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The role of an emerging wetland system in nutrient assimilation

by

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Supervisor: Marius Claassen

Co-supervisor: Liesl Hill

Declaration

I, Tarryn Valentia Hattingh, declare that the dissertation, which I hereby submit for the degree Master of Science in Environmental Management at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: Hattingh DATE: 12 June 2019

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Summary

This study aimed to examine the role of an emerging wetland system in nutrient assimilation as well as the improvement of water quality. The extent of the wetland was assessed over a period of eight years using Google Earth imagery, and it was noted that permanent ponding developed over the course of the eight years. Water samples were collected upstream of the wetland, in the west and east ponds of the wetland and downstream. The chemical water quality assessment indicated that the wetland may play a role in the assimilation of phosphate. However, it was a source of sulphates to the downstream environment. Toxic effects of water quality were noted on both *Daphnia magna* and *Selenastrum capricornutum* (renamed *Raphidocelis subcapitata*). However, the wetland remains a functional part of the ecosystem, providing food sources and habitats for birds, insects, molluscs, amphibians, fish and various plant matter.

Acknowledgements

I would first and foremost like to thank my supervisors, who have been supportive and encouraging from day one. Thank you for repeating yourselves when I asked the same questions time and time again and being there to talk through ideas with me.

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Introduction

Water quality is of paramount importance throughout the world. However, South Africa faces the further challenge of being a water scarce country. South Africa receives minimal rainfall across an increasing gradient from west to east (Nicholson *et al.,* 2018). As a result of water scarcity in the region, water resources need to be maintained and protected to ensure water security. Currently, only one third of South Africa's main rivers are considered to be in good condition (Donnenfeld *et al.*, 2018). This is influenced by a host of upstream activities which often only manifest, or are recorded, downstream in rivers.

There are a range of possible sources of water contamination that ultimately leads to a decrease in water quality. This further threatens the integrity of water resources. Industrial and mining activities, discharging effluent into water systems, agriculture, population growth as well as climate change all play a role in the quality of water resources. In South Africa, a major threat to water resources is salinization (Integrated Water Resource Studies, 2014). This is largely due to poor irrigation methods and industrial and sewage effluent that often have high salinity (Flügel, 1995; Scherman *et al*., 2003). This, in conjunction with a geology in South Africa that lends itself to naturally saline water resources, and high rates of evaporation, result in high levels of saline water entering already scarce water resources (Palmer *et al.*, 2004).

Wetlands are the most threatened aquatic ecosystems in South Africa (SANBI, 2011). Wetlands provide many ecosystem services, such as flood attenuation and reduction in pollutants in water systems, in addition to the provision of cultural goods and services. As such wetland protection, maintenance and promotion is integral to ensuring South African water supply is of sufficient quantity and adequate quality (Hammer and Bastian, 1989).

Aim and objectives

The aim of this study is to assess whether the emerging wetland system in Sasolburg, Free State Province, is contributing to nutrient assimilation and the improvement of water quality. The study comprises four key objectives:

- To assess the extent of the emerging wetland
- To ascertain the water quality of the wetland system and how it changes as water moves through the wetland
- To ascertain the potential ecological impacts of the water through chemical water quality analysis and ecotoxicity testing

- To assess whether water quality, as well as the ability of the wetland to assimilate nutrients, changes seasonally.

Structure of this dissertation

The deductive model of the scientific method was followed in this investigation, and the reporting thereof (Whewell, 1989). The importance of using this process was to ensure that the work conducted followed a logical and repeatable progression, with the use of a recognised technique. This can be further explained through four goals in scientific research: description (what we know), prediction (what we think), explanations (why the prediction may hold true), and controlling the outcome through the way in which the research is used, its application or prevention, or the prediction of future phenomena etc. (Montello and Sutton, 2006). The scientific method provides a framework for scientists to analyse scientific theories and apply them to their own research. It requires the examination of current research and ideas, and the subsequent collection of data through experimentation and investigation to test existing theories. This research then informs or elaborates on present knowledge within the specific scientific field. Ecological research requires critical analysis of both what is already known, as well as the assessment of data to expand on this knowledge (Ford, 2000).

In this dissertation, the aim and objectives of the study have been defined (above). The underlying knowledge and theories regarding wetlands are expanded upon in the literature review (Chapter 1). The way in which research was conducted, through various methodologies and means of analysis, is explained in each chapter respectively. This data is then used to infer various processes within the wetland environment (with each chapter discussing individual topics). The research is then synthesised in Chapter 8, with reference to the existing knowledge. The flow diagram on the following page (Figure 1) illustrates this process, and will appear at the start of each chapter to direct the reader as to how the chapter (which is highlighted) fits into the greater understanding of the wetland environment.

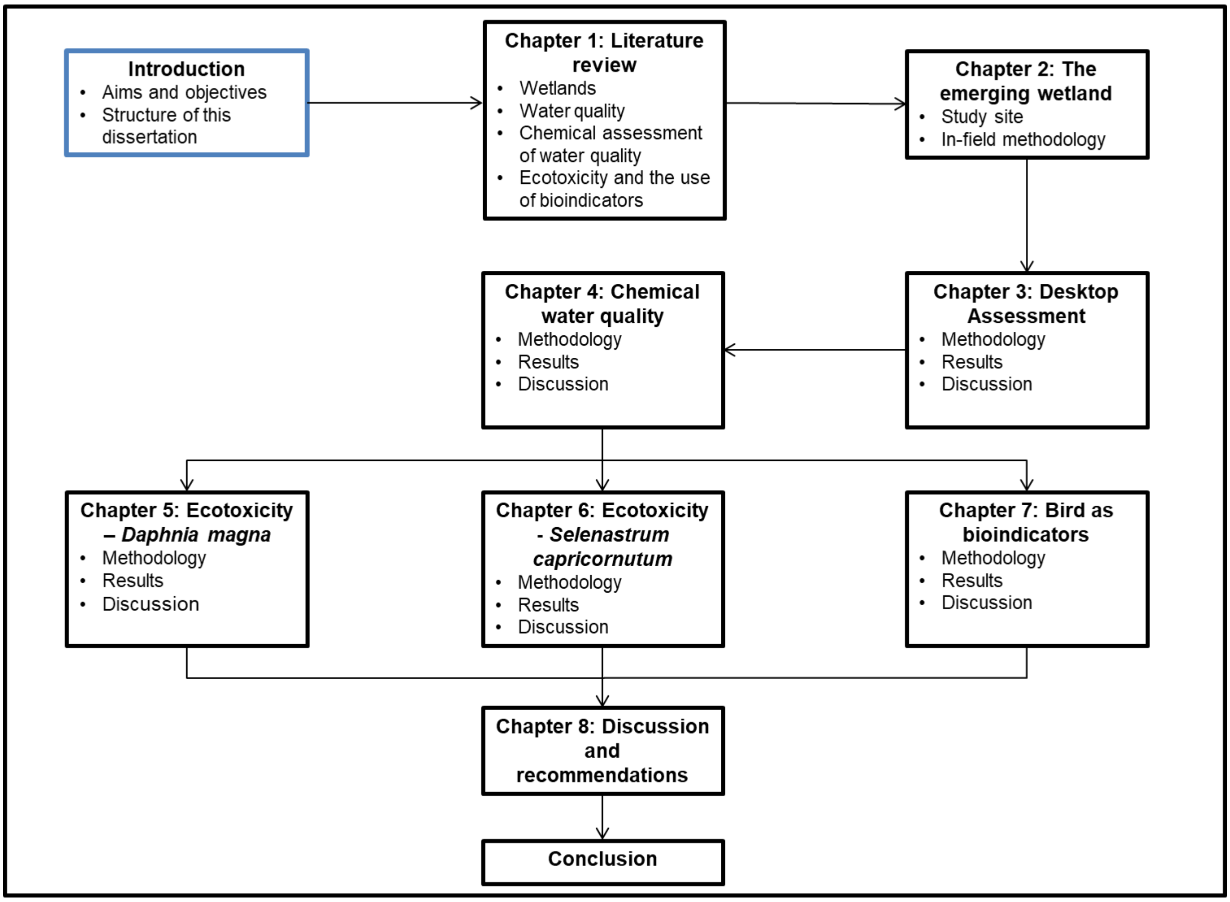

Figure 1: Structure of the dissertation

Chapter 1: Literature review

This dissertation examines the functionality of an emerging wetland system in changing water quality, specifically the changes in nutrient levels throughout the system. As such, wetland functionality and water quality indicators need to be assessed to better understand wetland dynamics and its effects on water quality. The flow diagram (Figure 2) shows the structure of the literature review which is highlighted.

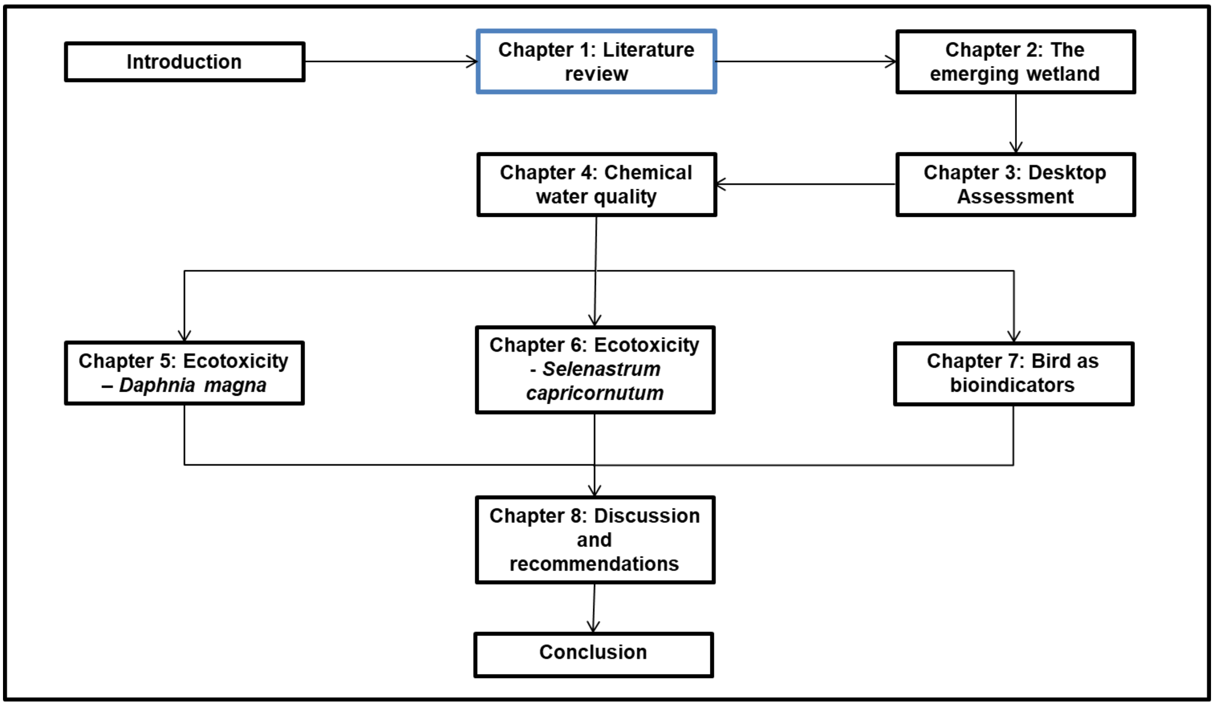

Figure 2: Structure of the dissertation - Chapter 1

Wetlands

Wetlands, according to the Ramsar Convention on Wetlands, can be defined as areas of “marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six metres”, and which are primarily controlled by water (Ramsar Convention Secretariat, 2016).

Wetlands exhibit a combination of three characteristics; wetlands have hydrophytic plants, hydromorphic soils which show anaerobic conditions (typically found in saturated settings), and a water table that results in saturation near the surface of the soil. This extended presence of water is the determining factor for the presence of hydrophytic plants and hydromorphic soils (DWAF, 2005).

The functionality of wetlands can be broadly categorised into three components. Wetlands have hydrologic functions, biogeochemical functions, and maintain habitats and sustain food webs (National Research Council (U.S.) and Committee on Characterization of Wetlands, 1995). All activities within wetlands fit into one of these three categories.

Ramsar estimates that more than a billion livelihoods are sustained in some way by wetlands (Ramsar Convention Secretariat, 2016). The key areas in which wetlands provide services are through water quality improvement, support of organisms and maintenance of biodiversity, mitigation of floods and carbon sequestration (Mitsch and Gosselink, 2000; Zedler and Kercher, 2005; Ramsar Convention Secretariat, 2016). Furthermore, wetlands are able to store both surface and subsurface waters, retain, transform and cycle nutrients and maintain plant and animal communities (National Research Council (U.S.) and Committee on Characterization of Wetlands, 1995; Mitsch *et al.*, 2009). One of the primary activities of wetlands, and principally a hydrologic function, is to process water and control runoff (DWAF, 2005; Scholz, 2006a). This is a vital operation that protects water resources, which is particularly important in South Africa which is plagued by water scarcity (DWAF, 2005). In this, and many other ways, wetland functions extend beyond the actual footprint of the wetland by influencing water quality and water levels in systems downstream. This is just one way in which the functionality of wetlands shows effects greater than that of the actual wetland footprint (National Research Council (U.S.) and Committee on Characterization of Wetlands, 1995). Wetlands form part of a greater environment, and as such can impact the greater environment. However, although wetlands can provide many functions to their direct and indirect environments they do not necessarily perform all functions (Davis, 1995).

Given that wetlands typically form the neighbouring area to surface waters, they often provide an opportune place for water filtration and transformation for the improvement of water quality before entering river systems (National Research Council (U.S.) and Committee on Characterization of Wetlands, 1995). Well-functioning wetlands accept, take-up, release and contribute to the cycling of nutrients from upstream waters (Hammer and Bastian, 1989; Reddy and DeLaune, 2008). These processes are important in understanding the changes in water chemistry and subsequent water quality.

Through various aerobic and anaerobic processes, wetlands can promote denitrification, where nitrites and nitrates are converted under anoxic conditions to produce nitrogen gas which is released into the atmosphere. This particular process is vital for protection of waterways from eutrophication. Eutrophication is characterised by the excessive growth of plant and algae due to an increase in one or more of the substances that typically limit growth (Chislock *et al.*, 2013). Nitrates are often one of the first terminal electron acceptors, which means that the redox reactions that allow the conversion of nitrates to nitrogen gas occur soon after oxygen levels are depleted in soils (Mitsch and Gosselink, 2000). It is apparent that denitrification is the preferred method of removing nitrogen from wetlands, as sequestration by algae or macrophytes does not permanently remove nitrogen from the system, especially important when the improvement of water quality to prevent eutrophication is of concern (Mitsch and Gosselink, 2000; Scholz, 2006a).

In natural environments, the main source of phosphorous in wetlands is from the weathering of minerals. However, the phosphorous concentrations found in wetlands cannot be attributed to natural processes alone but also the artificial increase due to agriculture and industry (Reddy and DeLaune, 2008). Biologically available phosphorous is in the form of ortho-phosphate. Given that the nutrient cycle for phosphorous is sedimentary and not gaseous (as nitrogen is), it generally forms complexes with organic matter, and as such is mostly inaccessible by plants (Ireland Environmental Protection Agency, 2001; Scholz, 2006a). Wetlands can form a buffer between uplands (the source of increased phosphorous loadings) and aquatic environments; however ultimate phosphorous loadings into the aquatic environment are largely dependent on the retention capacity of the wetlands acting as buffers (Reddy and DeLaune, 2008).

The cycling of sulphur in wetlands is a complex process due to the various forms of sulphur that can be present at any given time within the system. It can occur in both organic and inorganic forms, organic being present in plant tissues and animal tissues, and inorganic being in the form of pyrite, iron sulphide, hydrogen sulphide, monosulphides, sulphate and elemental sulphur (Reddy and DeLaune, 2008). Sulphur cycles in wetlands are driven by well-established oxidation-reduction reactions, with sulphur being removed from the cycle through both sedimentation and interactions with the atmosphere. Sulphur also contributes to the distinctive “bad egg” odour that is released by wetlands (Scholz, 2006a).

The ability of wetlands to function as nutrient processors and ‘nature’s kidneys’ in ecosystems has led to the creation of constructed wetlands, which mimic natural processes (Mitsch *et al.*, 2009). Moreover, wetlands have also been constructed to promote lost ecological functions due to the destruction of wetland habitats (Ghermandi *et al.*, 2010). Frequently, wetlands which are constructed for primary water quality benefits provide a host of secondary benefits not directly related to their construction, such as habitat provision for wildlife or aesthetic value in the urban environment (Scholz, 2006a).

