitation of 920 mm summer rainfall and mean annual temperature of 16.6°C (Mucina and Rutherford 2006). In KwaZulu-Natal, Southern KwaZulu-Natal Moist Grassland is found in interior valley basins from Howick (north of Pietermaritzburg) in the north, extending southwards towards the area between Underberg and Ixopo. This vegetation type receives summer rainfall with mean annual precipitation of approximately 920mm. Southern KwaZulu-Natal Moist Grassland is vulnerable a with conservation target of 23%, but with only 4% conserved in statutory Nature Reserves (Mucina and Rutherford 2006).
3.3 Geomorphology and drainage

The KwaZulu-Natal Midlands consists of an undulating region at an altitude ranging from about 1 000 to 1 800 m asl that constitutes mainly the Post-Africa I erosion surface that formed over the period from about 30 million to 5 million years following uplift of the southern African subcontinent (~30 m BP) and prior to a second uplift event of the same region (~5 my BP; Partridge and Maud 1989; McCarthy and Rubidge 2005). Most of the rivers draining the KwaZulu-Natal Midlands originate in the foothills of the Drakensberg Mountain Escarpment and often occur as incised valleys with interfluves making up the undulating land surface. Isolated remnants of the ancient
African erosion surface exist at a higher elevation that the Post-Africa I erosion surface within the Midlands of KwaZulu-Natal.

Being a region in southern Africa with a relatively high rainfall, there are many rivers draining the KwaZulu-Natal Midlands, the main ones being the uThukela, uMngeni, Mooi and uMkomazi Rivers. The study will focus on the uMngeni River catchment as most of the wetlands for this study occur within this catchment system.

The source of the uMngeni River is recognised as being a series of wetlands known as the uMngeni Sponge (Zunckel 2013). The 958 ha uMngeni Vlei Nature Reserve was registered as a wetland of international significance under the Ramsar Convention on the 13th of March 2013 as Site Number 2132 (Ramsar Convention, 2013). Approximately 270 ha of the site comprises the wetlands. The uMngeni River is approximately 230 km in length from its source to its mouth, with its catchment, estimated at 4 450 km², receiving an estimated mean annual precipitation of 930 mm (WRC 2002). In the upper region the uMngeni River passes mainly through natural and agricultural regions before reaching Pietermaritzburg, where most of the catchment land-cover is urban, consisting mostly of residential, industrial, and commercial development associated with the city. The approximate land-cover of natural, degraded bush/shrubland, agricultural and urban environments within the uMngeni catchment are 52%, 3%, 37% and 8% respectively (Zunckel 2013). The Midmar, Albert Falls, Nagle and Inanda Dams have been constructed within the river and regulate water storage and release in the uMngeni Catchment.

Given that the Midlands lies on the elevated Post-Africa I erosion surface, it is an environment that is characterised by erosion (McCarthy and Rubidge 2005). Erosion leads to the destruction of wetlands such that wetlands exist in settings that are protected from erosional incision. They are therefore typically associated with variation in the resistance to erosion of underlying lithologies. Wetlands in the Midlands are typically associated with lateral planing of softer Karoo sedimentary rocks such as shales, where these are the spatially most extensive lithologies upstream of a resistant lithology such as a dolerite dyke or sill (Tooth et al 2002; 2004; Grenfell et al 2008; 2009). Wetlands also occur due to tributary valley impoundment as a consequence of floodplain development (Grenfell et al 2008).
3.4 Climate

The Midlands receives relatively high summer rainfall of between 700 and 1 200 mm per year, with mean annual precipitation of 915 mm (Natural Resources Section 2012, Mucina and Rutherford 2006). The area experiences heavy and frequent occurrences of mist that adds to the moisture balance. In winter, spring and early summer, rain is associated with frontal activity, while thunderstorms are common in summer and autumn (Mucina and Rutherford 2006). The mean annual evapotranspiration is 1 463-1 797 mm and the mean annual temperature is approximately 15.8ºC. The region is subject to frequent drought spells during El-Nino events, and in winter moderate frost is common. Hailstorms are common during summer thunderstorm activity and hot, dry north-western berg winds are associated with exceptionally warm weather in the dry winter season, which are often associated with the incidence of extensive natural fires (Mucina and Rutherford 2006).

3.5 Socio-economic activities

Factors contributing to wetland degradation are highly variable, ranging from catchment based activities such as urbanization, obstruction of water and dam development to site-scale factors such as cultivation, drainage and over-grazing. The dominant factors impacting on wetland areas are different in different regions of the province, however Kotze (2004) argue that in agricultural areas such as the upper Mgeni catchment, primary impacts were attributed to cultivation & drainage and dams (74% and 19% respectively) whereas other impacts were quite limited. Midlands is dominated by agricultural landscape and most of the farms are privately owned, having been farmed since settlers arrived in the area during the 1800s. The most common land use is KZN Midlands is agriculture (annual crop production, dairy farming, poultry, hay harvesting and livestock) forestry and tourism.

Several studies have established that the conversion of wetlands to agriculture causes complex effects on the ecosystem. The need for irrigation water directly influences the wetlands, as water flows are regulated and diverted. Shallow and smaller wetlands in drier climates are more seriously affected as their water is used for irrigation compared to bigger wetlands in wetter places (Gopal 2000). Some of the effects of agriculture on wetlands includes 1) Direct loss of wetlands due to draining and conversion to agricultural land. 2) Indirect loss of wetlands area due to water withdrawal from rivers.
and streams for irrigation. 3) Runoff of fertilizers causing excessive eutrophication leading to fish kills, toxic algal blooms and negative impact on aquatic flora and fauna. 4) Loss of wetland area and function due to damming for water storage. 5) Loss of seasonal wetlands due to changed hydrologic cycle from water storage. 6) Loss of wetland functions due to salinization, sediment deposition, erosion and eutrophication. 7) Pollution from use of pesticides and other chemicals (Galbraith et al. 2005, Wiseman 2001). Agriculture is the most dominant form of land use in KZN Midlands, the landowners and their labourers depend on farming for their livelihoods. Wetlands have been drained and converted to croplands (mainly maize), and this has been confirmed to have high impacts on wetland characteristics and functioning (Kotze and Breem 2000). With significant amount of irrigated crops, landowners have built thousands of dames in KZN Midlands. Dams and water abstraction reduce the amount of water available to support wetland and river systems, and alter the properties of water flow downstream (Davies and Day 1998).

Forestry is widespread in KZN Midlands area and is mostly controlled by timber growing companies like Mondi and Sappi, which mainly grow wattle, gum and pine. There are also few townships in the KZN Midlands and are heavily populated with high un-employment rate. Residential areas have negative impact on the catchment level and on the wetlands themselves. Catchment management plays an important role in the health of aquatic ecosystems (Junk 2002). Wetlands are strongly influenced by their catchments, from which they receive water and dissolved and suspended materials, and with which they exchange organisms (Junk 2002). Run-off contaminated by fertilisers and biocides can drastically increase the nutrient levels of recipient wetlands, disrupting their ecosystem processes (Jeffries and Mills 1990, Gopal 2003).

### 3.6 Study sites

The study sites covered a wide range of wetlands ranging from pristine (Stillerust and Riverlea Wetlands) to those that are almost completely transformed (Cedara and Mpophomeni Wetlands). A total of 13 wetlands were selected for analysis in this study, extending westwards from close to the city of Pietermaritzburg in the east (Hilton wetland at an altitude of 1083 masl) to the foothills of the Drakensberg Mountains (Stillerust Wetland at an altitude of 1640 masl). The wetlands range in size from the
smallest being Ivanhoe communal wetland with an extent of 6 ha and the biggest being Stillerust Vlei at 129 ha. The most common land use in the impacted wetlands selected for this study was agricultural, with only Stillerust Vlei being in a protected area. Land ownership included both private and communal. In most of the wetlands there was one HGM unit, except Mpophomeni Wetland situated outside of this urban settlement, and Stillerust Vlei, which each had two HGM units.

3.6.1 Stillerust Vlei

Stillerust Vlei is on the foothills of the Drakensberg (S 29.38133, E 29.73075) and it is the biggest of the sampled wetlands (129 Ha). This wetland is on state owned land inside the Kamberg Nature Reserve. The catchment of this wetland is in good condition, with very few patches of alien trees. However there is a densely populated area, intensive farming and a diary upstream of this wetland. During the rainy season and through ground water return flow from urban and agricultural land, there is a degree of chemical pollution that ends up in the wetland.

3.6.2 Cedara Wetland

Cedara Wetland is heavily degraded given that it is dammed and ploughed for crop production. The wetland is located near Howick (S 29.53224, E 30.26144) about 40 km north-west of Pietermritzburg and the wetland is approximately 38 hectares in size. The catchment of this wetland is completely transformed to mostly maize fields, which extend into the wetland itself. There is also significant commercial forestry in the catchment. There is considerable input of chemical fertilizer to the crops growing in the wetland, and also via runoff and return flow from agricultural activity in the catchment. The flow of water into the wetland has also been reduced by the construction of dams upstream of the wetland. There is also residential development on the eastern side of the wetland, which influences both the quantity and timing of water inputs to the wetland, as well as influencing water quality.