Water quality

Water quality involves a number of aspects, with taste, smell and appearance historically providing clues as to the quality of water. Today, however, more objective methods are used which can assess levels of both microbial and chemical characteristics which influence water quality (Scholz, 2006b).

The assessment of water quality, especially in the context of complex industrial wastewater discharge, encompasses both substance-specific methodologies as well as ecotoxicology studies. The revision of general authorisations under the National Water Act defines complex industrial wastewater as water that contains “a complex mixture of substances that are difficult or impractical to chemically characterise and quantify” (RSA, 2013).

Because of the difficulty in ascertaining what substances are present in complex discharge wastewater, substance-specific assessments are often found to be lacking (Slabbert, 2004). Due to the vast number and complex nature of compounds found in discharge water, the list of hazardous substances, although comprehensive, cannot provide sufficient information as to the quality of water. Ecological toxicity effects are most often not as a result of a single substance, but rather the combination of these substances acting together (DWAF, 2003). Therefore, a range of methods need to be employed to best understand water quality and the impact thereof on aquatic ecosystems.

Chemical assessment of water quality

The chemical constituents of water can come from a variety of sources. Naturally occurring sources include chemical constituents from the weathering of rock as well as influence from climate and soils. Industrial sources and human dwellings can contribute to the addition of chemicals to aquatic environments, such as those from manufacturing, processing, solid wastes and urban runoff. Agricultural activity can contribute to the chemical make-up of aquatic environments due to fertilizers, pesticides, manure, as well as intensive farming practices. Water treatment and transport can also alter water chemistry. This can be through the addition of coagulants, the use of various piping materials and disinfection by-products. In addition, treatment may include the use of pesticides for human health concerns, such as those used for the control of insect vectors and diseases (WHO, 2017). There are a variety of chemical parameters that can be assessed which are expanded upon below:

Electrical conductivity, total dissolved solids and salinity

Electrical conductivity (µS/cm) is the measure of current conduction in a sample as a result of the ions present in the sample (Moore *et al.*, 2008). As such, electrical conductivity indicates the amount, or concentration, of total dissolved salts, or ions, present in water samples (Pal *et al.*, 2015). When the concentration of ions increases in a water sample, so does the electrical conductivity. However, it is important to note that this relationship is not linear, as the nature of the ions in solution (their concentration, charge and mobility) and their interaction with other ions can hinder their movement, and as a result, the conduction of current (Moore *et al.*, 2008).

Electrical conductivity is widely used as an indicator to determine the total dissolved solids (TDS) within a water sample (Pal *et al.*, 2015). Dissolved salts in water form ions. As such, the concentration of ions will not only affect the electrical conductivity, but also the TDS and salinity (Palmer *et al.*, 2004). All dissolved materials found in water, whether organic or inorganic, will be considered as part of the TDS measurement (Roos and Pieterse, 1995). However, salinity is the measure of the amount of inorganic salts only in a water sample (in parts per thousand or ppt). Salinity is an important indicator of water quality as most organisms are adapted to either freshwater (salinity < 5 ppt), or saline water (salinity > 35 ppt). Only some species are adapted to a wider range of salinities. Salinity is influenced by rain events, evaporation, as well as local geology and soils. It is often highest at low flow and typically decreases as water levels increase (Jeppesen et al., 2015). TDS can also be an indicator of salinity. Total dissolved solids (mg/L) values of less than 1000 mg/L indicate freshwater. TDS values above 1000 mg/L, but less than 10000 mg/L indicate brackish water. Saline water produces TDS values of between 10000 mg/L and 30000 mg/L. Brine water is considered water where the TDS value is above 30000 mg/L (Pal *et al.*, 2015).

The presence of dissolved ions in ecosystems can influence reproduction, growth and survival of different organisms. This is because most species operate within defined tolerance ranges. The response of different taxa to TDS is largely based on their specific ability to maintain internal ion-water balance. This process is energetically expensive, which can influence overall physical fitness of species (Olson and Hawkins, 2017). As a result, when there is a change (an increase or decrease) in the total dissolved solids within an ecosystem, either due to flooding, evaporation or pollution, species present in the ecosystem may not be able to endure the change.

Nutrients

Nutrients are often not viewed as pollutants. However, increased nutrient concentrations found in ecosystems often leads to reduced species diversity, and dominance of specific species (Furness, 1993). This can have cascading affects throughout an ecosystem, damaging the ecological integrity of the environment. Furthermore, nutrients can be toxic to some species in certain concentrations and aquatic conditions (Daigger, 2004). Nutrients are increasingly being introduced into aquatic systems through agricultural and industrial activity, wastewater effluents as well as increased anthropogenic pressure on water resources (Smith *et al.*, 1999; Kremser and Schnug, 2002). This presents one of the major concerns facing aquatic environments, as productivity is typically limited by nutrient availability. Although an increase in this productivity may be viewed as a positive in light of ecosystem health, significant increases in aquatic nutrient levels can lead to eutrophication (Water Environment Federation: Nutrient Removal Task Force, 2011).

Chlorophyll a

Chlorophyll *a* is the main chlorophyll type found in algae, and allows the algae to photosynthesize. Chlorophyll *a* can be used to determine the amount of algae present in the water system (Boyer *et al.*, 2009). This is important in assessing water quality as high levels of chlorophyll *a* indicate large amounts of algae in the water system. Algae can influence the clarity and the amount of oxygen available in the water, as well as the amount of food available in an ecosystem, which impacts on biological communities (Sharma *et al.*, 2016). The availability of nitrogen and phosphorous play a co-limiting role in the growth of chlorophyll *a* (Tolotti *et al.*, 2012).

pH and temperature

The pH of water sources is an important factor in water quality. The acidity or alkalinity of water is given by the pH value. This is measured as the concentration of hydrogen ions (H^+) (calculated as the negative logarithm of H^+). It influences the solubility of chemical components in the water, as well as the bioavailability thereof (Gambrell *et al*., 1991; Michaud, 1991). Temperature has a key influence on the types of organisms present in water sources as well as biological activity. It is accepted that higher temperatures generally result in greater rates of chemical reactions and biological activity, up to a point. In addition, just as species have a range of salinities in which they can survive, they also have a range of temperatures in which they can survive (Michaud, 1991). Temperature of water can affect water chemistry, with some compounds potentially being more toxic at higher water temperatures, or oxygen availability depleting at higher temperatures (Jankowski *et al.*, 2006).

Metals

Metals enter water sources through both natural and anthropogenic means (Nordberg *et al*., 2007). These numerous elements are ever present components of aquatic environments, and range from being essential to the biological functioning of organisms to being highly toxic to species. However even essential metals, such as magnesium (Mg) or calcium (Ca), can be detrimental to organism health should they occur in higher concentrations. Metals are of concern to water quality as they are persistent in the environment, can bioaccumulate and biomagnify, and may have toxic effects on organisms (Nordberg *et* al., 2007; Gheorghe *et al*., 2017).

Ecotoxicity and the use of bioindicators

Ecotoxicity is the study of how organisms, communities and ecosystems respond to, and are negatively affected by chemicals in the environment (Moiseenko, 2008). Consequently, ecotoxicity results provide the link between the physico-chemical results obtained in water quality measurements (including those discussed above, concentrations of metals and various complexes that form) and specific biological tolerances of organisms (Scherman *et al.*, 2003). Aquatic organisms are adapted to specific habitats. Therefore they reflect particular characteristics of that habitat. This is useful when attempting to ascertain the health of a system, and the ability of a system to respond to changes in the environment. Furthermore, by using ecotoxicity as an indicator for the level of conservation of different species, the reliability of the provision of their specific ecosystem services can be inferred (Marzullo *et al.*, 2018). For this reason, when doing ecotoxicity testing, it is advantageous to look at more than one trophic level. By assessing single organisms, the toxicity of the aquatic environment on that particular population can be ascertained. In order to assess the impacts of toxicants on communities, various trophic levels need to be considered (Moiseenko, 2008).

Ecotoxicity is conducted using biological indicators, which are able to show the cumulative impacts of both chemical changes to the ecosystem, as well as possible changes to the environment and habitat of the different species being studied (Maciorowski and Sims, 1981). Furthermore, by using bioindicators, the indirect effects of toxicants can be identified. This is especially possible when looking at communities within the ecosystem. Cairns Jr. *et al.* (1993) outline a number of factors that are important to consider when selecting biological indicators. Effectively, good bioindicators are those which are biologically relevant to the overall goal of monitoring and relatively easily measured. They should be responsive to change, and reflect that change in a proportionate manner. Good indicators are also abundant and well-studied (Holt and Miller, 2010).

Daphnia magna

The crustacean, *Daphnia magna*, of the order Cladocera is a species of water flea commonly used in ecotoxicity studies (Tonkopii and Iofina, 2008). They are filter-feeders, grazing on phytoplankton. For this reason, *D. magna* provide the crucial link between primary producers and fish and other invertebrate predators that feed on the water fleas (Taylor, 2010). *D. magna* are highly sensitive to toxicants and therefore make effective bioindicators (Tyagi *et al*., 2009). Their use in the assessment and monitoring of industrial effluents and chemical pollutants is widely accepted due to their broad habitat range and sensitivity, as well as their reasonably short life cycle (Persoone *et al.*, 2009). Because of their critical role in aquatic ecosystems, any negative effects on *D. magna* populations can adversely impact the functioning and structure of the aquatic ecosystem in which it operates (Taylor, 2010).

Selenastrum capricornutum

Selenastrum capricornutum (renamed *Raphidocelis subcapitata* - refer to Chapter 6 for discussion regarding the naming of the species), a species of green algae, are essential primary producers in well-functioning aquatic ecosystems (Ma *et al.*, 2006). Algae are particularly sensitive to chemical and physical modifications in their environments. Ecotoxicity testing using algae can identify not only toxicants that result in changes to abundance, but also substances that may inhibit or stimulate the growth of the algae (Dokulil, 2007). As algae occupy the lowest trophic level, changes to algal populations in aquatic systems can modify the overall structure and functioning of an ecosystem (Van Coillie *et al.*, 1983). As the primary producers of oxygen and organic material for the environment, their absence can lead to oxygen depletion and a decrease in food sources, which can lead to variations in the structure of ecosystems (Fargašová and Kizlink, 1996). These changes can be attributed to various factors, including industrial and agricultural activity (Shubert, 1984).

Birds as bioindicators

Birds make effective ecosystem indicators because they are clearly visible, and establishing presence and abundance is relatively easy (Ormerod and Tyler, 1993). As conservation has increased during the late 20th century, so too have bird populations in managed areas (Tozer *et al.*, 2018).

Availability of food resources for birds can limit the prevalence and presence of different bird species. As such, birds can be used as indicators of the occurrence of invertebrates, which have long been used as bioindicators in ecotoxicity studies. This provides further insight into the incidence of toxicants in an ecosystem without having to monitor invertebrates directly. A further advantage of using birds as indicators of invertebrates is that many sensitive areas, which often become areas of concern, have long term bird census data already available. This is seldom the case for invertebrates (Furness, 1993).

Given that birds, like many vertebrates, reflect not only singular changes in an ecosystem, but rather can be indicative of water quality as well as many other factors, using birds as indicators can be used to corroborate ecotoxicity finding from lower trophic levels (Ormerod and Tyler, 1993).

Chapter 2: The emerging wetland

The emerging wetland system that forms part of this study is discussed below, including the location of the study site, the climatic conditions present in the area, the land use directly surrounding the wetland as well as the catchment as a whole. The methodology employed in the field is also elaborated on. Figure 3 indicates the placement of Chapter 2 within the greater dissertation.

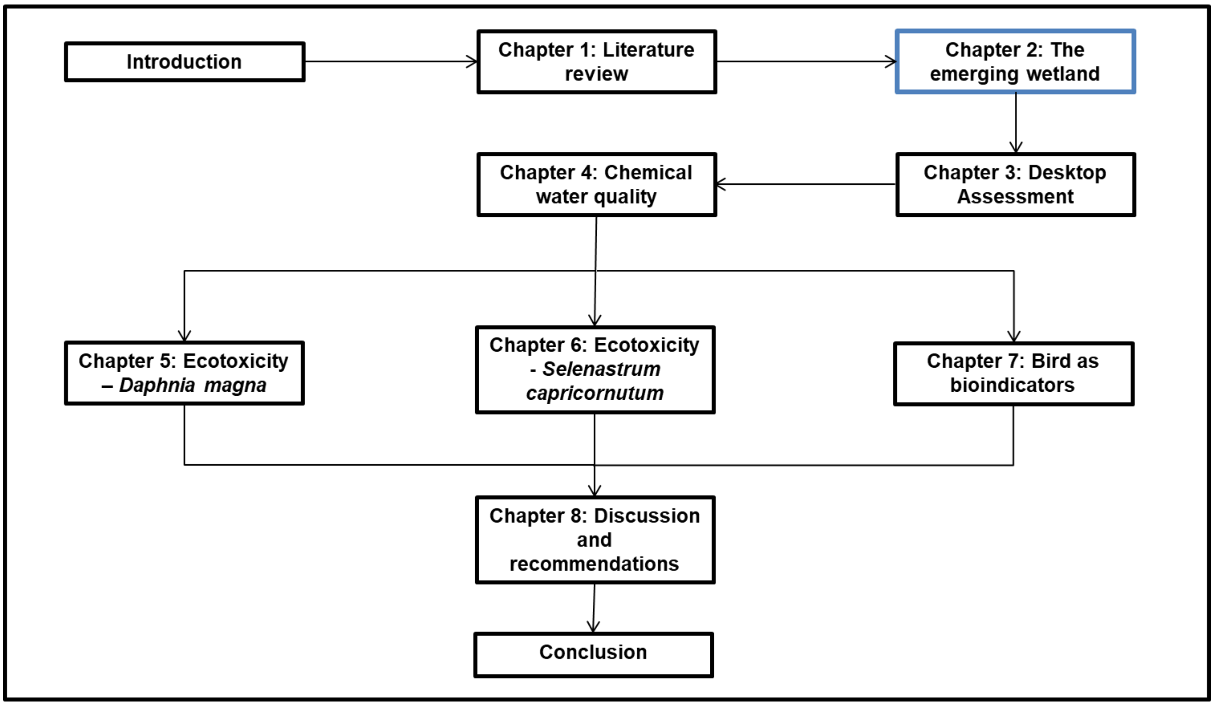

Figure 3: Structure of the dissertation - Chapter 2

Study site

Location

The emerging wetland system (26°47'24.79"S; 27°53'54.09"E) is situated in Sasolburg, on the northern edge of the Free State province in South Africa (Figure 4).

Sasolburg was founded in the 1950s along with the development of the first Sasol plant in South Africa. As such, the town's existence is closely related to and largely reliant on the industry present in the area.

The emerging wetland is situated adjacent to the Taaibosspruit, and drains into this river system. The Taaibosspruit in turn drains into the Vaal River system. The Vaal River provides water for civil services, such as drinking water, agriculture, industry and mining (Jury, 2016; Groffen *et al.*, 2018).

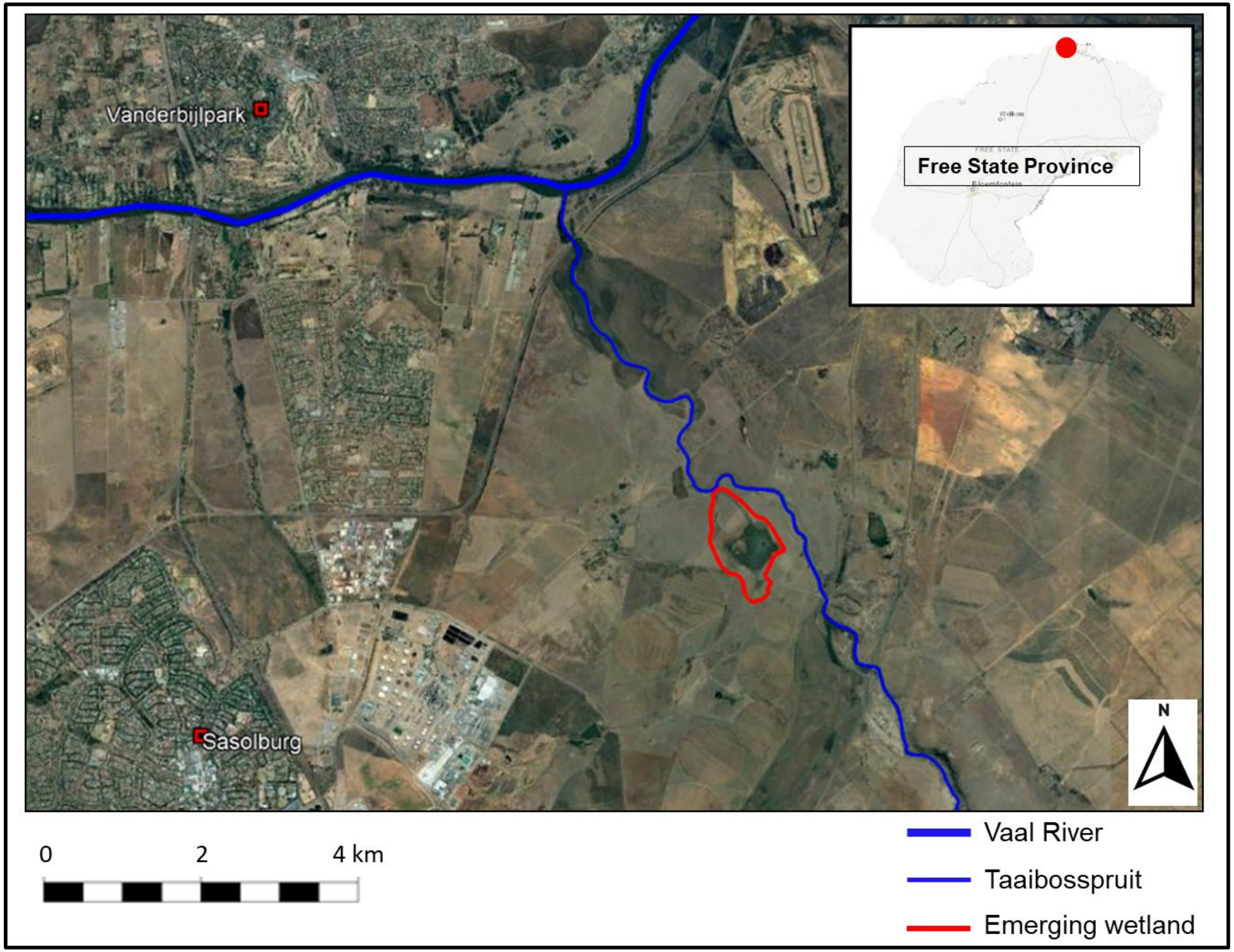

Figure 4: Map of the emerging wetland system

Climate

Sasolburg is situated in a summer rainfall area. It is approximately 1500 m above sea level, with a mild climate. The average precipitation is approximately 659 mm per annum, with the most rain falling in the summer months, particularly December and January. Highest temperatures generally occur in January (±21.5°C) and the coldest month is typically June (±9.2°C) (CLIMATE-DATA.ORG, 2019).

Land use

The land surrounding the wetland is currently predominantly used for grazing of cattle. The land upstream of the wetland and adjacent to the properties that the wetland is situated on is cultivated. Various agricultural activities are present along the Taaibosspruit downstream of the wetland.

Catchment activities

The catchment is characterised by a range of industrial and agricultural activities. However, most prevalent are the numerous chemical industries, including inland crude oil refining, the production of catalysts, fertilisers, and mining chemicals and high-density polyethylene and polypropylene manufacturing, amongst many others.

The emerging wetland system (seen in Figure 4 and Figure 5) is subject to inputs of complex industrial wastewater from a number of sources within the catchment. Over time the wetland has become a permanent feature of the landscape, being inundated with water (or flooded) throughout the year. This is likely due to constant wastewater discharge from upstream industry, thereby ensuring the presence of water in the system even during the dry season.

In-field methodology

Field work was conducted at the Sasolburg emerging wetland system (26°47'24.79"S; 27°53'54.09"E). It consisted of collecting water samples as well as the observation of birdlife present at the wetland. Water samples were collected in both the winter and summer seasons, whilst birdlife observation were done on a monthly basis for a six month period, from August 2018 to January 2019. The birdlife survey methodology will be expanded on in Chapter 7.

Four sample sites were selected to represent the various areas of the wetland. Figure 5 shows the emerging wetland system. Highlighted are the four sample sites that were used for the investigation. These four sites allow for the sampling of water from the input point of the wetland (A – upstream), the water within the wetland (B – west pond; C – east pond), as well as water from the output point of the wetland (D - downstream).

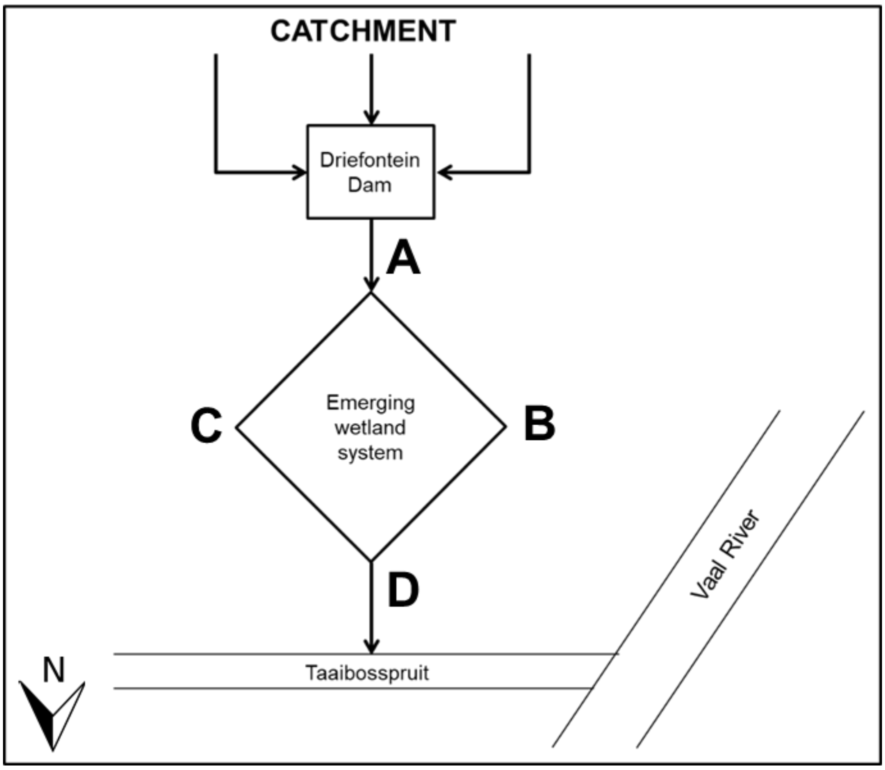

Figure 5: Schematic showing water flow in the region surrounding the wetland

The winter sampling effort took place on the morning of 20/08/2018. The summer sampling effort took place on the morning of 03/12/2018. Water samples were collected in sterile one litre sample bottles. Samples were collected in such a way so that the sediment was disturbed as little as possible. Disturbing sediment may impact on turbidity that was not naturally present in the water. To this end, sampling at points A and D, where water was actively flowing, were done at points where the water had uniform flow (sufficiently far above the divergence and below the confluence of the wetland) and is of sufficient depth to collect water without disturbing the surrounding environment (USGS, 2005). Sampling of points B and C, where water is largely stagnant or ponded, were inaccessible by foot. As such these samples were taken from the centre of the pond using a weighted sampling bottle attached to an unmanned aerial vehicle (UAV) (Koparan *et al.*, 2018).

The sampling sites were located as follows:

- A - upstream – 26°47'35.81"S; 27°53'55.68"E
- B - west pond – 26°47'19.72"S; 27°53'39.08"E
- C - east pond – 26°47'22.06"S; 27°54'1.04"E

- D - downstream – 26°47'2.44"S; 27°53'44.45"E

Water samples were labelled and stored in a cooler box for transport, and subsequently refrigerated before analysis took place in the CSIR laboratory.

It was noted that during the winter sampling, the west pond was almost entirely isolated from the rest of the wetland system. Although water from the wetland was flowing through the west pond, it was largely stagnant. The water was also highly turbid, likely due to the presence of algae in the water.

In contrast, the west pond was not as isolated from the wetland system during the summer sampling. The visual turbidity of the water sample from the west pond was consistent with the other three samples taken during the summer season.

Chapter 3: Desktop assessment

The desktop assessment encompasses a high-level investigation into the ever-changing presence of the wetland over time. The flow diagram below (Figure 6) highlights the role of the desktop assessment in this dissertation.

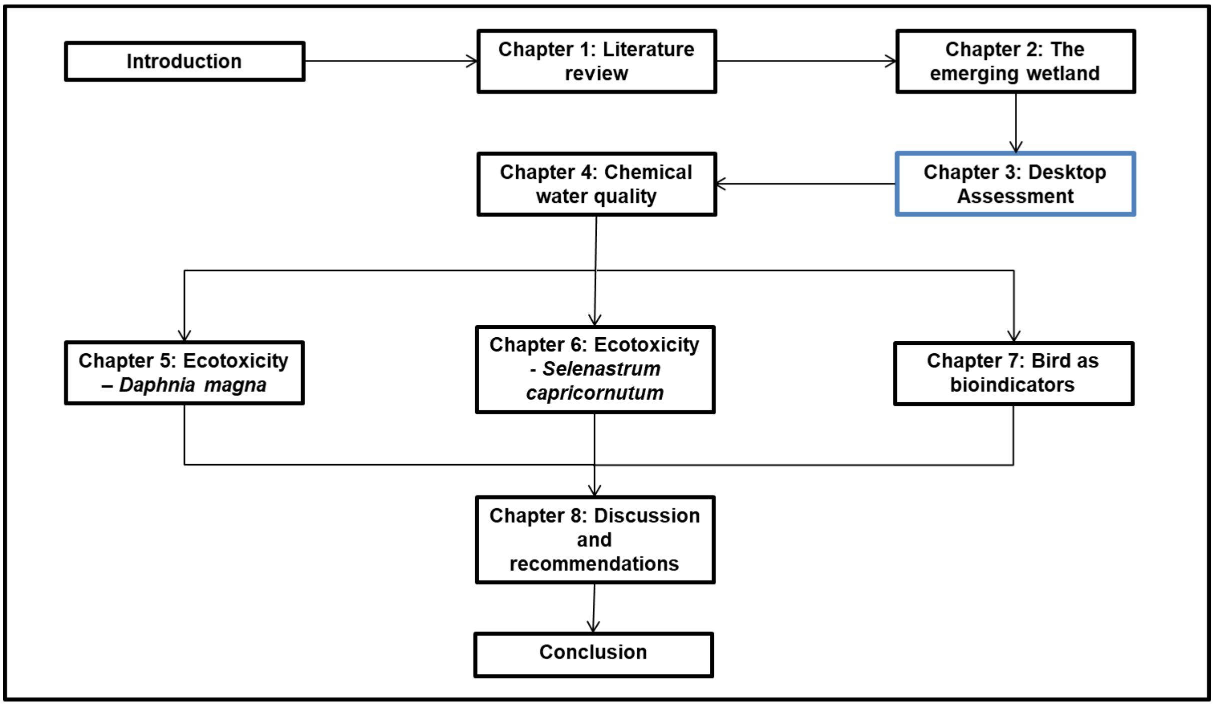

Figure 6: Structure of the dissertation - Chapter 3

Methodology

The extent of the emerging wetland was assessed using Google Earth imagery. This was examined over a period of eight years, from the first imagery in 2010 to the most recent satellite imagery in 2018. This allows for greater understanding of the development of the wetland over time.

Data analysis

The imagery was examined for changes to the extent of the wetland over time, as well as seasonal changes.

Results

The different satellite images of the wetland are shown below in Figures 7 to 12, with a total of eight years being chronicled.