3.6.3 Hilton Wetland

The Hilton Wetland (S 29.52353, E 30.3075) is approximately 30km north-west of Pietermaritzburg in the Hilton area and it is divided into two sections by the N3 National Road. The Hilton wetland is situated on private land owned by a large multinational commercial timber producer (Mondi Ltd). It is 20 hectares in size with the catchment
area of approximately 60 ha. The main activities on the wetland’s catchments are gum plantations and urban residential development. There are artificial drains within the wetland and two dams, all of which are in the lower part of the wetland.

### 3.6.4 Rifle Range Wetland

The Rifle Range wetland (S 29.54698, E 30.20402) is on private land near the town of Howick. It is 14 hectares in size with most of its catchment in good condition. There is a dam upstream of the wetland and maize fields on the northern side of the catchment. The apparent impacts on the wetland are from reduced flows from the dam upstream. The R103 Provincial Road crosses the Rifle Range Wetland and there is damming that takes place in the lower section of the wetland associated with the R103 Road.

### 3.6.5 Hlatikhulu Vlei

Hlatikhulu Vlei is a portion of an extensive wetland system with a floodplain on the northern margin and with several northward-flowing tributary arm valley-bottom wetlands that flow into the floodplain. For the purpose of this study the upper section of the easternmost tributary, the Northington Tributary (S 29.27977, E 29.69776), was examined. It covers an area of 17 hectares. Hlatikhulu Vlei is located on privately owned land that is being developed as a private game farm. The catchment associated with this wetland area is largely untransformed in the upper reaches, but the lower reaches have large areas under cultivation, including areas of irrigated agriculture. A small portion of the catchment is infested with alien plants and plantations also occur in the mid-reaches of the wetland catchment. Within the wetland itself, drainage channels, erosion gullies and dams have the most significant impacts.

### 3.6.6 Ivanhoe Farm Wetland

The Ivanhoe farm wetland occurs in the upper reaches of the UMngeni Sponge (S 29.52530, E 29.84773) and is 18 hectares in extent. This wetland is on privately owned land and is heavily impacted by a deep and dense series of agricultural drains, with most flows now effectively bypassing much of the wetland unit. The wetland unit itself is planted to artificial pasture and grazed as part of a commercial livestock operation with moderate and well managed stocking rates. Large areas of gentle topography
adjacent to the wetland are used for intensive agriculture, principally potatoes, while steeper slopes are used for extensive livestock grazing. Only a small portion of the wetland is affected by erosion of the agricultural drains and associated sediment removal. The steep head of the catchment is dominated by untransformed grassland, with limited plantations.

3.6.7 Ivanhoe Community Wetland

The Ivanhoe community wetland is on communal land on the outskirts of Impendle town (S 29.56717, E 29.86804). This is a small wetland (6 ha) that is in good condition. About 95% of its catchment is untransformed and it is mountainous with extensive dolerite rock in the form of a very large dolerite sill. Impacts to the wetland include compaction from the district road that passes through the upper part of the wetland, with a damming effect upstream of the road. There is also water abstraction from the wetland by the nearby households, which rely on a water pump to deliver water to a single house built adjacent to the wetland.

3.6.8 Mount Park Wetland

The Mount Park wetland is on privately owned land (S 29.54663, E 29.97774). This wetland is 20 hectares in size, with most of its catchment transformed to tree plantations and agricultural fields (mainly potatoes). There are also a small number of dams upstream of the main wetland. The wetland itself is drained and under agricultural fields. There is also a dam in the wetland.

3.6.9 Mount Shannon Wetland

The Mount Shannon Wetland is on the outskirts of Mpendle town (S 29.66945, E 29.91498) and covers an area of 48 ha. There is a dam in the mid-section of the wetland. This wetland is on privately owned land and the catchment of this wetland is completely transformed as commercial plantations are the primary land use. There were limited cattle grazing in this wetland as most of the former grazing land is under plantation. There are also dense stands of alien plants in the wetland.
3.6.10 Mpophomeni Community Wetland

The Mpophomeni Community Wetland is on communal land adjacent to the Mpophomeni Township (S 29.55730, E 30.18764) outside the town of Howick. The wetland is 55 ha in size and its catchment comprises urban residential development, which occurs to the north and east of the wetland. The western side of the catchment is mostly dominated by steep mountainous terrain dominated by natural grassland, which is heavily overgrazed by largely uncontrolled communal livestock. The impacts on the wetland include water pollution and the construction of a dam in the lower reaches of the wetland. There is also a massive gully present in this wetland that occurs along most (70%) of its length.

3.6.11 Mpophomeni Urban Wetland

The Mpophomeni Urban Wetland is within the developed urban area of this town (S 29.56724, S 30.18239). The wetland is treated as communal land, with livestock grazing, sports fields and waste disposal sites present in the wetland. The wetland is 15 hectares in size and its entire catchment is under formal and informal housing development. There is a sewage treatment plant in the wetland that discharges wastewater into the wetland.

3.6.12 Riverlea Wetland

Riverlea wetland is on privately owned land on a farm just outside Underberg (S 29.76445, S 29.44492). Riverlea is a small wetland (7 ha) and is considered to be in very good condition. The prominent land use around this wetland is agricultural fields (mainly maize) and there are a small number of dams in the catchment around this wetland. The catchment is small and it is largely untransformed, dominated by natural grasslands.

3.6.13 Meshlyn Wetland

The Meshlyn Wetland is on privately owned land, between Nottingham Road and Kamberg (S 29.32951, E 29.72891). The wetland is 41 hectares in size, with its entire catchment transformed. The most prominent land use around this wetland is agricultural fields (maize) and a number of commercial plantations. Livestock grazing characterises those parts of the catchment that are not under formal cultivation or
commercial tree plantations. The wetland itself is drained and cultivated, and appears to be completely transformed. Livestock grazing also takes place in the part of the wetland that is not cultivated.
Chapter 4: MATERIALS AND METHODS

4.1. Wetland selection and sampling

Wetlands of the same hydrogeomorphic (HGM) type (unchannelled valley bottom) were chosen such that they included a range of temporally, seasonally and permanently flooded zones. The wetlands chosen ranged in altitude from 1084 to 1640 masl, and varied in size from 6 ha to almost 129 ha in size. Their distribution in the Midlands of KwaZulu-Natal is shown in Figure 3.1.

The health of selected wetlands was assessed by independent practitioners using WET-Health. The assumption is that the health score as determined by WET-Health was a reasonably good reflection of the health of the wetland. Details of the size and health of wetlands, as determined using WET-Health (Macfarlane et al. 2009) that were examined in this study are presented in Table 4.1. Only the overall impact score was used in the analysis in this study, as attempts were made to also use hydrology, vegetation and geomorphology scores were unsuccessful.

Table 4.1: The size, health score and health class for the wetlands sampled in the present study.

<table>
<thead>
<tr>
<th>Wetland name</th>
<th>Size (ha)</th>
<th>Hydrology score</th>
<th>Geomorphology score</th>
<th>Vegetation score</th>
<th>Overall Impact score</th>
<th>Health class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cedara</td>
<td>38</td>
<td>7.3</td>
<td>1.3</td>
<td>3.5</td>
<td>4.5</td>
<td>D</td>
</tr>
<tr>
<td>Hilton</td>
<td>20</td>
<td>5.3</td>
<td>0.3</td>
<td>3.2</td>
<td>3.2</td>
<td>C</td>
</tr>
<tr>
<td>Hlatikhulu</td>
<td>17</td>
<td>4.3</td>
<td>0.4</td>
<td>3.5</td>
<td>2.9</td>
<td>C</td>
</tr>
<tr>
<td>Ivanhoe communal</td>
<td>6</td>
<td>1.1</td>
<td>1.3</td>
<td>3.5</td>
<td>1.8</td>
<td>A</td>
</tr>
<tr>
<td>Ivanhoe private</td>
<td>18</td>
<td>4</td>
<td>1</td>
<td>4.5</td>
<td>3.2</td>
<td>C</td>
</tr>
<tr>
<td>Meshlyn</td>
<td>41</td>
<td>7.5</td>
<td>0.1</td>
<td>7.1</td>
<td>5.2</td>
<td>D</td>
</tr>
<tr>
<td>Mpophomeni urban</td>
<td>15</td>
<td>6.5</td>
<td>2.7</td>
<td>4.2</td>
<td>4.8</td>
<td>D</td>
</tr>
<tr>
<td>Mpophomeni rural</td>
<td>55</td>
<td>5.5</td>
<td>3.1</td>
<td>4.2</td>
<td>4.4</td>
<td>D</td>
</tr>
<tr>
<td>Mt Park</td>
<td>20</td>
<td>5.7</td>
<td>0.5</td>
<td>3.5</td>
<td>3.5</td>
<td>C</td>
</tr>
<tr>
<td>Mt Shannon</td>
<td>48</td>
<td>3.6</td>
<td>0.4</td>
<td>3.7</td>
<td>2.7</td>
<td>C</td>
</tr>
<tr>
<td>Rifle Range</td>
<td>14</td>
<td>3</td>
<td>1</td>
<td>1.3</td>
<td>1.9</td>
<td>A</td>
</tr>
<tr>
<td>Riverlea</td>
<td>7</td>
<td>0</td>
<td>0.3</td>
<td>1.2</td>
<td>0.4</td>
<td>A</td>
</tr>
<tr>
<td>Stillerust</td>
<td>129</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>0.02</td>
<td>A</td>
</tr>
</tbody>
</table>
The assessment of the selected wetlands was done in order to cover a range of wetland health scores. Wetland health scores were based on the combined scores for vegetation, geomorphology and hydrology (Macfarlane et al. 2009). For budgetary reasons, wetlands that were within an hour’s drive of Pietermaritzburg were selected. The selected wetlands were then sampled for dragonflies, frogs, macroinvertebrates and plants. Water samples were also collected for analysis of a number of chemical variables (ammonia, nitrate/nitrite, pH, phosphate and sulphate).

4.2. Macroinvertebrate sampling

The sampling protocol derived for palustrine wetlands was followed, which uses a standard SASS net to collect macroinvertebrates (Bowd et al. 2006). A standard SASS net is a square-framed sweep net with a 23.5 cm mouth and 1 mm mesh. The most suitable sampling areas for invertebrate taxa is on emergent vegetation dominated by sedges, as this biotope contains greater numbers of families in comparison to open water areas that lack submerged vegetation (Bowd et al. 2006). The sweep net was dragged strongly from the surface, down through the water column at a 45° angle, until it touched the substrate and was then brought to the surface with the mouth facing upwards, ensuring that macroinvertebrates were not lost. There were three sampling points per wetland along its length (top, middle and bottom of the wetland) and six sweeps were taken at each sampling point.

Samples were processed in the field by removing all the vegetation and other unwanted material, such that macroinvertebrates could be handpicked using pipettes and forceps. Samples of all invertebrates thus collected were fixed in 70% ethanol and taken to the laboratory for identification. Macroinvertebrates were identified to the family level using the guide “Aquatic Invertebrates of South African Rivers” (Gerber and Gabriel 2002) and “A Guide to the Aquatic Invertebrates Families of Southern African East Coast Rivers” (Baxter 2004), which has a library with specimens for cross referencing.

4.3. Dragonfly sampling

Dragonfly sampling followed the technique described by Simaiika and Samways (2011). Equipment needed was a butterfly net, close-focus binoculars and plastic bottles in which to keep samples. Sampling was done on warm and windless days
between 09:00 and 17:00. Adult male dragonflies were counted within the wetland because these are easier to identify than females. Corbet (1999) states that males tend to set up territories around water in wait for females, because females leave the water to mature and only return to mate.

Sampling was conducted all over the wetland but concentrated around the permanent zone of the wetland. The sampling was standardised by sampling for one hour by two people, and male dragonflies were collected and later released to avoid duplication and for accurate identification. Voucher specimens for each species were collected for reference. If there was a high abundance of the same species, estimates were made of their abundance. Adult male dragonflies were identified using Samways (2008; “Dragonflies and Damselflies of South Africa”) and Tarboton & Tarboton (2002; “A Field Guide to the Dragonflies of South Africa”).

4.4. Vegetation sampling

A two meter radius plot was measured and sampled for plant species and for estimation of the cover-abundance of each species. Twenty plots per wetland were sampled, spread out evenly in the wetland to ensure coverage of the entire wetland. A maximum of 15 minutes was spent searching for new species in each plot to standardise the effort in each plot. For each wetland, species accumulation and species richness were recorded. To determine species accumulation samples were arranged in the order of samples along Axis 1 of an ordination, starting with the sampling point with the lowest axis 1 ordination score and working along axis 1 to the sampling point with the highest axis 1 ordination score. Species richness was determined by counting the number of different species recorded in each wetland.

4.5. Frog sampling

Frogs were sampled in summer between 17:00 and 19:00 since most frogs start calling from 17h00 and two hours was deemed adequate for sampling frogs in this study. Frog calls were recorded using a Samsung GT-N7100 cell phone, which was held with a stretched arm ensuring that the cellphone speaker was facing the direction of the frog that was calling. The use of a poor recording equipment was due to the fact that a proper recording equipment was very expensive. Another limiting factor in frog sampling was the absence of a frog specialist present during sampling. The recording
device was carried throughout the entire wetland. Before individual recordings were made it was necessary to stand still for about 5 minutes, because some frogs stop calling when they hear nearby noises. The frog calls were sent to a frog specialist for identification.

### 4.6. Environmental variables

At each site, pH, water temperature, electrical conductivity and dissolved oxygen were measured using a hand held multiparameter water quality meter. Water samples were collected in 0.5l bottles and were filled completely to avoid oxidation taking place. In each wetland two water samples were taken, one from the top of the wetland (where water enters the wetland), the other at the bottom (where water exits the wetland). The two recordings in each site were added together and divided by two, to get the average concentration for each solutes sampled. The water samples were placed in a cooler box with ice and were transported to the laboratory for analysis within 6 hours of sampling. The water was tested for ammonia, pH, nitrate/nitrite, sulphate, and phosphate by uMngeni Municipality laboratories. Sulphate was the only major ion that was sampled because of financial constraints. A GPS was used to measure the location where each water sample was collected.

### 4.7. Data analysis

Principal Component Analysis (PCA) in the 'VEGAN' package (Oksanen et al. 2010) was used to summarize differences in environmental characteristics (Physical and water quality data). PCA is a multivariate analysis technique applied to large data sets with numerous variables, with the goal of extracting the most important axes of variation in the data (Abdi and Williams 2010). Thus, the dimensionality of the data is reduced, while still summarising variation within the data set (Singh et al., 2004). These axes of variation were displayed as a scatter plot along the first two axes of the ordination such that samples with similar environmental characteristics are displayed close together while samples that are dissimilar are displayed far apart (Abdi & Williams 2010). The R statistical environment, version 2.14.2 (http://www.r-project.org) was used to test for differences in key environmental variables among the different wetlands.
For testing the differences between wetland health classes in relation to their variation in DBI, species richness and abundance scores, analysis of similarities (ANOSIM) was assessed. ANOSIM provides a way to test statistically whether there is a significant difference between two or more groups of sampling units. ANOSIM operates directly on a dissimilarity matrix, which is produced by the analysis. This method was used in conjunction with the non-metric multidimensional scaling (NMDS) ordination, which was used to graphically represent this variation. NMDS uses only the rank order of the dissimilarity values, such that if two groups of sampling units are really different in their species composition, then compositional dissimilarities between the groups ought to be greater than those within the groups. The ANOSIM statistic in the R environment is based on the difference of mean ranks between groups and within groups.

NMDS is a method of data analysis that represents a set of elements on the basis of a table of 'proximities' that defines the relations between the elements (Young et al. 1995). Community structure patterns were visualized by non-metric multidimensional scaling (NMDS) based on Bray-Curtis distances using the metaMDS function in 'VEGAN' (Oksanen et al. 2010). To satisfy the assumptions of normality and homogeneity of data, some environmental variables were logarithmic or arcsine-transformed (Clarke and Warwick 2001).
Chapter 5: RESULTS

This chapter displays the relationships between a range of biotic indices and the Health Classes of 13 wetlands as determined using WET-Health (Macfarlane et al. 2009). Taxa used in the determination of biotic indices were dragonflies, frogs, invertebrates and plants. The expectation is that there will be systematic variation in one or more of the biotic indices co-incident with variation in the Health Class of the wetland.

5.1 Dragonflies

5.1.1 Frequency of dragonfly species occurrences

There was considerable variation in the number of wetlands in which individual dragonfly taxa were present (Figure 5.1). There were 19 different taxa collected in this study (Appendix 1). *Nesciothemis farinosa and Trithemis dorsalis* were found in all 13 wetlands sampled. This suggests that the two species are tolerant of a wide range of variation in habitat conditions across wetland health classes. There were eight dragonfly species that occurred in just a single wetland, suggesting that these species are sensitive to variation in habitat conditions. The remaining species were found in two to five wetlands each.
5.1.2 The DBI scores in relation to Health Classes

The DBI scores varied from about 0.3 to 2.1, which suggests that habitat condition as determined by dragonfly taxa was variable (Figure 5.2). The Ivanhoe wetlands (communally and privately owned and managed (“communal” and “private” respectively) had the highest DBI scores (greater than 2.0) and the Stillerust and Riverlea wetlands had scores between 1.5 and 2.0. The Cedara and Mpophomeni (urban and rural) wetlands had the lowest DBI scores of less than 0.5. The remaining wetlands had DBI scores between 0.8 and 1.3. There was a significant degree of agreement between the DBI scores and the WET-Health scores ($F= 8.7612, p= 0.0129$) as the wetlands with the highest DBI scores were in wetlands with high wetland health scores, while the wetlands with the lowest DBI scores were generally in wetlands with the low health scores. There were some exceptions such as Ivanhoe wetland (farm) with a high DBI score but a low WET-Health score that indicated it was highly impacted. In contrast, the Rifle Range wetland had a WET-Health score that showed it to be near-pristine, but the DBI score was low.
5.1.3 Dragonfly species richness in relation to Health Classes

The overall dragonfly species richness varied between 3 and 9. Dragonfly species richness generally increased with increasing wetland health score (Figure 5.3), as all the wetlands in Class A had high species richness (between 6 and 9), although Ivanhoe farm with a C wetland Health Class had the highest dragonfly species richness (9). The lowest number of species recorded was three species per wetland, which included most of the wetlands in the D Health Class.
5.1.4 Dragonfly abundance in relation to Health Classes

Dragonfly species abundance varied from low to high with Mpophomeni urban wetland (Health Class D) having 6 individuals and Hilton wetland (Health Class C) having 50 individuals. The minimum number of species in wetlands in Health Class A, C and D was 19, 16 and 6 respectively (Figure 5.4). There was a significant difference in dragonfly species abundance with different WET-Health classes (global R = 0.246, p = 0.043).
There was a clear difference in the DBI scores of the wetlands in different Health Classes. Wetlands in Class A generally had the highest DBI scores, wetlands in Class C generally had intermediate scores and wetlands in Class D had the lowest scores (Figure 5.5a). None of the quartiles for these different Health Classes overlapped. Species richness also showed clear differences between the three classes, with wetlands in Class D having the lowest species richness of the three classes, and species richness increased with increasing Health Class. There was moderate overlap of the quartiles in Health Classes A and C (Figure 5.5b). Figure 5.5c also shows differences in terms of species abundance in the different Health Classes.