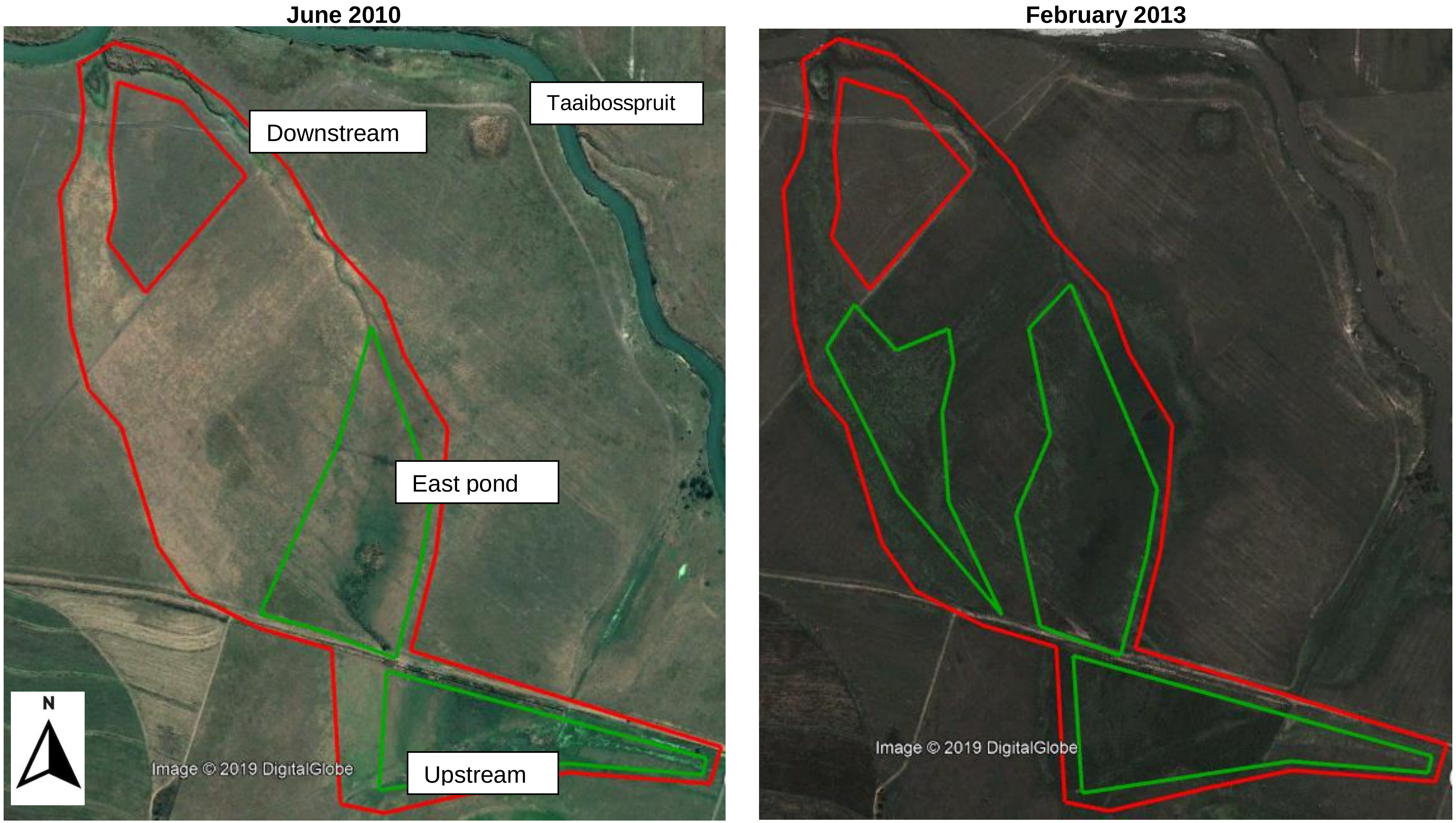

Figure 7: The wetland in 2010 and 2013

August 2014

December 2014

Figure 8: The wetland in 2014

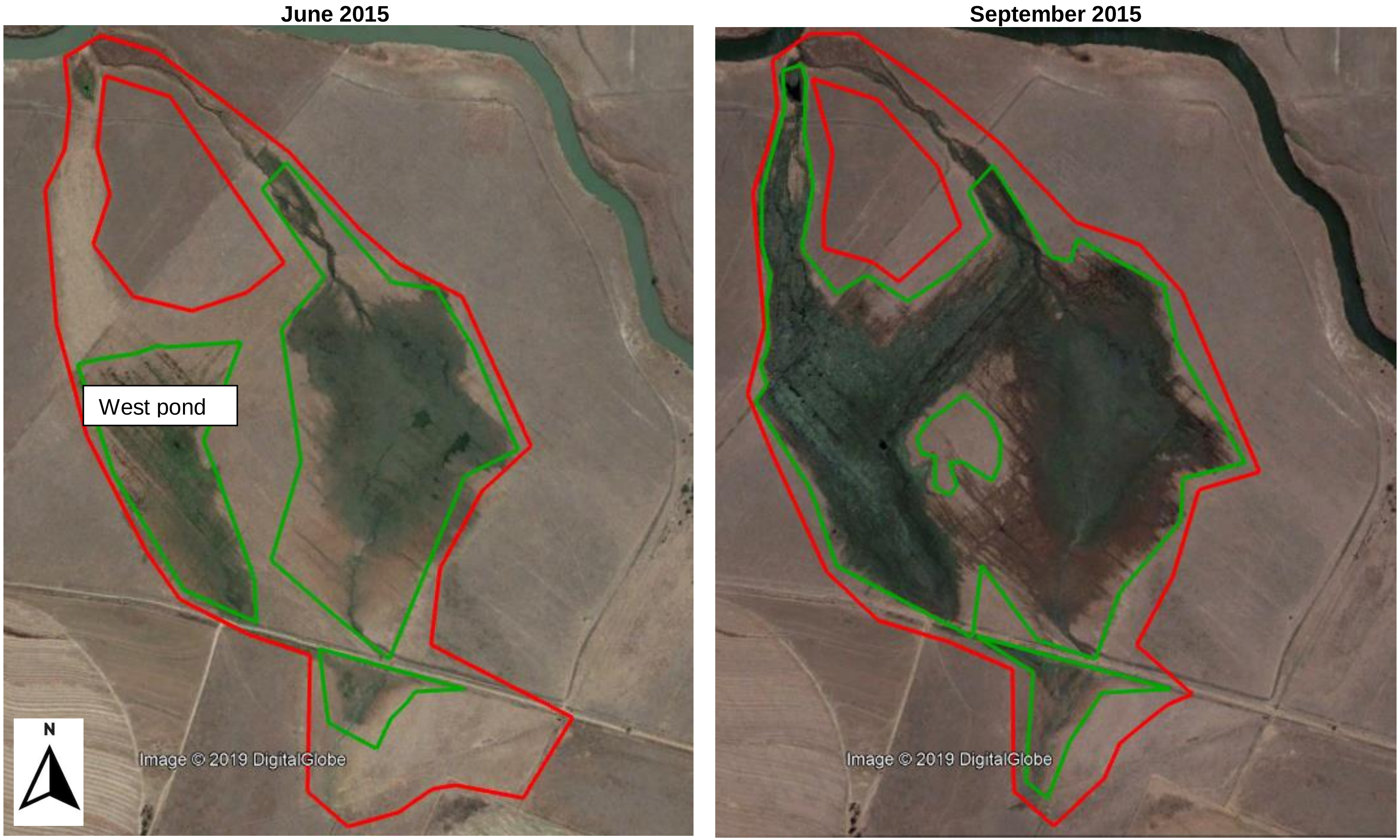

Figure 9: The wetland in 2015

May 2016 **August 2016**

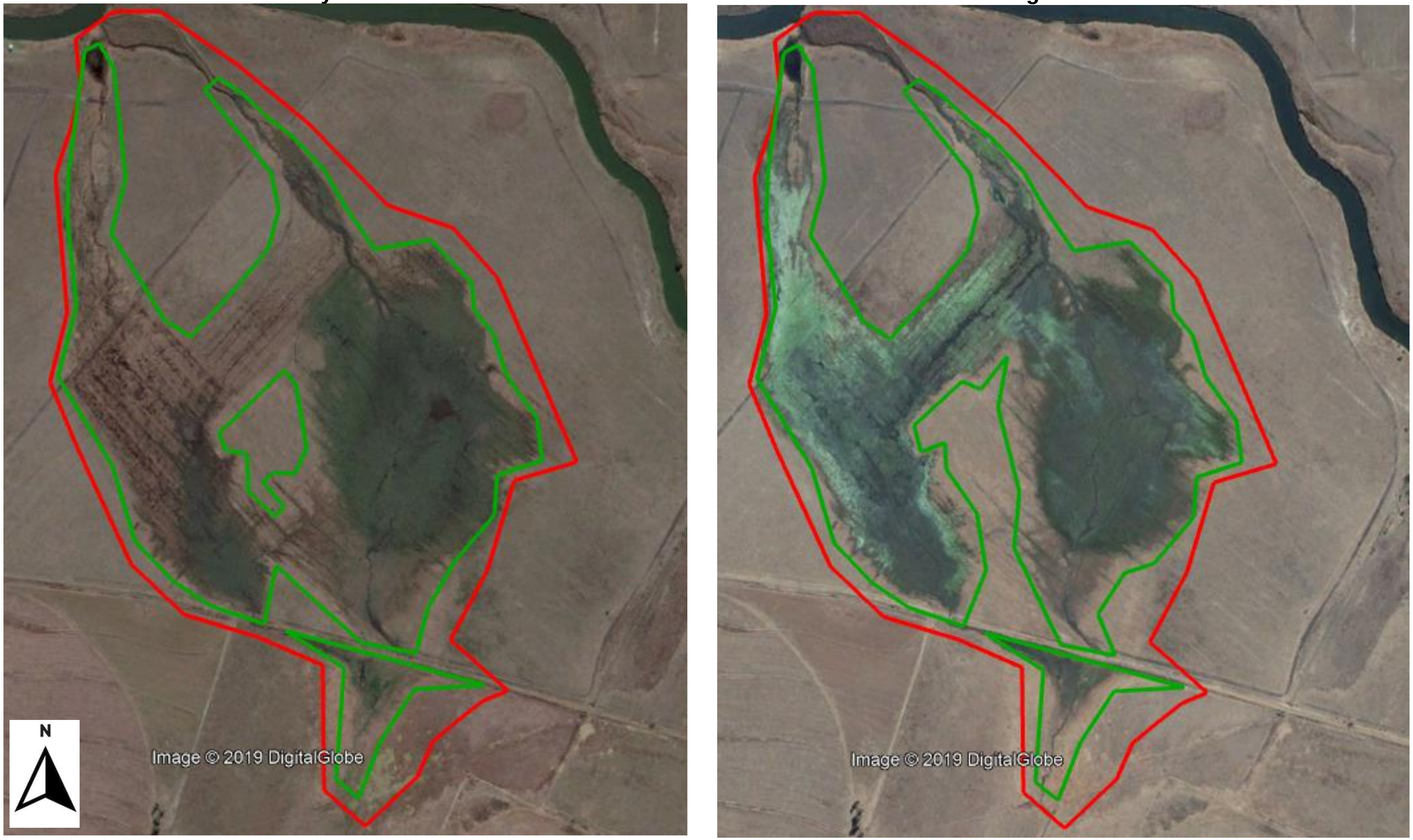

Figure 10: The wetland in 2016

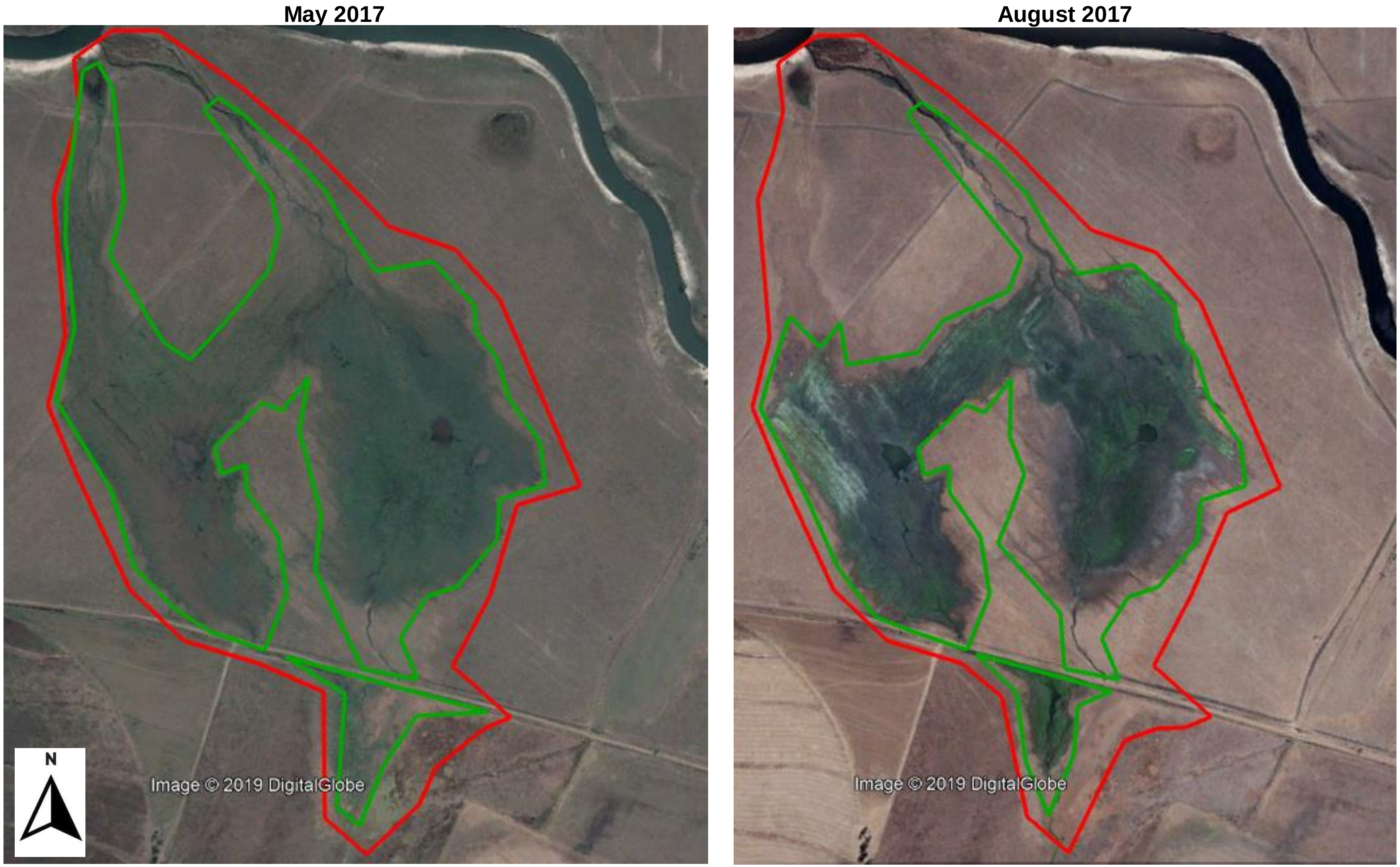

Figure 11: The wetland in 2017

Figure 12: The wetland in 2018

The wetland area, outlined in red in all the satellite images, depicts the general wetland area, where there is evidence of wetting at some point in time. The actual wetted zone was outlined in green.

In 2010, the inundated area of the wetland amounted to 20 ha. This was made up of two separate wetland zones. As can be seen, the wetland drains into the Taaibosspruit in one area. The east pond is also identifiable, and is indicated on Figure 7 along with the upstream and downstream points. The Taaibosspruit is also shown.

The next satellite imagery available on Google Earth is in 2013. The wetted zone in February of 2013 totalled 31 ha. It was made up of three separate wetland areas.

In 2014, a winter (August) satellite image and summer (December) satellite image was available. The inundated zone in winter was significantly smaller than that of the summer zone, from 19 ha in winter to 35.3 ha in summer.

June 2015 (winter) illustrates the development of the west pond (indicated on Figure 9). Once again, the wetted zone is larger in summer (September 2015) than in winter. The sizes of the inundated areas are 30.9 ha in winter and 47.5 ha in summer. The two separate wetland areas above the road crossing merge in the summer of 2015 to form a continuous wetland area.

In 2016 the previous years' merging remains visible. The east pond is more clearly developed in May of 2016. May and August both occur in the winter season, however it can be seen that the wetland's wetted zone decreases from May 2016 to August 2016.

In August of 2017, both the west and east pond can be clearly seen. Once again, a clear seasonality to the inundated wetland area can be seen, with a reduction in area as the winter season continues.

The year of the beginning of this study, 2018, provided a clear indication of three ponds; two ponds to the east and one to the west. Interestingly, in September the green colouration of the west pond is in accordance with the samples collected from the west pond which were distinctly green in colour.

Discussion

The results of the desktop study indicate that the wetland has changed significantly over the past eight years. This is evidenced by the development of ponds and the changes in the way water moves through the wetland. Wetlands have been shown to change in extent, from increasing in size when more water from the catchment is input into the system, to decreasing in size due to upper catchment changes and development (Zhuang *et al.*, 2015; Mellink *et al.*, 2018).

Changes to extent were accompanied by changes to shape and water movement. A clear seasonality was seen in the satellite imagery. In the summer months, which coincide with the rainfall season in Sasolburg, the wetland increased in extent. In the winter months, the wetlands extent decreased noticeably.

Wetlands are dynamic systems which are reflective of their broader environment. Changes in wetland extent can have an impact on the way in which they function, as hydrology is often times impacted by these changes in extent (Zhuang *et al.*, 2015; Mellink *et al.*, 2018).

Chapter 4: Chemical water quality

Three different methodologies were used to gather data on the chemical water quality of the wetland. By looking at a number of different methods of chemical water quality assessment, insight into various methodologies could be gained. Water quality examination is done for a number of different reasons. It can be done for monitoring, which is a long term effort to define the status of the aquatic environment and identify trends in water quality. It can also be done in terms of surveillance, to direct management and operational activities. Lastly, water quality can be surveyed, usually done as a finite effort to measure water quality for a specific purpose (Meybeck *et al.*, 1996). It is often conducted using a number of different available technologies, and is largely dependent on the type of monitoring or assessment required, the cost involved as well as the time available for assessment. The figure below (Figure 13) gives an indication of the examination of chemical water quality in relation to the study as a whole.

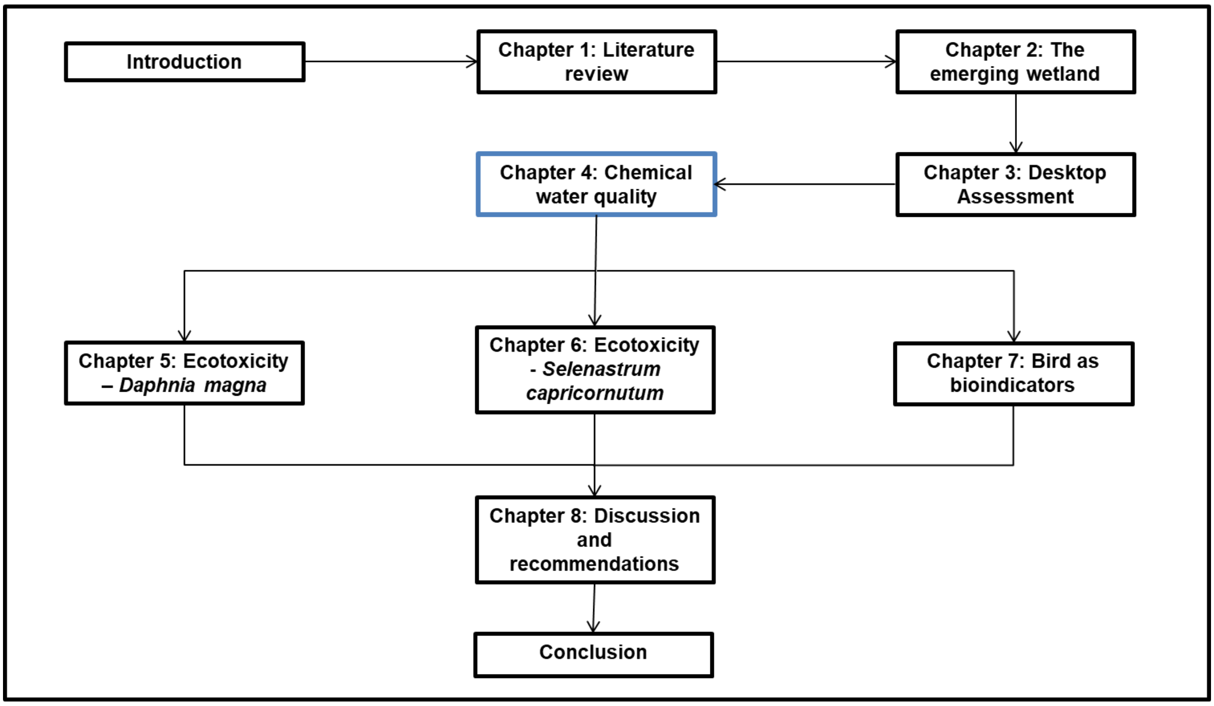

Figure 13: Structure of this dissertation - Chapter 4

Methodology

The analysis of the physico-chemical parameters was conducted using three separate methods, i.e. the use of the Hydrolab DS5, the utilisation of a colorimeter, as well as third party laboratory analysis.

Hydrolab DS5

With the use of the HYDROLAB® DS5, pH, electrical conductivity (EC), salinity, nitrate concentration, chlorophyll and phycocyanin concentrations were determined. The upstream sample was placed in the test chamber and the probe inserted into the sample. The measurements were recorded. The sample chamber was rinsed with clean water and the process repeated for the other three samples (east pond, west pond and downstream). This was done for the winter sample only.