Figure 5.4: Dragonfly species abundance in each wetland
5.1.6. Dragonfly community composition

Figure 5.6 illustrates the similarity and dissimilarity of individual wetlands in different Health Classes to each other as determined using NMDS analysis based on dragonfly species composition and abundance in each wetland. Wetlands with low WET-Health scores had high Axis 1 scores, and wetlands with high health scores had the lowest Axis 1 scores. Based on species composition and abundance, it seemed possible to distinguish wetlands in the D Health Class from those in the A and C Health Classes, with Class D having higher Axis 1 scores than both Class A (ANOSIM, p=0.029, r=0.542) and Class C (ANOSIM, p=0.008, r=0.494). However there was no statistical difference between Axis 1 scores of wetlands in Health Class A and Health Class C (ANOSIM, p=0.278, r=0.984).
The SIMPER analysis revealed that there was a 56% difference in dragonfly taxa in wetlands in Health Classes D and C (Table 5.1). *Crocothemis erythraea* contributed 14% of the total dissimilarity between wetlands in Health Classes D and C, followed by *Trithemis stictica* (13%), *Palpopleura jucunda* (11%) and *Trithemis dorsalis* (10%).

There was a 58% difference between wetlands in Health Class D and those in Health Class A, with *Trithemis stictica*, *Crocothemis erythraea* and *Nesiothemis farinosa* contributing about 13%, 12% and 10% respectively to the difference. There was no clear difference between wetlands in Health Class A and those in Health Class C, as the average dissimilarity was less than 50%. Only two species contributed to minor differences: *Trithemis stictica* and *Palpopleura jucunda* at 14% and 10% respectively.
Table 5.1: Results of the SIMPER analysis of dragonfly distribution for the different wetland health classes

<table>
<thead>
<tr>
<th>Classes D &amp; C</th>
<th>Average dissimilarity = 56.42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Av.Abund</td>
</tr>
<tr>
<td>Crocothemis erythraea</td>
<td>1.12</td>
</tr>
<tr>
<td>Trithemis stictica</td>
<td>0</td>
</tr>
<tr>
<td>Palpopleura jucunda</td>
<td>0</td>
</tr>
<tr>
<td>Trithemis dorsalis</td>
<td>1.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Classes D &amp; A</th>
<th>Average dissimilarity = 58.15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Av.Abund</td>
</tr>
<tr>
<td>Trithemis stictica</td>
<td>0</td>
</tr>
<tr>
<td>Crocothemis erythraea</td>
<td>1.12</td>
</tr>
<tr>
<td>Nesciothemis farinosa</td>
<td>1.45</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Classes C &amp; A</th>
<th>Average dissimilarity = 46.22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Av.Abund</td>
</tr>
<tr>
<td>Trithemis stictica</td>
<td>1.05</td>
</tr>
<tr>
<td>Palpopleura jucunda</td>
<td>0.86</td>
</tr>
</tbody>
</table>

5.2 Frogs

5.2.1 Frog species occurrence

Ten different frog species were found in this study (Appendix 2). Cacosternum nanum was the most common frog found in 11 (about 85%) of the wetlands sampled (Figure 5.7). Kassina senegalensis and Strongylopus grayii were recorded in 5 and 3 different wetlands respectively. Amietophrynus gutturalis, Afrixalus spinifrons and Amieta queckettie were only recorded once in separate wetlands. The rest of the frog species were recorded in two wetlands. It is worth noting that most of the frog species that were recorded were common species with a widespread distribution.
**Figure 5.7:** The frequency of occurrence of individual frog species in all the wetlands sampled.

### 5.2.2 Frog species richness

Hilton wetland had the highest species richness with five different frog species. There was only one frog species recorded in Meshlyn, Ivanhoe (private) and Riverlea wetland (Fig.5.8). Most of the wetlands in Health Class D (80%) were amongst the wetlands with high frog species richness. There was no systematic variation in frog species richness and wetland Health Classes, such that frogs do not seem to be useful bioindicators of wetland health (Figure 5.8).
5.3 Macroinvertebrates

5.3.1 Macroinvertebrate occurrence

There were 47 macroinvertebrate families recorded in this study (Appendix 3), with the Gyrinidae family occurring in the greatest number of wetlands (n=11; Figure 5.9). The Physidae family was recorded in 9 wetlands, with Aeshnidae and Dytiscidae both recorded in 8 wetlands. The majority of the macroinvertebrate families were found in 3 to 7 wetlands. About 30% of the total macroinvertebrate families sampled were recorded in only a single wetland (e.g. Dixidae, Psychodidea).
Figure 5.9: The number of wetlands in which each macroinvertebrates family was recorded.
5.3.2 Macroinvertebrates species richness

Stillerust (Health Class A) had the highest macroinvertebrate species richness (22 species) followed by Mpophomeni (urban) and Meshlyn, both of which were in Health Class D, with 19 and 16 species respectively (Figure 5.10). The rest of the wetlands had macroinvertebrate species richness ranging from 9 to 16. Three out of four wetlands in Health Class A were among the wetlands with low macroinvertebrate species richness of about 10 species in each wetland. There was no relationship between the macroinvertebrate species richness and the Health Class.

![Macroinvertebrate species richness in each wetland](image)

Figure 5.10 Macroinvertebrate species richness in each wetland. The letters indicate the wetland Health Class.

5.3.3 Macroinvertebrate indices

There was no clear separation amongst the different health classes based on the SASS score, the ASPT, overall invertebrate abundance or invertebrate species richness (Figure 5.11a, b, c, d). There was considerable overlap in the quartiles of these variables across all Health Classes, and where there were distinct trends, such
as for invertebrate abundance and richness (Figure 5.11 c and d), the relationships were contrary to what would be expected.

![Box-and-whisker plots](image)

Figure 5.11: Box-and-whisker plots of macroinvertebrate SASS scores (a), average score per taxon (ASPT; b), macroinvertebrate abundance (c) and richness (d) for wetlands in different Health Classes.

5.3.4. Macroinvertebrate community composition

Based on an analysis of similarity (ANOSIM) there was no statistical difference between community composition and wetland Health Classes (Global $R=0.081$, $p=0.274$). A similar result was evident based on an analysis using NMDS, where there was considerable overlap between wetlands in different Health Classes along Axes 1 and 2 (Figure 5.12).
5.4 Vegetation

5.4.1 Plant species richness

Plant species richness generally decreased with decreasing WET-Health score. The Hilton wetland had the highest species richness with over 50 species recorded (Figure 5.13), but it had a Health Class of C. However, all the wetlands in Health Class A had high species richness ranging from 38 to 48. Mpophomeni rural wetland in Health Class D had species richness of about 35, which was higher than all the other wetlands in Health Class C (Figure 5.13). The rest of the wetlands in Health Class C and in Health Class D had average species richness counts of 30 and 25 respectively. Species richness therefore seemed to decrease with decreasing wetland Health Class.
5.4.2 Plant species accumulation curve for each wetland

Species accumulation curves for each wetland generally show a relationship that increases rapidly as sample number increases initially, but that saturates at higher sample numbers as fewer and fewer novel species are encountered in the wetland as the number of samples increases. The accumulation curve showed that all the wetlands in class A had higher accumulation rates than the wetlands in class C and D as all the wetlands in class A showed saturation at higher sampling efforts than the other two classes. However the Hilton wetland in class C was still increasing at a high sampling effort when all the other wetlands accumulation curves had started saturating (Figure 5.14).
Figure 5.14: The cumulative number of species in relation to the number of sample plots for each of the wetlands sampled in this study.
5.4.3 Plant species accumulation rate in each wetland class

Plotting the curve gives a way of estimating the number of additional species that will be discovered with further sampling effort. This was done by fitting the logarithmic function on each species accumulation curve. This relationship is well known in ecology and it is considered useful to examine the relationship between species accumulation rate and wetland health. Logarithmic trendlines were fitted in each accumulation curve and all the wetlands in class A had higher species accumulation than the other two wetland classes in general (Figure 5.15) Hilton wetland in class C had the highest accumulation rate (14.57) and Meshlyn wetland in Health Class D had the lowest accumulation curve (5.14) (Figure 5.15). There was a significant difference in terms of species accumulation and wetland classes \( (F = 79.461, p = 4.41 \times 10^{-9}) \).