Colorimetric analysis

The colorimetric analysis was done using a portable colorimeter (HACH® DR/890 Colorimeter), which assesses the concentration of various compounds or elements with the aid of a colour reagent (Hach, 2013 and Nikolov et al., 2016). Concentrations of sulphate (SO_4), phosphorous (PO_4^{3-}), nitrate (NO_3^- N) and nitrite (NO_2^- N) were measured for the winter sampling effort. All four samples (upstream, east pond, west pond and downstream samples) were centrifuged. Thereafter the samples were filtered through a 0.22 µm filter in preparation for the below tests.

To determine the concentration of sulphate (SO_4) in the water samples the SulfaVer4 Method (US EPA accepted method for reporting wastewater analysis) or method 8051 in accordance with the HACH® DR/890 Colorimeter Procedures Manual was used (Hach, 2013). This procedure was repeated for each of the samples in order to assess sulphate concentration.

Phosphorous (or orthophosphate) concentration was measured using the Ascorbic Acid or PhosVer3 Method (method 8048). This method is equivalent to the US EPA method 365.2 for wastewater (Hach, 2013). This method was used for all four samples to determine the concentration of orthophosphate in the water.

The concentration of nitrate (NO_3^- N) was determined using the Cadmium Reduction Method (method 8039), as stipulated in the HACH® DR/890 Colorimeter Procedures Manual (Hach, 2013). This high range test was conducted with the use of a NitraVer5 Nitrate Reagent Power Pillow. This was repeated for all four water samples, and the nitrate concentration was recorded.

In order to test for the concentration of nitrite (NO_2^- N) a low rage Diazotization Method (approved by the US EPA for reporting wastewater analyses), in accordance with the HACH® DR/890 Colorimeter Procedures Manual (Hach, 2013). This method (method 8507) makes use of the NitriVer 3 Nitrate Reagent Powder Pillow. All four water samples were assessed with the use of this method.

CSIR Water Quality Laboratory

The summer water samples were analysed by the CSIR Water Quality Laboratory in Stellenbosch, which is SANAS (South African National Accreditation System) accredited. Water was analysed for concentrations of potassium, sodium (Na^{+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), ammonia (NH_3), sulphate (SO_4^{-2}), chloride (Cl^{-}), nitrates (NO_3^{-}), orthophosphates (PO_4^{-3}) and total dissolved salts. Also included in the analysis were the concentrations of cations and anions found in each water sample.

Data analysis

The results of each of the above assessments were examined to identify changes in the aforementioned parameters from the inflow point of the wetland to the outflow point.

The results from the three above analyses were compared to both national and international water quality guidelines. This was done by ascertaining the skewness of the data (upstream, downstream, west pond and east pond) for each parameter and then selecting the most appropriate measure of central tendency of each water quality parameter. If the data was deemed to be skew (skewness <-0.5 or >0.5) the most appropriate measure of centrality was the median. If the data was calculated to be negligibly skew or not skew (-0.5 < skewness < 0.5), then the mean was used to indicate central tendency.

National water quality guidelines have been prescribed by the Department of Water Affairs and Forestry (DWAF, 1996a-e) for a host of different uses. This study compares the chemical water quality at the wetland to that of South Africa's domestic water use limits, category 4 industrial water use process limits, agriculture limits (both for irrigation and livestock rearing) as well as the water quality guidelines for aquatic ecosystems. The international guidelines used were the World Health Organisation Water Quality Guidelines (WHO, 2017).

Domestic water use is defined as water that is used within the domestic environment, and includes drinking water, food preparation, water for personal use (bathing and hygiene), as well as water for washing and laundry and garden use (DWAF, 1996a). Category 4 industrial water use processes, as outlined in the DWAF Water Quality Guideline Volume 3, are defined as processes that, within reason, can make use of any quality of water without creating problems or the need to further treat the water before use (DWAF, 1996b). Irrigation water is water used for the watering of crops and plants over and above rainwater. Included here is water for commercial crops, irrigation application and distribution schemes, home gardening, floricultural crops and potted plants (DWAF, 1996c). Livestock watering requires a large range of different water qualities due to the vastly diverse nature of differing production schemes. As such, the no effect target water quality is such that there should be no impact on any livestock in terms of toxicological effect or palatability of the water, no matter the species (DWAF, 1996d). Aquatic ecosystems are considered the foundation to which all other water sources are derived, and as such need to be protected as an important water resource (DWAF, 1996e). All values considered from the guidelines are for within target water quality ranges.

The cation and anion results from the laboratory analysis were examined for their relative concentrations and major constituents.

Results

Colorimeter

Colorimetric analysis was conducted within seven days of the collection of the winter water sample. The assessment included nitrate and nitrite, phosphorous and sulphate concentration analysis. The results are shown below (Table 1).

Table 1: Results of the colorimetric analysis of the winter sample

Parameter measured (mg/L)	**Upstream**	**West pond**	**East pond**	**Downstream**
NO_3^- - N (nitrate)	286	287	280	254
NO_2^- - N (nitrite)	0,89	4,2	3,17	8,6
PO_4^{3-} (phosphate)	162	87	183	104
SO_4^{2-} (sulphate)	190	440	190	500

As can be seen above, nitrate concentration decreases by 11.2% from the inflow point to the outflow point of the wetland. That being said, nitrate concentration remained relatively consistent throughout the wetland, with a notable decrease at the downstream sample point. Nitrite, in contrast, increases through the wetland, with the highest concentration of nitrite being found at the downstream sample point. Phosphorous decreases by 35.8% throughout the wetland. The lowest phosphorous concentration was found in the west pond. Sulphate increased from in the inflow to the outflow of the wetland, and as with nitrite the highest concentration was found at the downstream sample point.

Hydrolab

The Hydrolab analysis was conducted within 72 hours of the winter water samples being collected. The outcome of the analysis is tabulated below (Table 2).

The measurement of pH indicated that the pH remained relatively consistent throughout the wetland. Electrical conductivity (EC) maintained the same consistency; however a gradual increase in EC can be seen throughout the wetland, with a noteworthy increase of 36.39% from the inflow to outflow of the wetland. Salinity and total dissolved solid concentrations follow the same pattern as that of EC. Nitrate measurements indicate that the concentration increases throughout the wetland by 44%. Chlorophyll *a* was found to be highest in the west pond, and lowest downstream.

Table 2: Results of the Hydrolab analysis of the winter sample

Parameter measured (mg/L unless otherwise stated)	**Upstream**	**West pond**	**East pond**	**Downstream**
pH (pH units)	7,12	7,34	7,16	7,13
Electrical conductivity (µS/cm)	6015	6524	6769	8204
Salinity (ppt)	3,32	3,62	3,76	4,59
Total dissolved solids	3800	4200	4300	5300
NO_3^- (nitrate)	706,7	762,83	834,1	1018,95
Chlorophyll *a*	270,05	460,75	152,72	19,09
Phyco-cyanin (cells/mL)	0	0	0	0

Laboratory analysis

Laboratory analysis was completed on the 31/01/2019 on the summer water sample. The results are tabulated below (Table 3).

Table 3: Results of laboratory analysis of the summer sample

Parameter measured (mg/L)	**Upstream**	**West pond**	**East pond**	**Downstream**
Potassium as K Dissolved	67.0	89.00	103.0	122.0
Sodium as Na Dissolved	39.0	54.0	75.0	82.0
Calcium as Ca Dissolved	128.0	177.0	227.0	218.0
Magnesium as Mg Dissolved	35.0	45.0	60.0	64.0
Ammonia as N	558.0	651.0	572.0	515.0
Sulphate as SO_4 Dissolved	189.0	234.0	332.0	344.0
Chloride as Cl Dissolved	100.0	111.0	173.0	182.0
Nitrate + Nitrite as N	626.0	772.0	589.0	613.0
Ortho-phosphate as P	39.0	32.0	17.0	8.7
Total dissolved salts (Calc)	3968.0	4736.0	4480.0	4352.0

The major cation concentrations (potassium, sodium, calcium and magnesium) all increase as water moves through the wetland. Ammonia concentrations remained consistent, with the exception of the west pond which was found to be considerably higher than the other sample points. Of the major anions measured (chloride and sulphate), both concentrations increased from the inflow to outflow point of the wetland. The concentration of nitrate and nitrite, measured together, indicates a slight decrease in concentration through the wetland (2% decrease). However the highest nitrate and nitrite concentration was found in the west pond.

The cation and anion concentrations (meq/L) were also assessed during the laboratory analysis. The relative concentration of the ions, as well as total cation and anion presence has been tabulated in Table 7. This is illustrated below (Figure 14).

The dominant cation throughout the wetland system is calcium, followed by magnesium. All cations show an increasing trend as water moves through the wetland. Of the three major anions, only sulphate and chloride were measured. The concentration of sulphate was greater than that of chloride, and the presence of anions increases from the inflow point of the wetland to the outflow point. The west pond contained marginally higher concentrations of both anions and cations.

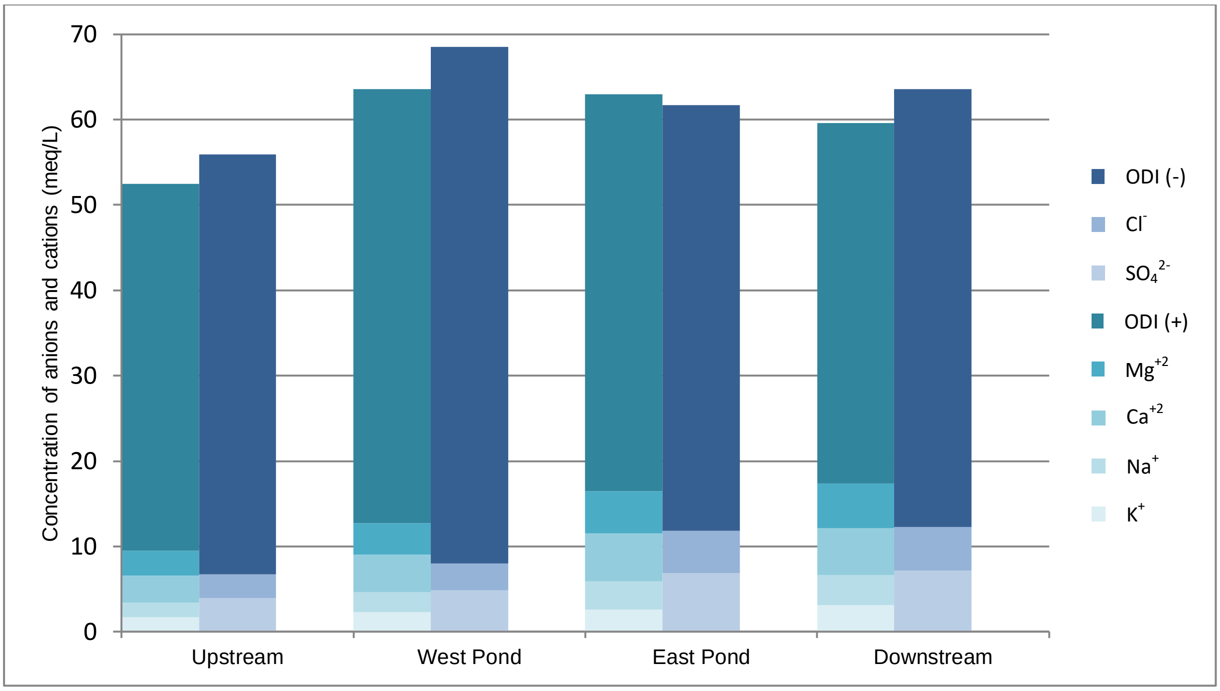

Figure 14: Major cations and anions in the wetland during summer (ODI – other dissolved ions, either cation/anions).

Comparison to guideline values

According to the colorimetric assessment and guideline values, depicted in Table 4, all measured chemical water quality parameters exceeded at least one water quality limit as set by the guidelines. The nitrate concentration exceeded all available limits, that being for livestock watering and for drinking water according to the World Health Organisation. The median concentration of nitrite exceeded the WHO limit by 0.685 mg/L. The combined nitrate and nitrite value exceeded domestic water use limits by over 47 times. The only available ortho-phosphate limit was for aquatic environments. The winter water sample exceeded the stipulated concentration. The sulphate concentration (at 330 mg/L) meant that it was not suitable for drinking water; however it may be suitable for category 4 industrial uses and livestock watering.

The results of the hydrolab analysis and comparison to guideline values are shown in Table 5. The pH measurement complied with all water use limits. The electrical conductivity, temperature compensated to 25°C, was substantially above all available compliance limits. The domestic, category 4 industrial, irrigation and livestock watering uses were exceeded by the total dissolved solid measurement. The value prescribed by aquatic environmental water use guideline states that the TDS measurement should not be a greater than 15% change from natural background conditions. These background conditions were not established, and thus a comparison could not be made. The nitrate concentration of 798.47 mg/L was 7.98 times greater than the livestock watering limit and 15.97 times greater than the WHO drinking water limit. The chlorophyll *a* limit of less than 1 mg/L for drinking water was exceeded by the winter water sample. However, phyco-cyanin is well within all given bounds.

The results from the laboratory analysis, along with guideline values have been tabulated (Table 6). The concentration of potassium exceeded the only available target water quality limit, for domestic water use. Sodium concentration was below all available compliance limits. Calcium, magnesium and sulphate values were suitable for livestock watering, and sulphate was also suitable for industrial use; however they were not considered suitable for drinking water. Ammonia levels were non-compliant to the two available limits, i.e. domestic use and livestock watering. Chloride levels in the water sample exceeded drinking and irrigation water limits. Nitrate and nitrite levels exceeded the drinking water target water quality by 103 times, and ortho-phosphate was non-compliant with aquatic water limits (the only available limit for ortho-phosphate). The concentration of total dissolved salts was greater than all national water quality limits, with the exception of aquatic environmental limits which requires water Total Dissolved Salts to differ by no more than 15% from natural conditions. As this value was not determined, a comparison could not be drawn.

Not all parameters measured during the chemical analysis were included in the guidelines, and as such could not be compared to stipulated concentrations, for example the measure of salinity (Table 5).

Table 4: Results (mean/median) from colorimetric assessment of the winter samples compared to guideline values

Parameter measured (mg/L)	**Median (M) or Mean ($\bar{x}$)**	**National water quality guidelines**					**International water quality guidelines**
		Domestic water use (DWAF, 1996a)	**Industrial water use (DWAF, 1996b)**	**Irrigation (DWAF, 1996c)**	**Livestock watering (DWAF, 1996d)**	**Aquatic ecosystems (DWAF, 1996e)**	**World Health Organisation (WHO, 2017)**
NO_3^- - N (nitrate)	M = 283	≤ 6	-	-	≤ 100	-	≤ 50
NO_2^- - N (nitrite)	M = 3.685		-	-	-	-	≤ 3
PO_4^{3-} (phosphate)	$\bar{x}$ = 134	-	-	-	-	< 5	-
SO_4^{2-} (sulphate)	$\bar{x}$ = 330	≤ 200	≤ 500	-	≤ 1000	-	-

Table 5: Results (mean/median) from Hydrolab analysis of the winter samples compared to guideline values

Parameter measured (mg/L unless otherwise stated)	**Median (M) or Mean ($\bar{x}$)**	**National water quality guidelines**					**International water quality guidelines**
		Domestic water use (DWAF, 1996a)	**Industrial water use (DWAF, 1996b)**	**Irrigation (DWAF, 1996c)**	**Livestock watering (DWAF, 1996d)**	**Aquatic ecosystems (DWAF, 1996e)**	**World Health Organisation (WHO, 2017)**
pH (pH units)	M = 7.145	6.0 - 9.0	5.0 - 10.0	6.5 – 8.4	-	< 5% difference from background value	-
Electrical conductivity (µS/cm)	M = 6646.5	≤ 700	≤ 2500	< 400	-	-	-
Salinity	M = 3.69	-	-	-	-	-	-
Total dissolved solids	M = 4250	≤ 450	≤ 1600	≤ 260	≤ 1000	< 15% difference from natural conditions	-
NO_3^- (nitrate)	M = 798.465	-	-	-	≤ 100	-	≤ 50
Chlorophyll *a*	$\bar{x}$ = 225.6525	≤ 1	-	-	-	-	-
Phyco-cyanin (cells/mL)	0	≤ 50	-	-	≤ 2000	-	-

Table 6: Results (mean/median) from laboratory analysis of the summer samples compared to guideline values