**Figure 5.15:** Logarithmic plant species accumulation rates in relation to health classes for wetlands sampled in this study.
5.5 Physical and chemical parameters

5.5.1 Water quality results

Values for pH were close to neutral and varied from slightly acidic (pH = 6.28 in Ivanhoe communal wetland) to slightly basic (pH = 7.82 in Rifle Range wetland; Table 5.2). Ammonia was high in the Mpophomeni Urban wetland, but otherwise values can be considered low at less than 0.2 mg/l. Total nitrogen concentration was generally low at less than 1 mg/l, but it was higher than this in the cases of Hlatikulu (1.36 mg/l), Stillertust (2.39 mg/l) and Mpophomeni Urban (3.32 mg/l). Phosphate concentration was also generally low at less than 70 μg/l, but higher than 100 μg/l at Hlatikulu (127.1 μg/l) and Mpophomeni Urban (247.4 μg/l). Phosphate was very high at more than 2500 μg/l at the top section of the Stillertust wetland and it was drastically reduced at the bottom of the wetland where water exits the wetland (426 μg/l) (Table 5.2). Sulphate concentration was generally low at less than 5 mg/l, but at Hilton the concentration was almost 18 mg/l. There was no relationship between the pH or concentration of major solutes and the wetland Health Class, but it was notable that all the wetlands in Health Class A were associated with low solute concentrations for most solutes from the inflow to the outflow of the wetland. This was not necessarily the case for wetlands in other Health Classes.
Table 5.2: Values for pH, ammonia, sulphate, total nitrogen and phosphate concentrations for wetlands examined in the study taken at the head ("Top") and toe of the wetland ("Bot"). The Health Class of each wetland is also shown.

<table>
<thead>
<tr>
<th>Wetland name</th>
<th>Ammonia (mg N/L)</th>
<th>pH</th>
<th>Sulphate (mg SO4/L)</th>
<th>Nitrogen (mg N/L)</th>
<th>Phosphate (μg P/L)</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivanhoe com. Top</td>
<td>&lt;0.04</td>
<td>6.09</td>
<td>&lt;0.16</td>
<td>&lt;1.00</td>
<td>19.5</td>
<td>A</td>
</tr>
<tr>
<td>Ivanhoe com Bot</td>
<td>&lt;0.04</td>
<td>6.47</td>
<td>&lt;0.16</td>
<td>&lt;1.00</td>
<td>&lt;15.0</td>
<td></td>
</tr>
<tr>
<td>River Lea Top</td>
<td>0.04</td>
<td>6.77</td>
<td>&lt;0.16</td>
<td>&lt;1.00</td>
<td>51.5</td>
<td>A</td>
</tr>
<tr>
<td>River Lea Bottom</td>
<td>&lt;0.04</td>
<td>6.87</td>
<td>&lt;0.16</td>
<td>&lt;1.00</td>
<td>28.2</td>
<td></td>
</tr>
<tr>
<td>Riffle Range Top</td>
<td>&lt;0.04</td>
<td>7.66</td>
<td>2.27</td>
<td>1.27</td>
<td>25.4</td>
<td>A</td>
</tr>
<tr>
<td>Riffle Range Bottom</td>
<td>&lt;0.04</td>
<td>7.98</td>
<td>&lt;0.16</td>
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</tr>
<tr>
<td>Stillerust Top</td>
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<td>0.32</td>
<td>1.30</td>
<td>2861</td>
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<tr>
<td>Stillerust Bottom</td>
<td>&lt;0.04</td>
<td>6.53</td>
<td>&lt;0.16</td>
<td>3.48</td>
<td>426</td>
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</tr>
<tr>
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<td>1.38</td>
<td>&lt;1.00</td>
<td>21.2</td>
<td>C</td>
</tr>
<tr>
<td>MT Park Bottom</td>
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<td>6.75</td>
<td>0.24</td>
<td>&lt;1.00</td>
<td>38.8</td>
<td></td>
</tr>
<tr>
<td>Ivanhoe private Top</td>
<td>&lt;0.04</td>
<td>6.77</td>
<td>0.97</td>
<td>&lt;1.00</td>
<td>25.3</td>
<td>C</td>
</tr>
<tr>
<td>Ivanhoe private Bottom</td>
<td>&lt;0.04</td>
<td>6.48</td>
<td>0.95</td>
<td>&lt;1.00</td>
<td>20.3</td>
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</tr>
<tr>
<td>Hilton Top</td>
<td>&lt;0.04</td>
<td>6.55</td>
<td>3.61</td>
<td>&lt;1.00</td>
<td>16.2</td>
<td>C</td>
</tr>
<tr>
<td>Hilton Bottom</td>
<td>&lt;0.04</td>
<td>6.25</td>
<td>32.2</td>
<td>&lt;1.00</td>
<td>&lt;15.0</td>
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<tr>
<td>Hlatihulu Top</td>
<td>&lt;0.04</td>
<td>6.29</td>
<td>0.59</td>
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<td>Hlatihulu Bottom</td>
<td>0.04</td>
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<td>MT Shannon top</td>
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<td>7.01</td>
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<td>43.6</td>
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<td>&lt;0.16</td>
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<td>1.00</td>
<td>&lt;1.00</td>
<td>53.5</td>
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</tr>
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<td>Cedara Bottom</td>
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<td>7.30</td>
<td>4.56</td>
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<tr>
<td>Mpops rural -Top</td>
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<td>7.63</td>
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<td>&lt;1.00</td>
<td>52.2</td>
<td>D</td>
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<td>Mpops rural bottom</td>
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<td>2.63</td>
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<td>75.6</td>
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</tr>
<tr>
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<td>6.59</td>
<td>456</td>
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<td>0.04</td>
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<td>0.50</td>
<td>&lt;1.00</td>
<td>33.6</td>
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<tr>
<td>Meshlyn bottom</td>
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<td>6.73</td>
<td>1.59</td>
<td>1.24</td>
<td>36.6</td>
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</tr>
</tbody>
</table>
5.5.2 Chemical parameters

The Principal Component Analysis (PCA) of all chemical variables revealed that 78% of the variability across all sites was accounted for by the first three axes of the ordination (35%, 26% and 17% respectively Table 5.3). Given that most of the variation in the data was explained by the first two components, only these will be considered.

Table 5.3: The first three axes of Principal Component Analysis (PCA) results as they explained 78% of variability for the chemical parameters.

<table>
<thead>
<tr>
<th></th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue</td>
<td>3.1175</td>
<td>2.3342</td>
<td>1.5253</td>
</tr>
<tr>
<td>Proportion Explained</td>
<td>0.3464</td>
<td>0.2594</td>
<td>0.1695</td>
</tr>
<tr>
<td>Cumulative Proportion</td>
<td>0.3464</td>
<td>0.6058</td>
<td>0.7752</td>
</tr>
</tbody>
</table>

Ammonia, nitrogen and pH were highly correlated variables with axis 1 scores, they had correlation co-efficients of (0.7163, 0.6722 and 0.6708) respectively (Table 5.4). In PC1 only sulphate had a negative correlation. However phosphate was the most important chemical variable as it showed the highest correlation in PC 2 (1.03123), followed by a total area of the wetland at 0.86678. All other physiological data accounted for less than 50% in PC 2, with ammonia, pH and sulphate having negative correlation (Table 5.4).
Table 5.4: Physical and chemical parameters of the first two axes of a standardised PCA.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PC 1</th>
<th>PC 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td>0.7163</td>
<td>-0.06678</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.6722</td>
<td>0.38314</td>
</tr>
<tr>
<td>pH</td>
<td>0.6708</td>
<td>-0.46044</td>
</tr>
<tr>
<td>Area</td>
<td>0.3260</td>
<td>0.86678</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.2216</td>
<td>1.03123</td>
</tr>
<tr>
<td>Sulphate</td>
<td>-0.3139</td>
<td>-0.16358</td>
</tr>
</tbody>
</table>
Chapter 6: DISCUSSION

Biomonitoring has been focused on rivers and it is only recently that wetlands are receiving attention with respect to biomonitoring. Biological assessment supports wetland management by highlighting pressures and threats that limit biodiversity and ecosystem services or place them at risk. It can also provide feedback on the effectiveness of management interventions in sustaining or restoring wetland values. Biomonitoring can provide reliable, quantitative characterizations of ecological condition, and there is a growing need to identify effective bioindicators for use in wetland management and protection (Sifneos et al. 2010).

6.1 Dragonflies as a potential bioindicator of wetland integrity

Bioassessment tools based on dragonflies have been developed and tested in Europe and South Africa. Odonata are already established as focal organisms for freshwater conservation (Samways 2008) and as good indicators of site value and habitat quality for ponds, lakes, rivers, and streams (Silva et al. 2010, Raebel et al. 2012, Rosset et al., 2013). In Austria, Chovanec and Waringer (2001) combined species-specific abundance classes, niche width, and habitat preference into an Odonata Habitat Index meant to classify the ecological status of river-floodplain systems. In this study, DBI, as developed by Simaika and Samways (2009), combined species' geographical range, risk of extinction, and sensitivity to habitat change. Such a DBI has been effective for assessing river condition in South Africa (Simaika & Samways 2011) and the conservation value of ponds and small lakes in Europe and South Africa (Rosset et al. 2013).

Chwala & Waringer 1996 argue that dragonflies play an essential role in bioindication, and surveys of dragonfly communities have become essential tools for the ecological assessment of aquatic systems, particularly of standing waters, wetlands and floodplain areas. Kutcher (2014) established that conservatism of adult Odonata averaged across species may provide a robust indicator of freshwater wetland condition. Adult dragonflies are easy to identify to the species level while flying over water (Kutcher 2014), making them especially well-suited for rapid assessment methods (Fennessy et al. 2007) and addressing the increased focus on wetland quality and not just quantity in the United States (Scozzafava et al. 2011). Dragonflies are
reliable indicators of the ecological integrity and hydrological processes of wetland areas, as well as of the ecological quality of the land–water interface and its connectivity. The key factors for understanding this indicator potential are highly specific sets of habitat requirements reflected by distinct patterns of niche differentiation (Schmidt 1985).

In this study dragonflies were demonstrated to be a suitable taxonomic group to be used as a bioindicator of wetlands health, because DBI score decreases with decreasing wetland health score. Almost all of the wetlands in health class A had higher DBI scores than wetlands in classes C and D, except for the Rifle Range wetland (class A), which had a surprisingly low DBI for an A class wetland. However, the Rifle Range wetland was one of the most naturally dry wetlands encountered in this study with infrequent and limited surface water inundation compared with the other class A wetlands, which were permanently or semi-permanently flooded. Furthermore, it also had a higher hydrological impact that the rest of the A class wetlands examined. There was a small patch of open water on this wetland with short vegetation present at the time of sampling for the DBI, which reduced suitable habitat for dragonflies and contributed to the low DBI score. This supports the suggestion by Macfarlane et al. (2007) that assessment of wetland health requires understanding of wetland systems beyond examining a wetland at face value. This also highlights the need to examine wetlands in the same HGM class separately, and within each of these, one needs to consider the natural and altered hydrological regime in respect of duration of flooding.

The lack of statistically significant differences between WET-Health scores and DBI scores could also be explained by the fact that the WET-Health assessments were conducted by different specialists and that the wetlands might have been scored differently by different specialists. No assessment has been undertaken of variation in WET-Health scores between different individuals. Another possible explanation could be the difference in wetland size, but this would need to be investigated further as Ivanhoe farm wetland had higher species richness than all the wetlands and it was one of the smallest wetlands.

The dragonfly species *N. praetorius* and *A. miniscula* were the most sensitive species in respect of having the highest DBI scores (5). These two species were found in wetlands that were in health class A and both were recorded in Ivanhoe farm wetland...
There may have been localised areas of suitable habitat in the Ivanhoe farm wetland in an otherwise degraded system, which contributed to these species being present and therefore yielding a high DBI score.

All the wetlands in class D have the site DBI score of 0.33 except for Meshlyn, which had a site DBI score of 1.3, which was higher than most wetlands in class C. Zosteraeschna miniscula was recorded in the Meshlyn wetland and it has a species DBI score of 5. Once again, the presence of a single species resulted in a higher site DBI score. Zosteraeschna miniscula is found sporadically in marshes with reeds and pools along streams running through grassland environments (Samways 2008). This type of habitat was not present at Meshlyn wetland, as the wetland is completely transformed and drained. It could be that the species was encountered whilst flying from other nearby wetlands or from the river downstream into which the wetland drained.

This study clearly demonstrated that dragonflies are very promising bioindicators of wetland health as the DBI scores for individual wetlands were found to be closely correlated with WET-Health scores. The reasons for this were that:

- Species that are common, widespread, non-threatened and highly tolerant of disturbance (Simaika and Samways 2008), and therefore with the lowest species DBI scores – such as N. farinose, C. erythraea and T. dorsalis with DBI scores of 1, 0 and 0 respectively – were the only species recorded in three of the four wetlands in class D.
- The near pristine wetlands had greater diversity of dragonflies, including species that have high DBI scores.

Chovanec (2000) highlighted the following advantages of using dragonflies as bioindicators:

1) A long tradition of ecological work on dragonflies has led to advanced knowledge of the ecological requirements of a large number of odonate species.

2) The good indication potential of dragonflies is based on a high correlation between given structural components of the habitat and the presence of certain species.

3) Many dragonfly species depend on structurally heterogeneous aquatic and terrestrial microhabitats; therefore, they are good indicators of the ecological conditions of aquatic/terrestrial transition zones and of landscape patterning.
4) The relatively small number of species; most of which can be identified in the field, which corresponds to the requirements of conservation strategies.
5) The relatively long life cycles of odonate species meets the requirements for medium or long-term monitoring.
6) Dragonflies are susceptible to specific types of habitat changes induced by human activities (e.g. manipulation of riparian vegetation) and they rapidly react by appearance or disappearance or by changes in abundances. Because of their migration behaviour, new habitats can be rapidly colonized.
7) In biodiversity conservation, dragonflies serve as umbrella species representing land–water areas and specific biotic assemblages.

There are some disadvantages of using dragonflies as a bioindicator:
1) Dragonflies do not fly if the weather is windy and or if the weather is not sunny (in this study sampling was done on sunny and windless days). However, this might present practical challenges, especially if one has tight deadlines or budget constraints.
2) Different dragonfly species fly at different times of the year (in this study sampling was done in summer when most of the dragonfly species were flying). To work around this problem it would be ideal to extend the sampling period to cover different seasons.
3) Some species fly fast and they have huge home ranges (they can visit different wetlands in which they do not reside).

To help reduce misrepresentation of the DBI score, one needs to look at the habitat requirements of the species to make an informed decision about whether or not the species resides there or it was just flying within its broader home range.

6.2 Frogs as a potential bioindicators of wetlands

Results of this study showed that frogs are not a reliable bioindicator species of wetlands, which is contrary to what others have found (Stebbins and Cohen 1995, Jansen and Healey 2003). Jansen and Healey (2003) reported that species richness increased significantly with wetland condition scores. Other studies globally have showed that clearing of vegetation for grazing and cropping have reduced frog species
richness, abundance and restricted movements of adults across floodplains (Sadlier and Pressey 1994). Domestic cattle and sheep cause soil compaction and erosion while reducing plant biomass, species richness and water quality (Robertson 1997) and together these have a negative impact on frog community composition and species richness.

Stebbins and Cohen (1995) state that frogs are the best aquatic indicators because they have complex life cycles encompassing both terrestrial and aquatic habitats, and they also have highly permeable skin at all life history stages. Aquatic stages of frogs have highly specialised usage of microhabitats for foraging and cover from predators, and wetlands are important habitats for many frog species as they provide the critical habitat for breeding and larval development (Jansen and Healey 2003). It must be noted that different frog species have different seasonal and daily peaks of activity based on their biology and the weather. To increase the likelihood of detecting target species, one should conduct surveys at the time of year and day when those species are most likely to be active and during weather conditions favouring activity. Individual species were not targeted in this study, which increased the likelihood that some species were not calling during sampling. Alternatively, the season or weather may not have been suitable for sampling. Furthermore, use of a cell phone to record the frogs was viewed as a limitation and also the absence of the frog specialist during sampling. The frog specialist highlighted the poor quality of the recordings as there was so much ambient noise, which resulted in difficulty identifying some frogs. There were also restrictions in terms of accessing some wetlands. In the case of Riverlea wetland, frog recording was conducted too early on this wetland at a time when most of the frogs would unlikely to be calling. Snodgrass et al. (2000) state that wetland size does not influence frog species richness, but the cycle of wetting and drying of wetlands has been shown to influence community structure and species richness. Most of the wetlands in this study were drained, which might have contributed to the low number of frog species recorded.

6.3 Macroinvertebrates as a potential bioindicator of wetland integrity

Literature shows that invertebrate community composition can be indicative of wetland integrity or used as a biomonitoring tool, although many macroinvertebrate bioindicator
indices (such as SASS5) are designed for low/moderate flow conditions. Such tools are not viewed as applicable in wetlands, impoundments, estuaries and other aquatic habitats with stagnant water. SASS5 works best when the diversity of habitats is wide and includes riffles or rapids, but it also produces valuable results from habitats that might lack some of the required features such as riffles or rapids (Dickens & Graham 2002). Given this it is necessary to interpret the data in relation to habitat quality, availability and diversity, and also in relation to the ecological region in which sampling was undertaken (Dickens & Graham 2002). Data should also be interpreted in relation to the season of collection, as some natural variation in invertebrate populations will occur during the course of the year and between years (Dickens & Graham 2002).