Parameter measured (mg/L)	**Median (M) or Mean ($\bar{x}$)**	**National water quality guidelines**					**International water quality guidelines**
		Domestic water use (DWAF, 1996a)	**Industrial water use (DWAF, 1996b)**	**Irrigation (DWAF, 1996c)**	**Livestock watering (DWAF, 1996d)**	**Aquatic ecosystems (DWAF, 1996e)**	**World Health Organisation (WHO, 2017)**
Potassium as K Dissolved	$\bar{x}$ = 95.25	≤ 50	-	-	-	-	-
Sodium as Na Dissolved	$\bar{x}$ = 62.50	≤ 100	-	≤ 70	≤ 2000	-	≤ 200
Calcium as Ca Dissolved	M = 197.50	≤ 32	-	-	≤ 1000	-	-
Magnesium as Mg Dissolved	$\bar{x}$ = 51.00	≤ 30	-	-	≤ 500	-	-
Ammonia as N	M = 565.00	≤ 1	-	-	-	≤ 7	-
Sulphate as SO4 Dissolved	$\bar{x}$ = 274.75	≤ 200	≤ 500	-	≤ 1000	-	-

Chloride as Cl Dissolved	$\bar{x}$ = 141.50	≤ 100	≤ 500	≤ 100	≤ 1500	-	≤ 250
Nitrate + Nitrite as N	M = 619.50	≤ 6	-	-	-	-	-
Ortho-phosphate as P	$\bar{x}$ = 24.18	-	-	-	-	≤ 5	-
Total dissolved salts (Calc)	M = 4416.00	≤ 450	≤ 1600	≤ 260	≤ 1000	< 15% difference from natural conditions	-

Table 7: The cation and anion concentrations for the summer sampling effort from laboratory analysis

	Upstream	**West pond**	**East pond**	**Downstream**
CATIONS (meq/L)	52.5	63.6	63.0	59.6
ANIONS (meq/L)	55.9	68.5	61.7	63.6
Abs Difference	3.35	4.84	-1.33	3.95
Relative concentrations of the major cations (meq/L)	$Ca^{2+} > Mg^{2+} > K^{+} > Na^{+}$	$Ca^{2+} > Mg^{2+} > Na^{+} > K^{+}$	$Ca^{2+} > Mg^{2+} > Na^{+} > K^{+}$	$Ca^{2+} > Mg^{2+} > Na^{+} > K^{+}$
Relative concentrations of the major anions (meq/L)	$SO_4^{2-} > Cl^{-}$	$SO_4^{2-} > Cl^{-}$	$SO_4^{2-} > Cl^{-}$	$SO_4^{2-} > Cl^{-}$

Discussion

Chapter 4 of this study examines chemical water quality through the use of three different methodologies in order to assess spatial changes in the concentration of nutrients within the wetland. Water quality is defined by the Department of Water Affairs and Forestry guidelines for water quality as the physical, chemical, biological and aesthetic characteristics of water that impact on its fitness for use, as well as for the protection of the aquatic environment (DWAF, 1996a-e). Chemical water quality refers specifically to any chemical constituents of the water, and their concentrations are often measured against guideline values.

The results of the chemical water quality assessment indicate that there are some spatial differences in nutrient concentrations throughout the wetland. This varies from nutrient to nutrient, however a number of similar trends were seen. Of main concern to this study are the major nutrients found in systems; i.e. nitrogen, phosphorous and sulphur.

Although the different methods used rendered differing results, nitrate and nitrite decreased marginally as water moved through the wetland (with the exception of the Hydrolab results that suggested nitrate concentration increased). The capability of the wetland to assimilate nitrogen is largely influenced by anaerobic processes which encourage the reduction of nitrates and nitrites and the formation of nitrogen gas (denitrification). Nitrates and nitrites are the first electron acceptors to be reduced under conditions of oxygen depletion (Mitsch and Gosselink, 2000; Collins, 2005; Scholz, 2006a). However, the nitrate concentration differs negligibly from upstream to downstream and it may indicate that the wetland is not influencing the presence or absence thereof to any substantial extent, or is only able to function as a denitrifier to a limited extent.

Phosphorous is considered a significant limiting nutrient in ecosystems, including wetlands (Mitsch and Gosselink, 2000). The results suggest that there is a decrease in phosphorous from the inflow point of the wetland to the outflow point, as shown by both the colorimetric and laboratory analysis. This could be attributed to wetland processes which convert available phosphorous into unbound forms thereof (Mitsch and Gosselink, 2000; Scholz, 2006a). Wetlands contribute to the sorption and physical burial of phosphorous through interactions with the redox potential, pH, presence of Fe, Al, Ca and phosphorous levels of the soil (Faulkner and Richardson, 1989). However, the values for phosphorous concentration need to be carefully considered as it is difficult to completely separate the analysis of bound and unbound forms of the phosphate. Notwithstanding the aforementioned caution, the concentrations of phosphates in the water sample still give an important indication of the presence of the nutrient in the water source. This is especially important when dealing with waste discharge and water management (Ireland Environmental Protection Agency, 2001).

The results of the analysis of sulphate concentration indicate that the wetland is not contributing to the assimilation thereof. The increase noted in the results (Tables 1 and 3) may be due to lack of continuous anaerobic conditions. Under anaerobic processes sulphate is typically removed from the system through the reduction thereof. Unlike nitrogen however, sulphate reduction takes longer to occur once anaerobic conditions have been established, and requires a more reduced environment (Mitsch and Gosselink, 2000; Scholz, 2006a). Anaerobic conditions may not be sufficiently maintained to ensure significant conversion of sulphate to gaseous sulphur.

The results above do not correspond with the typical assertion that wetlands act as nutrient sinks, where water entering the system slows and surrenders nutrients to chemical processes and plant uptake (Dodds and Whiles, 2010). All three major nutrients occur in abundance within the wetland; however there is no evidence to suggest that the wetland assimilates all these nutrients to any great extent. In fact, in the case of sulphates, the concentration increases considerably as water moves through the wetland. The role of evapotranspiration needs to be considered as one of the possible reasons for the lack of assimilation of the nutrients. As water in the wetland moves slowly through the system, ponding in areas, evapotranspiration results in the concentration of various compounds (Humphries *et al.*, 2011).

Interestingly, the capacity of wetlands to immobilise nitrates and nitrites is greater than that of phosphorous. This is because phosphorous cannot be released into the atmosphere, as with nitrogen gas after denitrification, and is instead stored by the wetland in sediments and organic matter (Mitsch and Gosselink, 2000; Dodds and Whiles, 2010). This means that the wetland can become saturated with phosphorous whilst the nitrogen cycle continues unencumbered. Whilst right now it would appear that phosphorous is being assimilated by the wetland, this process may not continue in the same fashion as phosphorous loading continues to inundate the wetland environment.

The results for chlorophyll *a*, assessed during the winter sampling using the Hydrolab, indicate that the west pond had the most chlorophyll *a* present. Chlorophyll *a* is used as an indication of the amount of algae present (Sharma *et al.*, 2016). This is consistent with the visual turbidity of the water (discussed in Chapter 2), where the sample for the west pond was found to have a green colouration in contrast to the other samples taken during the same effort. The presence and abundance of algae is typically limited by the presence of nutrients, particularly nitrogen and phosphorous. The Hydrolab results for nitrogen (as NO_3^-) show that nitrogen content is second lowest in the west pond (at 762,83 mg/L). However, examination of nitrogen concentration using the colorimeter shows that nitrates and nitrites were highest in the west pond. This difference may be attributed to the time from which the sample was assessed using each method, as well as the method themselves. Phosphorous concentration was lowest in the west pond, as indicated by the colorimetric analysis (Table 1). This suggests that there are other mechanisms not investigated that may be contributing to the noticeably greater concentration of algae in the west pond.

The Department of Water Affairs and Forestry suggest that the relative concentrations of cations and anions in aquatic systems are predictable as they tend to follow a select pattern (DWAF, 1996e). However, the results of the relative abundance of the summer sample cations and anions (as given by laboratory analysis) indicate that the wetland system does not conform to DWAF aquatic cation and anion expectations.

In contrast to the assertions of the Department of Water Affairs and Forestry, the presence and relative abundance of ions in freshwater could be considered as highly variable. This is largely due to the differing environments that freshwater systems occur in. Ionic composition of freshwater sources can be influenced by atmospheric conditions, in particular that of sea spray, the weathering of rock, which introduces various ions into solution, and the evaporation and precipitation dynamics of the area (Kalff, 2002). Given that the area of concern is relatively small, differences due to underlying rock (and the weathering thereof) and atmospheric conditions are unlikely to impact on the spatial differences noted in ion composition of the wetland. The effect of evaporation may be of significance however. In particular, the west pond is somewhat isolated from the rest of the wetland (more so during the winter than in the summer season, likely due to increases in rainfall in the region). This slight isolation may increase the effect of evaporation in the west pond which may result in evapoconcentration of compounds (Eary, 1998; Nickson *et al.*, 2005; Anderson and Stedmon, 2007). This could account for the increase in ionic composition seen.

The assessment of the results against both national and international guidelines was carefully considered. Given that the wetland drains into the Taaibosspruit, which feeds into the Vaal River (as discussed in Chapter 2), the water that is present in the wetland may have an impact on drinking water quality. This is because the Vaal River forms part of the provision of domestic water for the region. As such, both the domestic use guidelines and the WHO guidelines were used with drinking water quality in mind (DWAF, 1996a; WHO, 2017). The selection of category 4 industrial water use was due to the category being for the most basic or rudimentary water uses in industry (i.e. the poorest water quality). This includes water for rough washing of floors, apparatus etc., fire fighting, dust suppression, irrigation and use as a transport agent (DWAF, 1996b). All other categories (categories 1 – 3) require water of domestic quality or above, and the water is often subject to costly processing to ensure that it is suitable for use in the industrial processes. Target water quality for this purpose is primarily to ensure that the use of the water does not damage equipment or cause a negative impact on the environment should it becomes waste water (DWAF, 1996b). The impact of the wetland water quality on agriculture was assessed using the agricultural guidelines (DWAF, 1996c-d). As discussed in Chapter 2, the surrounding land uses include both livestock rearing (cattle specifically) as well as the cultivation of crops, which may be directly influenced by water quality of the wetland. The impact of the wetland on the surrounding aquatic environment is also a concern given its placement within a greater aquatic system. As a result, the guidelines for aquatic ecosystems were used to assess the impact of water quality on the integrity of the ecosystem (DWAF, 1996e).

Extended exposure to water that contains chemical constituents in excess of the various requirements set out by the guidelines is usually required before health concerns manifest. Importantly though, high nitrate concentrations in water are an exception to this. This is because nitrates found in excess can cause rapid onset of negative health impacts. Often seen in bottle-fed infants, methaemoglobinaemia (blue baby syndrome) arises shortly after repeated exposure to excess nitrate levels (WHO, 2017).

Phosphorous and nitrogen are considered limiting reagents in natural environments. When not limiting, they can promote undesirable levels of primary productivity, resulting in algal blooms. These algal blooms can lead to a eutrophic environment, severely impacting on water quality and ecosystem integrity (Smith *et al.*, 1999; Wang *et al.*, 2019)

It is imperative to note that different analytical methodologies give differing results. However, all the methodologies employed are widely used measures of chemical water quality. Each approach has positive and negative attributes associated with their use.

Colorimeters are useful for testing water in the field, as they are easily portable (Chua, 2019). However, the turbidity of the water may impact the results. In the field, water is not easily filtered prior to use if the water is highly turbid, whilst conducting the experiment in a laboratory allows for filtration to ensure accurate results. Colorimetric assessment can just as easily be done in the field as in a laboratory. Furthermore, colour development in the sample using the various reagents follow very specific methodologies and are highly sensitive to changes in this method. For example, even minor changes in the length of time reagents are shaken, or changes to the manner in which it is shaken can play a role in variation of results. As such, the use of a colorimeter demands extreme exactness throughout the process. Although this accuracy results in highly accurate results, the sensitivity is likely to result in unavoidable differences due to human error (Hach, 2013).

The Hydrolab is considered to be one of the foremost water quality assessment tools available for in-field monitoring. Its durability and design allows for the long-term placement of the device in the environment, where upon retrieval data can be downloaded and examined. However, the Hydrolab is expensive to purchase, which makes accessibility of instrumentation the limiting factor in its usage. As with all probes, the Hydrolab requires regular calibration to ensure accuracy in the results (Hach, 2006).

External laboratory analysis, however, is often time-intensive as samples need to be sent for analysis, and the lag time from sampling to analysis may influence the results. However, accuracy of the results may be more pronounced as testing is done under laboratory conditions.

The analysis of chemical water quality involved a number of techniques as well as chemical constituents. It is important to note that these constituents are not the only chemicals found in the wetland, nor are they the only chemicals that may be impacting on water quality. Chemical water quality testing is limited by the abundance of different chemicals, time to measure all these constituents as well as the cost involved.

Chapter 5: Ecotoxicity – *Daphnia magna*

As can be seen below (Figure 15), the desktop and water quality assessments have been discussed, which is followed by the assessment of ecotoxicity using *Daphnia magna*.

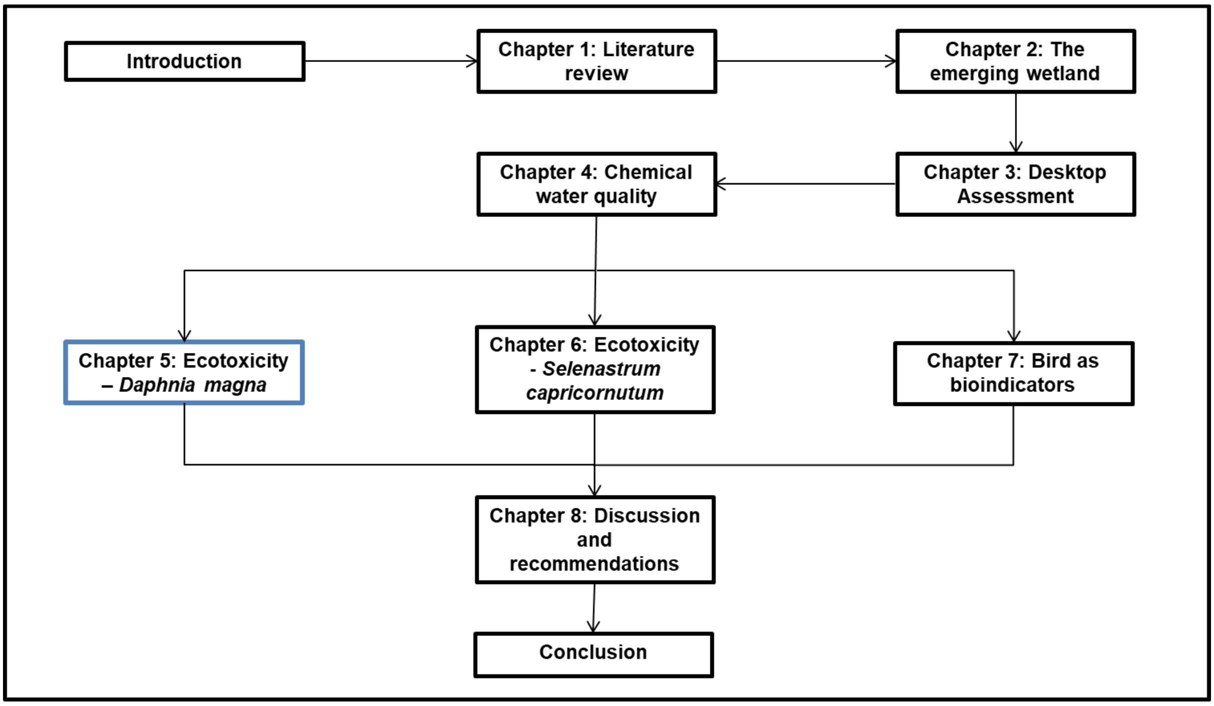

Figure 15: Structure of the dissertation – Chapter 5

Methodology

The *D. magna* acute toxicity test was conducted in accordance with the guidelines set out by the U.S. Environmental Protection Agency's Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (USEPA, 2002).

In preparing for the acute *D. magna* toxicity assay, water samples were evaluated in terms of their electrical conductivity, the dissolved oxygen content, the pH and the temperature. Physico-chemical assessments were done using a bench top probe fitted with the appropriate probes (Hach HQ40d Multi Reader) (Appendix A). All samples were filtered in order to remove large impurities in the sample, as well as any organisms that may have been present. Samples were filtered as the samples were turbid and not conducive to the test being performed without filtration.

As per the USEPA guidelines, the test consisted of the undiluted sample and five dilutions as well as a control. The water samples were diluted using moderately hard, dechlorinated tap water. The recommended dilution factor for the test was ≥0.5. As such, dilutions of 50%, 25%, 12.5%, 6.25% and 3.125% of each sample were made (USEPA, 2002). All physico-chemical readings were repeated for each dilution.

Four test chambers (50mL glass beakers) were prepared for each sample (undiluted and dilutions) and the control. Twenty-five millilitres of each sample and the control were placed in their respective test chambers. Laboratory-reared *D. magna*, less than 24 hours old, were transferred into each test chamber using a Pasteur pipette (five test organisms per test chamber).

The number of *D. magna* mortalities was counted after 24 and 48 hours. After 48 hours, the *D. magna* acute toxicity test concluded. The physico-chemical readings were repeated at the conclusion of the test. Table 8, below summarises the conditions of the toxicity test.

Table 8: Test conditions for the *D. magna* acute toxicity test (USEPA, 2002)

Test conditions for the *Daphnia magna* acute toxicity test	
Test species: *Daphnia magna* Age of test organism: less than 24 hours old Test type: static non-renewal	
Parameter	**Condition to be maintained during test**
Water temperature	20 ºC ± 1 ºC; or 25 ± 1 ºC
Light quality	Ambient laboratory illumination
Photoperiod	8 hours dark:16 hours light
Feeding regime	Feed algae and commercial fish flakes while in holding prior to test
Aeration	None
Size of test chamber	50 mL
Volume of test sample	25 mL
Number of test organisms per chamber	5
Number of replicate chambers	4
Total number of test organisms per sample	20
Control and dilution water	Moderately hard dechlorinated water, or moderately hard reconstituted water
Test duration	48 hours
Effect measured	Percentage lethality (no movement on gentle prodding), calculated in relation to the control
Test acceptability	90% or greater survival in control
Interpretation	Lethality >10% indicates toxicity, provided that control lethality is ≤10%

Data analysis

At the conclusion of the winter and summer tests, the total number of *D. magna* mortalities was recorded per sample. The criterion for mortality of *D. magna* was no movement or no response to gentle prodding.

These values where then used to calculate the LC_{50} of the sample. The LC_{50}, or Lethal Concentration, is the point at which 50% of the test organisms (*D. magna*) die. The LC_{50} is calculated using the Probit model. The Probit model is a regression model that assumes a dichotomous response variable (dependent variable). In this case the response is mortality (dead).

The Mann-Whitney U Test (which is equivalent to the Wilcoxon Rank Sum Test) was used to determine if there was a significant difference in the LC_{50} values from the winter and summer sampling effort. This test was chosen as it is non-parametric, and is suitable for a small number of outcomes (in this case, four winter LC_{50} values and four summer LC_{50} values) (D'Agostino Sr *et al*., 2006).

The Mann-Whitney U Test makes use of the median rather than the mean as small samples are represented. The values of each season were ranked from smallest to largest to test the hypothesis:

H_0: there is no significant difference in the LC_{50} from winter to summer
H_1: there is a significant difference in the LC_{50} from winter to summer.

This same methodology was employed to analyse if there was any statistical difference in the electrical conductivity of the samples from winter to summer, testing the hypothesis:

H_0: there is no significant difference in electrical conductivity from winter to summer
H_1: there is a significant difference in electrical conductivity from winter to summer.

Validity of the D. magna acute toxicity test

In order for the acute *D. magna* toxicity test to be valid, one criterion needed to be fulfilled: the control group was required to have a mortality of less than or equal to 10% (USEPA, 2002).

Results

The results of the winter and summer *D. magna* 48-hour acute toxicity test are tabulated below (winter – Table 9; summer – Table 11). The mortalities were used to calculate the LC_{50} for each sample site per season (Table 10 (winter) and 12 (summer)).

Winter sampling

Table 9 depicts the results from the winter ecotoxicity test. As can be seen, at the 100% concentration (undiluted sample water) as well as the 50% dilution, *D. magna* mortality was 100% within the 48 hours of the test throughout the wetland.

Table 9: The results of the winter D. magna acute toxicity test - mortality after 48 hours

Winter sampling	Replicate A No. of mortalities	Replicate B No. of mortalities	Replicate C No. of mortalities	Replicate D No. of mortalities	Total mortalities	Percentage (%)
Control	0	0	0	0	0	0
			Upstream			
3.125%	0	1	1	0	2	10
6.25%	0	1	1	0	2	10
12.5%	0	2	0	1	3	15
25%	4	3	5	4	16	80
50%	5	5	5	5	20	100
100%	5	5	5	5	20	100
			West pond			
3.125%	0	0	1	0	1	5
6.25%	0	0	2	0	2	10
12.5%	1	3	0	1	5	25
25%	5	4	3	5	17	85
50%	5	5	5	5	20	100
100%	5	5	5	5	20	100
			East pond			
3.125%	0	0	0	0	0	0
6.25%	0	0	0	2	2	10
12.5%	0	1	2	3	6	30
25%	2	2	2	2	8	40
50%	5	5	5	5	20	100
100%	5	5	5	5	20	100
			Downstream			
3.125%	0	0	0	0	0	0
6.25%	0	1	0	0	1	5
12.5%	0	0	1	1	2	10
25%	5	4	3	4	16	80
50%	5	5	5	5	20	100
100%	5	5	5	5	20	100

The LC_{50} was calculated after 48 hours using the Probit model. This is illustrated in Table 10 and Figure 16 below. The lowest LC_{50}, and thereby the water that requires the most dilution in order to maintain *D. magna* populations, was in the west pond. The upstream and downstream sample sites maintained a similar LC_{50} of ~18 %. A notable increase in LC_{50} was present in the east pond.

Table 10: The LC50 (mg/L) values of D. magna calculated for the winter sampling

Winter sampling	LC_{50} (%)	95% confidence interval		R^2
		Lower limit	Upper limit	
Upstream	18,188	12,108	27,32	0.691
West pond	14,842	10,496	20,987	0.884
East pond	31,445	19,141	51,656	0.9307
Downstream	18,073	12,108	27,32	0.8569

Summer sampling

In all but the upstream summer sample, the 100%, 50% and 25% samples all incurred a 100% *D*. magna mortality after 48 hours (Table 11).

Table 11: The results of the summer *D. magna* acute toxicity test - mortality after 48 hours

Summer sampling	Replicate A No. of mortalities	Replicate B No. of mortalities	Replicate C No. of mortalities	Replicate D No. of mortalities	Total mortalities	Percentage (%)
Control	0	0	0	0	0	0
			Upstream			
3.125%	0	0	0	0	0	0
6.25%	0	0	0	0	0	0
12.5%	0	0	2	0	2	10
25%	2	1	2	3	8	40
50%	5	5	5	5	20	100
100%	5	5	5	5	20	100
			West pond			
3.125%	1	0	0	0	1	5
6.25%	0	1	1	1	3	15
12.5%	4	5	5	5	19	95
25%	5	5	5	5	20	100
50%	5	5	5	5	20	100
100%	5	5	5	5	20	100
			East pond			
3.125%	0	0	0	0	0	0
6.25%	1	1	0	0	2	10
12.5%	5	4	4	5	18	90
25%	5	5	5	5	20	100
50%	5	5	5	5	20	100
100%	5	5	5	5	20	100

Downstream						
3.125%	0	0	1	0	1	5
6.25%	0	1	0	0	1	5
12.5%	3	2	3	3	11	55
25%	5	5	5	5	20	100
50%	5	5	5	5	20	100
100%	5	5	5	5	20	100

The summer sampling results were used in the Probit model to calculate the LC_{50} of *D. magna* (Table 12 and Figure 16). The LC_{50} values decreased from the inflow point of the wetland to the outflow point. The lowest LC_{50} value was found in the west pond, followed by the east pond and then downstream. This indicates that upstream waters require the least dilution to sustain 50% of *D. magna* in the wetland.

Table 12: The LC50 (mg/L) values of *D. magna* calculated for the summer sampling

Summer sampling	**LC_{50} (%)**	**95% confidence interval**		**R^2**
		Lower limit	**Upper limit**	
Upstream	29,656	21,591	40,734	1
West pond	6,777	5,198	8,836	0.8123
East pond	8,839	7,22	10,821	1
Downstream	13,87	9,85	19,53	0.75

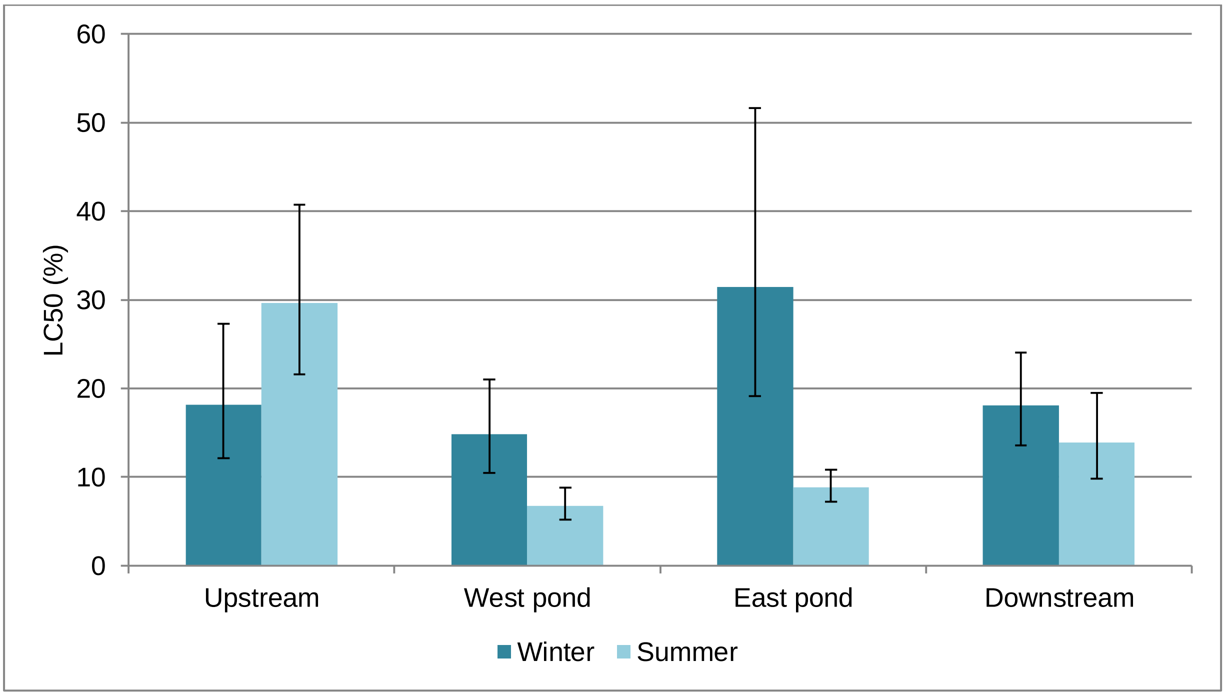

Figure 16: The seasonal difference in LC50 values for *D. magna*

Seasonal differences

Using the Mann-Whitney U Test, the winter and summer LC_{50} values were analysed. The U-score for the test, which makes use of the summed ranks and the number in each population, was calculated to be U = 3. At $\alpha = 0.05$, the critical value is 0. Given that $U > \alpha$, or $3 > 0$, we do not reject the null hypothesis (H_0: there is no significant difference in the LC_{50} from winter to summer), and as such there is no statistical evidence to believe that there is any difference in the LC_{50} values from winter to summer.

Electrical conductivity

The electrical conductivity was measured for the undiluted (100%) test sample for both winter and summer using the Hach HQ40d Multi Reader. These results are tabulated below in Table 13.

Table 13: Electrical conductivity of winter and summer samples

	Winter electrical conductivity (µS/cm)	**Summer electrical conductivity (µS/cm)**
Upstream	6340	6080
West pond	4990	7610
East pond	5220	7070
Downstream	4650	6880
Median	5105	6975

The Mann-Whitney U Test was used to ascertain if there were any significant seasonal differences in electrical conductivity. The Mann-Whitney U Test indicated that the electrical conductivity for winter (median = 5105 µS/cm) was statistically equivalent to that of the summer electrical conductivity (median = 6975 µS/cm), U = 1, $\alpha = 0.05$.

Validity of the test

The results of the validity checks show that there were no mortalities recorded in the controls. As such the test was deemed to be valid.

Discussion

The *D. magna* acute immobilisation or mortality test was conducted to ascertain the LC_{50} (%) of the species when exposed to the emerging wetland waters. *D. magna* is considered a good indicator species for water quality as it is widespread throughout the world and sensitive to environmental changes (Driesen, 2015). They also provide a critical link between primary production, such as phytoplankton on which they feed, and higher trophic levels. They are predated upon by fish and larger invertebrates (Dodson and Hanazato, 1995; Taylor, 2010).

The results indicate that *D. magna,* when exposed to undiluted wetland water, regardless of season, do not survive. This suggests that *D. magna* would not survive in the wetland waters if the conditions in the sample water prevail. According to test criteria, lethality greater than 10% indicates toxicity (USEPA, 2002). As such, the water in the wetland can be considered highly toxic to *D. magna*. This can have significant impacts on the ecosystem integrity.

The results of the winter LC_{50} point to decreased water quality in the west pond, whilst the east pond expressed the least toxicity. However, overall as water moves from upstream to downstream points, the toxicity of the water remains the same. The *Daphnia* ecotoxicity test shows a ‘birds-eye view’ of the various mechanisms within the wetland waters, without giving detail as to exact processes at play (as is the nature of ecotoxicity testing). Toxicity in the west pond may be greater due to the possible effects of evapoconcentration of various pollutants (Eary, 1998; Nickson *et al.*, 2005; Anderson and Stedmon, 2007). As a stagnant and relatively isolated water body, the greatest manifestation of evaporation is likely to occur in the west pond, where presumably primary water loss is in the form of evaporation.

In the summer sample the LC_{50} and electrical conductivity follow an inverse trend (high LC_{50} is coupled with lower EC, and vice versa), which is not seen in the winter sample. This can be viewed in conjunction with the results of the cation and anion concentrations (Chapter 4; Table 7) where the same pattern prevails. This may indicate that EC is impacting on the survival of *D. magna*, which has been noted in a number of other ecotoxicity test species (Bori *et al.*, 2016). Given that the wetland is situated in a summer rainfall area, it is possible that pollutants may be more prevalent in the summer months. Although higher influxes of water may result in the dilution of contaminants, it may also contribute to greater contaminant concentrations from upstream areas as runoff from the catchment is increased (Estèbe *et al.*, 1998).

Having said the above, the Mann-Whitney U Test indicated that the LC_{50} for the winter sampling (median = 18.1305) was statistically not different to that of the summer LC_{50}

(median = 11.3545), U = 3, α = 0.05. This was mirrored by the Mann-Whitney U Test to compare electrical conductivity from winter to summer, which was also deemed to statistically show no differences.

The presence of a daphnid species in the west pond during the winter season is of interest in examining the water quality of the wetland. Although not conclusively identified, it is believed to be either *D. pulex* or *D. longispina*. As discussed in Chapter 2, the west pond was almost entirely isolated from the rest of the wetland. This may account for the presence of the crustacean in the west pond whilst not being present in the other sample sites. The relative sensitivity of *D. magna and D. pulex* are believed to be similar (Elnabarawy *et al.*, 1986). However, the LC_{50} for the west pond during the winter sampling was 14.842%. There was no survival in *D. magna* at a 100% or 50% dilution, despite the survival of the second, naturally occurring daphnid species.

Although the ecotoxicity testing suggests that there is no statistical difference in toxicity between the two seasons, no daphids were present during the summer sampling of the west pond. This may be due to the west pond being actively part of the wetland system as a result of the increase in inundation or a deterioration in water quality in the wetland that is not yet shown by the results and may become more apparent should further testing be conducted. Another possibility is that the large amounts of phytoplankton, in the form of green algae, which were present in the west pond, made the west pond a suitable and food abundant environment. The improved nutrition provided by the nitrogen, sulphur or phosphorous (nutrient) enrichment may have had a positive effect on fitness of the daphnid species (Aalto *et al.*, 2013). As a result they may have been more able to prevail in conditions that otherwise would not have promoted survival. As algae concentrations decreased, so may have the presence of the *Daphnia*. Another possibility is that increased food allowed for greater sustenance for the daphnid species by the west pond (Cunha *et al.*, 2010). A decrease in food source by the summer meant that the *Daphnia* could not be sustained, and as such none were encountered. However, the role of nutrients in both the inhibition or promotion of population growth in *Daphnia* is not clear, as Aalto *et al.* (2013) contend that there is little correlation between increased food availability and increased abundance of *Daphnia*.