As was the case with Bowd (2005) and Bird (2010), this study suggests that macroinvertebrates appear to be unsuitable as a bioindicator of wetland integrity. Bowd et al. (2006) investigated the use of SASS5 for distinguishing levels of impairment among a set of palustrine wetlands in the Midlands of Kwazulu-Natal that were differentially impacted by dairy effluent. Their study showed that without modification, SASS5 could not be used to gauge levels of organic pollution for this wetland type. Batzer (2013) highlighted the lack of predictable patterns of wetland invertebrate distribution and abundance with environmental factors in several well-studied wetland systems. Focusing on smaller macroinvertebrate trophic groups did not produce stronger relationships between environmental characteristics and the sampled invertebrate assemblages (Batzer 2013). Vegetation is also cited as an important factor controlling wetland macroinvertebrate community structure and composition (Batzer 2013).

It was also highlighted in Bowd (2005), that macroinvertebrates are strongly influenced by vegetation availability. An attempt must be made to sample invertebrates in similar vegetation types. The wetlands sampled in this study had different contributions of submerged and emergent vegetation, which could explain the differences in abundance and diversity of invertebrate populations among wetlands. Davis and Bidwell (2008) argue that surrounding land use practices such as burning, grazing and mowing, had a greater influence on macroinvertebrate assemblages than wetland vegetation management techniques. Aerial stages of aquatic macroinvertebrates are important for species dispersal and macroinvertebrate aerial stages are more sensitive than the aquatic stages to land use practices around wetlands (Raebel et al. 2012,
Tangen et al. 2003). Wetland degradation and alteration of macroinvertebrate fauna can thus result from adjacent agricultural land use practices that result in water pollution, sedimentation and altered wetland hydrology (Schulz 2004).

These difficulties suggest that may be best to score wetland macroinvertebrates differently from SASS5, which is designed for rivers. There is thus a need to develop a SASS5 equivalent for wetlands, because not all of the biotopes that need to be sampled in SASS5 are present in wetlands. Therefore, the scoring assigned to each taxon will need to be adjusted accordingly. Chutter (1998) states that where biotope variation is low, the SASS score is low relative to ASPT score. It has come to light in this study that some wetlands had few species that have high score, but some impacted wetlands had a high number of tolerant species with low scores. However, when the ASPT and SASS5 score were calculated they were similar between impacted and non-impacted wetlands. Hence, there is a need to score macroinvertebrates for wetlands differently from the current scoring system in SASS5, which is used in rivers. Contrary to the results of this study, some studies have shown that macroinvertebrates are a useful bioindicator for aquatic and wetland ecosystems (Karr & Chu 1999, Wissinger 1999, Wright et al. 2000, Bonada et al. 2006, Ollis et al. 2006).

6.4 Vegetation as a potential bioindicator of wetland integrity

Vegetation has been shown to be a sensitive measure of anthropogenic impacts to wetland ecosystems, and can serve as a means to evaluate best management practices, assess restoration, prioritize wetland related resource management decisions, and establish aquatic life use standards for wetlands (USEPA 2002). Marcelino et al. (2012) argue that different levels of anthropogenic disturbance and management intensity correlate with measurable changes in the partitioning of plant species in both herbaceous and arborescent community types. Overall, a significant relationship between increased anthropogenic activities and decreased indigenous species diversity (i.e. native, endemic and threatened plants), has been observed (Marcelino et al. 2012). Conversely an increase in introduced species diversity (i.e. invasive, naturalized, and cultivated plants) has been observed in relation to increased anthropogenic activities in wetlands (Marcelino et al. 2012). Given this, many studies
have shown plants to be very good indicators of wetland ecological condition, including species richness.

Many human-related alterations to the environment that act to degrade wetland ecosystems cause shifts in plant community composition that can be quantified easily. Individual species show differential tolerance to a wide array of stressors (USEPA 2002). In this study plants proved to be reasonably good bio-indicators of wetland health. However there is a plant index (The floristic quality assessment index, FQAI) that was proposed in the late 1970s as a method for assessing habitat quality in the Chicago area of the USA (Swink and Wilhelm 1979). The FQAI uses measures of ecological conservatism and richness of the native plant community to derive an estimate of habitat quality (Miller and Wardrop 2006). In general, plants that are widespread with broad tolerances of environmental conditions (generalist species) are given lower values than plants with more narrow distributions and tolerances (conservative species). Ecological conservatism is expressed numerically as a “coefficient of conservatism”. The coefficient of conservatism is a subjective rating of individual plant species based on professional opinion, with reference to species preference for non-degraded natural communities (Miller & Wardrop 2006). Conservatism values range from 0 to 10 and are assigned a priori based on an individual plant species “fidelity” to specific habitat types and its tolerance to both natural and anthropogenic disturbance (Miller and Wardrop 2006). Conservatism values of different sites in the study area are averaged and weighted based on species richness, which proved good predictor of habitat condition (Miller and Wardrop 2006). This was also the case in South Africa, as illustrated by Cowden et al. (2014), who used FQAI (amongst other indices) to show that plants can be used to assess the response of vegetation following wetland rehabilitation. However, the index still needs to be modified further for use in South Africa.

Miller and Wardrop (2006), argue that since FQAI combines measures of richness with individual plant tolerances, the index should be responsive to these stressors and to landscape-level changes that cause them.

While the FQAI is gradually gaining acceptance as an effective evaluation tool, two fundamental issues remain that have been problematic for the index since its conception: the overwhelming influence of species richness in the equation and the
role of non-native species in assessing floristic quality (Miller and Wardrop 2006). However recent studies have suggested the inclusion of non-native species as an alternative to the traditional approach (Rooney and Rogers 2002, Bernthal 2003, Andreas et al. 2004, Rothrock 2004).

Bedford (1996) states that plants are important components of wetland ecosystems, which makes them one of the best indicators of factors that shape wetlands within their landscape. Some of these contributions include (Mitsch & Gosselink 2000; U.S.EPA 2002) who suggest that:

1) Wetland vegetation is at the base of the food chain and, as such, is a primary pathway for energy flow in the system.
2) Wetland vegetation provides critical habitat structure for other taxonomic groups, such as epiphytic bacteria, phytoplankton, and some species of algae, periphyton, macroinvertebrates, amphibians, and fish. The composition and diversity of the plant community is considered to influence diversity in these other taxonomic groups.
3) Strong links exist between vegetation and wetland water chemistry. Plants remove nutrients through uptake and accumulation in tissues, but they also act as a nutrient pump by moving compounds from the sediment into the water column.
4) Plants are found in all wetlands and are primarily immobile.
5) Plant taxonomy is well known, and excellent field guides are available for many regions and covering a great diversity of plant species. There is some understanding of differing responses of some species to human disturbance.
6) Sampling techniques are well developed and extensively documented, and can be used in both freshwater and saltwater systems.

There are also disadvantages in using plants as wetland bioindicators (USEPA 2002):

1) A delay may occur in the response time to stressors, particularly in long-lived species.
2) Plant identification to species level can be laborious, or restricted to narrow periods during the field season. Several assemblages such as the grasses and sedges, may be particularly difficult to identify to the species level.
3) Sampling techniques for some assemblages, such as the submerged species, can be difficult.
4) Vegetation sampling is generally limited to the growing season.
5) Research or literature on plant species responses to specific stressors is not well developed.

6.5 Wetland health and water quality

This study showed that water quality at top of the wetland (the main point where water enters the wetland) was consistently poorer than the water quality at the bottom of the wetland (main exit water point from the wetland). For example, the water quality improves by just over 85% in terms of phosphate concentrations in Stillerust Vlei (Health Class A, assessed using WET-Health tool). UrbancBercic and Gaberscik (1997) showed that *Phragmites australis* effectively removed water pollutants. It is worth reporting that there were extensive patches of *Phragmites australis* in the middle and lower sections of most of the wetlands where water quality improved from the top to the toe of the wetland. This suggests that wetlands in a good condition deliver ecosystem services to a greater degree than more degraded ones.

Other than wetlands in Health Class D, wetlands generally led to improved water quality. However, those wetlands in Health Class D were consistently associated with decreased water quality as water passed through the wetland. This was likely to be because wetlands in Health Class D were severely degraded in respect of vegetation composition and cover, which reduced the ability of these communities to remove solutes from inflowing waters. Shelef *et al.* (2011) showed that physiological parameters of plant performance are useful as bioindicators for water quality enhancement in wetlands. It must be noted that in this study only one set of water samples were collected in each wetland due to financial constraints.
Chapter 7: CONCLUSION

The motivation for this study comes from the lack of information regarding rapid assessment of wetland condition using biota as bioindicators. The complexity of wetlands requires development of user friendly and robust biomonitoring tools. In South Africa, and globally, wetlands continue to be degraded. Conservation entities are looking for quick biologically based assessment tools to help assess long term ecological changes of these systems. The literature review showed that little has been done to develop biomonitoring systems for wetlands, and where studies have been done, there has been limited success. The main focus of this thesis was to test four different taxa (dragonflies, macroinvertebrates, frogs and plants) to determine how they respond to wetlands impacted primarily by agricultural activities, and determine the degree of congruency with assessments of wetland condition as assessed by the WET-Health tool.

Dragonflies proved in this study to be the most useful of these four taxonomic groups, which might be explored further as a bioindicator of wetland condition. This taxonomic group showed sensitivity towards anthropogenic impacts and was well correlated with WET-Health scores. However the use of dragonflies for rapid wetland health assessments needs to be studied in more detail and far more widely in South Africa. In this study plants also showed promise as a bioindicator of wetland health, as there were clear differences in species accumulation and species richness that were systematically related to variation in wetland health classes.

The assessment of wetland health is still in its infancy, and because it has not been extensively or rigorously tested, comparison of bioindicator results with WET-Health scores may be nonlinear and therefore complex. However in South Africa, WET-Health is now widely applied. However, prior to this study, no research had been undertaken of the relationship of WET-Health assessment scores to various biotic indices. This study has shed light on how WET-Health classes relate to various biotic indices, but there were some limitations that need to be addressed in future research of this nature. It must be noted that this study was conducted in a single sampling season, and future research should make sure that sampling occurs in different seasons. In further studies it is also advised that alien species and indigenous wetland
plants should be differentiated, such that the relationship between the occurrence of native and alien plant species and wetland integrity can be determined.
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### APPENDICES

Appendix 1. List of dragonflies found in the Midlands area, and those found in this study

<table>
<thead>
<tr>
<th>No</th>
<th>Latin Name</th>
<th>Common Name</th>
<th>Found in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pinheyschna subpupillata</td>
<td>Stream Hawker</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Zosteraechna miniscula</td>
<td>Friendly Hawker</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Anax ephippiger</td>
<td>Vagrant Emperor</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Anax imperator</td>
<td>Blue Emperor</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Anax speratus</td>
<td>Orange Emperor</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>Notogomphus praetorius</td>
<td>Yellowjack</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>Paragomphus cognatus</td>
<td>Rock Hooktail</td>
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</tr>
<tr>
<td>8</td>
<td>Paragomphus elpidius</td>
<td>Corckscrew Hooktail</td>
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</tr>
<tr>
<td>9</td>
<td>Orthetrum abbotti</td>
<td>Little Skimmer</td>
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</tr>
<tr>
<td>10</td>
<td>Orthetrum caffrum</td>
<td>Two-striped Skimmer</td>
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</tr>
<tr>
<td>11</td>
<td>Orthetrum chrysostigma</td>
<td>Epaulet Skimmer</td>
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</tr>
<tr>
<td>12</td>
<td>Orthetrum Julia</td>
<td>Julia Skimmer</td>
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</tr>
<tr>
<td>13</td>
<td>Orthetrum trinacria</td>
<td>Long Skimmer</td>
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</tr>
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<td>14</td>
<td>Neciothemis farinosa</td>
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<td>15</td>
<td>Palpopleura jucunda</td>
<td>Yellow-veined Widow</td>
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</tr>
<tr>
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<td>Palpopleura lucia</td>
<td>Lucia Widow</td>
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<td>Crocethemis erythraea</td>
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<td>Trithemis arteriosa</td>
<td>Red-veined Drowning</td>
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</tr>
<tr>
<td>19</td>
<td>Trithemis dorsalis</td>
<td>Highland Dropwing</td>
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<tr>
<td>20</td>
<td>Trithemis furva</td>
<td>Navy Dropwing</td>
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<td>21</td>
<td>Trithemis kirby</td>
<td>Orange-winged Dropwing</td>
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<td>Trithemis stictica</td>
<td>Jaunty Dropwing</td>
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<tr>
<td>23</td>
<td>Zygonyx natalensis</td>
<td>Blue Cascade</td>
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<td>24</td>
<td>Zygonyx torridus</td>
<td>Ringed Cascade</td>
<td>No</td>
</tr>
<tr>
<td>25</td>
<td>Pantala flavescens</td>
<td>Pantala or Wandering Glider</td>
<td>No</td>
</tr>
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<td>26</td>
<td>Sympetrum fonscolombii</td>
<td>Nomad</td>
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<tr>
<td>27</td>
<td>Notiothemis jonesi</td>
<td>Eastern Forestwatcher</td>
<td>Yes</td>
</tr>
<tr>
<td>28</td>
<td>Ictinogomphus ferox</td>
<td>Common Tigertail</td>
<td>Yes</td>
</tr>
<tr>
<td>29</td>
<td>Acisoma panorpoides</td>
<td>Stout Pintail</td>
<td>Yes</td>
</tr>
<tr>
<td>30</td>
<td>Pantala flavescenes</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>31</td>
<td>Tramea basilaris</td>
<td>Keyhole Glider</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Appendix 2. List of frog species found in the Midlands area, as well as in this study

<table>
<thead>
<tr>
<th>No</th>
<th>Latin Name</th>
<th>Common Name</th>
<th>Habitat</th>
<th>Found in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Arthroleptis walhbergi</em></td>
<td>Bush Squeaker</td>
<td>Leaf litter</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td><em>Leptopelis xenodactylus</em> <em>EN</em></td>
<td>Long-toed Tree Frog</td>
<td>Mistbelt grassland, wetlands, marshes</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td><em>Breviceps adsersus</em></td>
<td>Bushveld Rain Frog</td>
<td>Fossorial, Grassland</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td><em>Breviceps bagginsi</em></td>
<td>Bilbo’s Rain Frog</td>
<td>Fossorial, Grassland, Grassy verges</td>
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<tr>
<td>5</td>
<td><em>Breviceps verrucosus</em></td>
<td>Plaintive Rain Frog</td>
<td>Fossorial, Forest, Grassland, Gardens</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td><em>Amietophrynus gutturalis</em></td>
<td>Guttural Toad</td>
<td>Ponds, dams, gardens</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td><em>Amietophrynus rangeri</em></td>
<td>Raucous Toad</td>
<td>Rivers, streams, dams, grassland</td>
<td>No</td>
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<td>8</td>
<td><em>Hadromophryne natalensis</em></td>
<td>Cascade Frog</td>
<td>Rocky streams, Forest, Grassland</td>
<td>No</td>
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<td>9</td>
<td><em>Afrixalus spinifrons intermedius</em> <em>NT</em></td>
<td>Natal Leaf-folding Frog</td>
<td>Wetlands</td>
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<td>10</td>
<td><em>Hyperolius marmoratus marmoratus</em></td>
<td>Painted Reed Frog</td>
<td>Wetlands, ponds</td>
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<td><em>Hyperolius pusillus</em></td>
<td>Water-lily Reed Frog</td>
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<td>12</td>
<td><em>Hyperolius semidiscus</em></td>
<td>Yellow-striped Reed Frog</td>
<td>Pans, pools, dams, savanna</td>
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<td>13</td>
<td><em>Kassina senegalensis</em></td>
<td>Bubbling Kassina</td>
<td>Ponds, gardens</td>
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<td>14</td>
<td><em>Semnodactylus wealli</em></td>
<td>Rattling Frog</td>
<td>Pans, vleis, grassland</td>
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<td>15</td>
<td><em>Phrynobatrachus natalensis</em></td>
<td>Snoring Puddle Frog</td>
<td>Open water, farmland</td>
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<td><em>Ptychadena oxyrhynchus</em></td>
<td>Sharp-nosed Grass Frog</td>
<td>woodland, Grassland, savanna, vleis, pans</td>
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<td><em>Xenopus laevis</em></td>
<td>Common Platanna</td>
<td>Any aquatic habitats</td>
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<td><em>Anhydrophryne hewitti</em></td>
<td>Natal Chirping Frog</td>
<td>Forest, Streams, Leaf-litter, Moss</td>
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<td><em>Anhydrophryne ngongoniensis</em> <em>EN</em></td>
<td>Mistbelt Chirping Frog</td>
<td>Mistbelt Grassland, Afromontane Forest</td>
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<td>20</td>
<td><em>Cacosternum boettgeri</em></td>
<td>Boettger’s Caco</td>
<td>Grassland, Thicket, Vleis, Farmland, Open areas</td>
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<td><em>Cacosternum nanum</em></td>
<td>Dwarf / Bronze Caco</td>
<td>Grassland, Vleis, Thicket, Forest, Ponds, Dams, Streams, Roadside puddles, Ditches</td>
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<td><em>Cacosternum rythhum</em></td>
<td>Rhythmic Caco</td>
<td>Temporary pools in grassland</td>
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<td>23</td>
<td><em>Amietia poyntoni</em></td>
<td>Poynton’s River Frog</td>
<td>Rivers, Streams, Grassland, Dams, Farmland,</td>
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<td><em>Amietia quecketti</em></td>
<td>Common River Frog</td>
<td>Ponds, Wetlands, Streams</td>
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<td>25</td>
<td><em>Strongulopus grayii</em></td>
<td>Clicking Stream Frog</td>
<td>Grassland, Forest, Streams</td>
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<td>26</td>
<td><em>Strongylopus fasciatus</em></td>
<td>Striped Stream Frog</td>
<td>Gardens, open water</td>
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<td>27</td>
<td><em>Tomopterna natalensis</em></td>
<td>Natal Sand Frog</td>
<td>farmland, shallow water</td>
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Appendix 3. List of macroinvertebrates found in this study

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<th>Number</th>
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<td>4</td>
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<td>Calopterygidae</td>
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