Given the small sample size, it is important to note that the median LC_{50} values for the winter and summer samples do differ ($Median_{SUMMER} - Median_{WINTER} = 11.3545 - 18.1305 = -6.776$). This may indicate that, although we do not reject the null hypothesis, a larger sample size could give greater insight into the seasonal differences in the LC_{50} of *D. magna.*

Chapter 6: Ecotoxicity - *Selenastrum capricornutum*

Selenastrum capricornutum, specifically the model bioassay strain *Selenastrum capricornutum* NIVA-CHL 1, is one of the most widely used bioassay alga worldwide. It was isolated by O.M. Skulberg in 1959 (Skulberg, 1964; Krienitz *et al.*, 2011). The bioassay strain was however incorrectly designated as *S. capricornutum*, and was subsequently reclassified to the genus *Raphidocelis* using molecular data (Krienitz *et al.*, 2011). For the purpose of this study, the name *S. capricornutum* will be maintained, as all standards and development of procedures have been designed using this nomenclature. Figure 17 places the ecotoxicity testing using *S. capricornutum* into the context of the overall study.

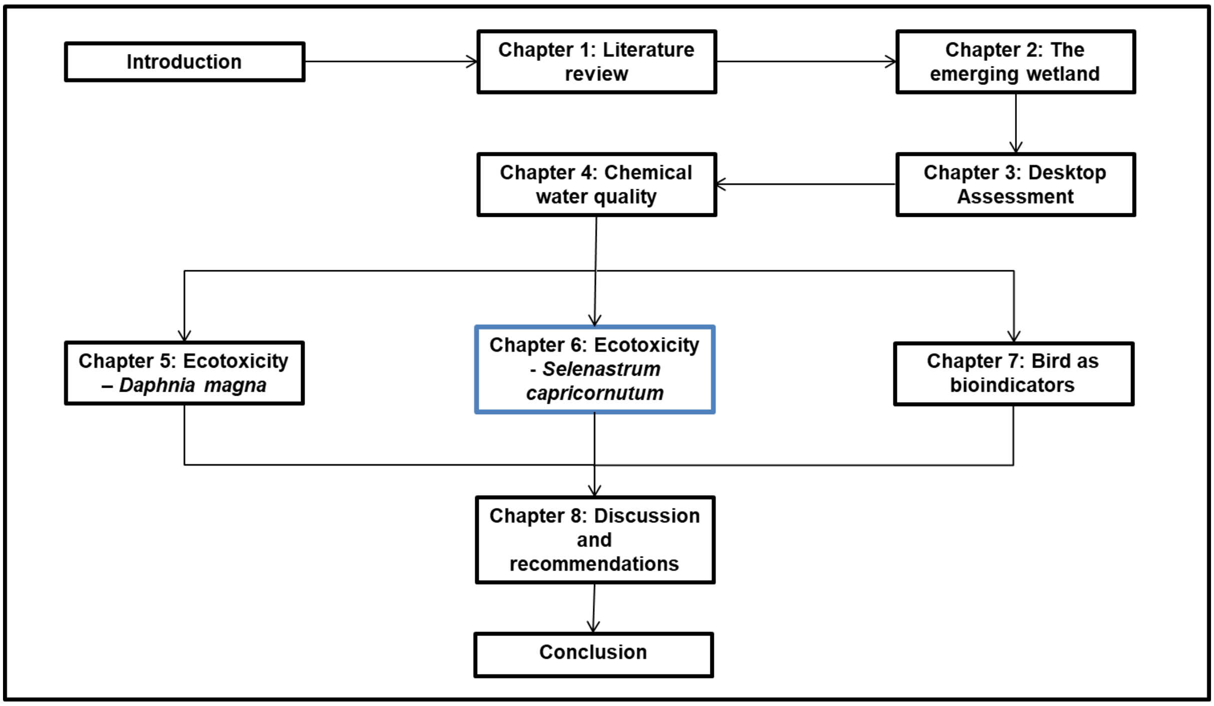

Figure 17: Structure of the dissertation - Chapter 6

Methodology

A 72-hour *S. capricornutum* growth inhibition test, which measures the response of the algal population in terms of changes in cell density (measured as optical density) was conducted (DWAF, 2006).

The four water samples that were collected during the winter and summer sampling effort were centrifuged at 8000 rpm for 5 minutes prior to the beginning of the test. This was done as the samples were very turbid with organic matter present. All water samples were then further filtered through 0.45 µm syringe filters into sterile 50 mL falcon tubes.

The test was performed according to the 24 well microplate method described by the Department of Water Affairs and Forestry (DWAF, 2006).

Cell density was measured spectrophotometrically at 670 nm at the start of the test (0 hours) and thereafter every 24 hours (i.e. at time = 24 hours, 48 hours and 72 hours). The table below (Table 14) summarises the test conditions.

Table 14: Test conditions for the *S. capricornutum* growth inhibition test (DWAF, 2006).

Test conditions for the *Selenastrum capricornutum* growth inhibition test	
Test species: *Selenastrum capricornutum* Age of test organism: 4 to 7 days Test type: non-renewal	
Parameter	**Condition to be maintained during test**
Temperature	24 ± 2° C
Light quality	"Cool white" fluorescent lighting
Light intensity	±4000 lux
Photoperiod	Continuous
Feeding	Algal growth medium
Size of test well	2 mL (24-well plate)
Volume of test sample	1.8 mL
Initial cell density in test chambers	200 000 cells/ mL
Number of replicate wells	6
Control/dilution water	Sterile Milli-Q water
Shaking rate	100 cpm continuous
Aeration	None
OD measurement	670 nm
Test duration	72 hours
Effects measured	Percentage inhibition (or stimulation) in terms of OD
Interpretation	Inhibition of ≥20% over controls indicates toxic activity, while growth of ≥20% over controls indicates stimulation.

Data analysis

The mean value, standard deviation and coefficient of variance for each sample and their dilutions was calculated using the initial (at time = 0 hours) and final (at time = 72 hours) blank corrected optical density.

The mean was subsequently used to calculate the percentage growth inhibition of the *S. capricornutum* using the following equation:

$$\%\, growth\, inhibition = \frac{(ODC_{72} - ODC_0) - (ODS_{72} - ODS_0)}{(ODC_{72} - ODC_0)}\, x\, 100$$

Where,

ODC_{72} = Mean of the three control wells after 72 hours;

ODC_0 = Mean of the three control wells at time 0;

ODS_{72} = Mean of the three sample wells after 72 hours; and
ODS_0 = Mean of the three sample wells at time 0.

The percentage growth inhibition for the winter and summer samples were investigated respectively to determine if the percentage growth inhibition of *S. capricornutum* differed at different points within the wetland. This was done by looking at the increase or decreases throughout the emerging wetland system from the upstream sample point to the downstream sample point. In addition, the different dilutions (50%, 25%, 12.5% and 6.25%) and the undiluted samples were compared.

The percentage growth inhibition between seasons (using the undiluted sample percentage growth inhibition – Table 15 and 16) was also examined using the non-parametric Mann-Whitney U Test to establish if any changes had occurred in the inhibition of *S. capricornutum*. The Mann-Whitney U Test is an appropriate statistical analysis for small sample sizes as it makes use of the median rather than the mean of the sample. In using this test, the following hypothesis was analysed:

H_0: there is no significant difference in percentage growth inhibition from winter to summer
H_1: there is a significant difference in percentage growth inhibition from winter to summer.

Validity of the test

In order to obtain a valid test for the chronic *S. capricornutum* growth toxicity test, two criteria need to be met (DWAF, 2006):

- The coefficient of variance for the control needs to be less than or equal to 10%; and
- The cell density for the control has to have increased at least 16 fold over the 72 hour period.

Results

At the conclusion of the test, the percentage growth inhibition for the winter and summer sampling efforts were calculated, and are shown in Table 15 and Table 16 respectively.

As can be seen below (Table 15), the percentage algal growth inhibition for the winter period (undiluted sample) increased by 36.97% from the input point of the emerging wetland (upstream) to the downstream outflow point. The west pond experienced the lowest percentage growth inhibition. The east pond and downstream percentage growth inhibitions are higher than that of the upstream and west pond.

Table 15: The growth inhibition (%) of *S. capricornutum* based on the winter 72-hour ecotoxicity test.

	Optical Density @ t = 0 hours	**Optical Density @ t = 72 hours**	**Growth inhibition (%)**
Control	0,01567	0,269	-
		Upstream	
6.25%	0,02333	0,269	3,02632
12.5%	0,02667	0,208	28,4211
25%	0,03467	0,156	52,1053
50%	0,01033	0,123	55,5263
Undiluted	0,01567	0,09	70,6579
		West Pond	
6.25%	0,02667	0,1947	33,6842
12.5%	0,015	0,1857	32,6316
25%	0,0273	0,181	39,3421
50%	0,01567	0,1527	45,9211
Undiluted	0,02	0,1117	63,8158
		East Pond	
6.25%	0,013	0,2767	-4,07895*
12.5%	0,02033	0,165	42,8947
25%	0,01267	0,145	47,7632
50%	0,00867	0,09167	67,2368
Undiluted	0,01333	0,002	104,4737
		Downstream	
6.25%	0,01767	0,2427	11,1842
12.5%	0,02533	0,1787	39,4737
25%	0,018	0,1063	65,1316
50%	0,00333	0,0463	83,02632
Undiluted	0,04867	0,02933	107,6316

*Growth stimulation

Table 16: The growth inhibition (%) of *S. capricornutum* based on the summer 72-hour ecotoxicity test.

	Optical Density @ t = 0 hours	Optical Density@ t = 72 hours	Growth inhibition (%)
Control	0,013	0,4323	-
	Upstream		
6.25%	0,01667	0,1673	64,06995
12.5%	0,01133	0,1473	67,5676
25%	0,017	0,2167	52,3847
50%	0,01533	0,2363	47,2973
Undiluted	0,01467	0,2097	53,4976
	West Pond		
6.25%	0,008333	0,3297	23,3704
12.5%	0,01067	0,3903	9,4595
25%	0,01567	0,3587	18,2035
50%	0,01833	0,1287	73,6884
Undiluted	0,02233	0,04567	94,4356
	East Pond		
6.25%	0,02067	0,2973	34,02226
12.5%	0,02333	0,3963	11,04928
25%	0,027	0,169	66,1367
50%	0,039	0,04167	99,3641
Undiluted	0,05133	0,02667	105,8824
	Downstream		
6.25%	0,025	0,409	8,4261
12.5%	0,022	0,3667	17,8060
25%	0,03067	0,249	47,9332
50%	0,033	0,01067	105,3259
Undiluted	0,04933	0,01433	108,3466

Table 15 also shows the effect of the dilutions on percentage growth inhibition for each winter sample point. The upstream, east pond and downstream samples show a decrease in growth inhibition from the undiluted sample through to the 6.25% sample. Interestingly, the east pond's 6.25% dilution shows algal growth stimulation. In the west pond, growth inhibition decreases from the undiluted sample to the 12.5% dilution. Subsequently there is a small increase in growth inhibition for the 6.25% dilution.

In Table 16 we note an approximately twofold increase in percentage growth inhibition from the undiluted upstream sample (the inflow point) to the downstream outflow point. The east pond percentage growth inhibition is similar to that of downstream, which is consistent with the winter results. Furthermore, it can be seen that there is a trend of decreasing growth inhibition from the undiluted sample to the 6.25% dilution in the west pond, east pond and downstream sample points. The west and east ponds do, however, indicate an increase in growth inhibition from the 6.25% dilutions. The upstream sample point shows a decreasing growth inhibition until 25% where growth inhibition subsequently increases slightly.

It is important to note that in both Table 15 and Table 16 the results of the percentage growth inhibition have been calculated using cell density (measured as optical density) that have been corrected against the control (or blank corrected).

The Mann-Whitney U Test was used to ascertain whether there was seasonal difference in percentage growth inhibition in *S. capricornutum*. The critical value at an $\alpha = 0.05$ level of significance for a sample size of eight (four per season) is zero (0). The null hypothesis (H_0: there is no significant difference in percentage growth inhibition from winter to summer) was not rejected as the calculated U value (the test statistic), which was calculated using the undiluted percentage growth inhibition values for both winter and summer, was 1.5 which is greater than the critical value ($1.5 > 0$). As such, there is no statistical evidence to suggest that there is any seasonal variation in percentage growth inhibition of *S. capricornutum*.

Validity of the S. capricornutum growth inhibition test

The coefficient of variance of the control at the end of the winter *S. capricornutum* test (time = 72 hours) was equal to 7.3704%, and the summer coefficient of variance at time = 72 hours was 4.6338%. Both coefficients of variance are below the required 10%, and as such fulfil the first criteria of validity.

The mean optical density measured for the control of the winter algal toxicity test increased 17.17 times (from 0.0157 to 0.2690). The mean optical density measured for the control of the summer algal toxicity test increased 33.26 times (from 0.0130 to 0.4323). As a result the test is valid as both values exceed the required 16-fold increase.

Discussion

The use of *S. capricornutum* as a test species in ecotoxicity testing is valuable in that algae (along with other autotrophs) form the base of the food webs found in environments. As the primary producers, any changes to their abundance may disrupt community dynamics within the food webs present in ecosystems (McCormick and Cairns Jr., 1994).

According to the test conditions (Table 14), toxic effects of the wetland water are present in the event that inhibition of algal growth is greater than 20% (DWAF, 2006). As evidenced by the results, the water in the wetland inhibits *S. capricornutum* growth (compared to the Control) during both winter ($\text{Median}_{\text{WINTER}}$ = 87.5658% growth inhibition) and summer ($\text{Median}_{\text{SUMMER}}$ = 100.159% growth inhibition) seasons to that of a toxic level.

Given the high levels of nutrients (Chapter 4; Tables 4-6) in the wetland, algal growth can either be stimulated or inhibited. Stimulation of algal growth is one of the primary concerns with regard to algal populations in aquatic systems. Just as the loss of the dominant primary producers in the aquatic environment can have detrimental effects on communities and ecosystem functioning (should algal growth be inhibited), so too can excessive algal growth (McCormick and Cairns Jr., 1994). Algal blooms, leading to eutrophication (with effects such as fish kills and oxygen depletion in water etc.) can greatly alter both the *in situ* and downstream aquatic environment (McCormick and Cairns Jr., 1994; Smith *et al.*, 1999). The *S. capricornutum* toxicity results indicate that all growth was inhibited throughout the wetland. This may indicate that the nutrient levels in the wetland exceed threshold values for growth and promotion of algae. This could also mean that other toxicants (such as heavy metals etc.) or a combination of toxicants acting together in the system are present which override the stimulation of growth in *S. capricornutum* (Gheorghe *et al.*, 2017). This is significant as the strength of ecotoxicity testing lies in its ability to react to a spectrum of possible interactions, and not just individual water quality parameter which so often do not act in isolation (Maciorowski and Sims, 1981; Valavanidis and Vlachogianni, 2015).

As was discussed in Chapter 2, during the winter sampling effort it was noted that the west pond water sample had a large amount of algal activity in comparison with the other three sample points. The west pond was also isolated to a certain extent, with very little movement of water through the pond. This is consistent with the slight minimisation in *S. capricornutum* growth inhibition in the ecotoxicity test for the west pond, which was lower than the other sample points. The west pond was not isolated from the wetland system to the same extent during the summer sampling, and as such may not have experienced the same conditions that provided for large amounts of algae to be present. There are numerous different algal species, and the measurement of chlorophyll *a* (Chapter 4; Table 5) gives a good indication of overall algal growth, and not species composition or abundances thereof. The composition of the algae was also not assessed specifically. Different algal species also have different sensitivities to compounds. For example, *S. capricornutum* is generally most sensitive to different herbicides, followed by *Chlorella* species and then *Chlamydomonas* species (Fairchild *et al.*, 1998; Rojíčková and Maršálek, 1999). This indicates that although *Selenastrum* was inhibited by the wetland water in the west pond, other algal species may be better adapted to the conditions and as a result can proliferate.

The percentage growth inhibition of the various dilutions for winter and summer expressed in Tables 15 and 16 indicate an overall decrease in growth inhibition throughout the dilution series, with the exception of the upstream summer sample where inhibition increases slightly from the 25% dilution to the 6.25% dilution. Given that toxicity is noted when inhibition is greater than 20% (Table 14) the dilution series for the winter upstream, east pond and downstream, and summer downstream only show evidence of no toxicity at the 6.25% dilution. The winter west pond and summer upstream sample points show toxicity at all dilutions. Due to the increases in inhibition seen in the summer west pond and east pond, the 12.5% dilution limits toxic activity; however the 6.25% dilution does not. This shows that although the overall downward trend in inhibition is noted, only very low concentrations of sample water limit the toxicity of the water. The increases in inhibition at low concentrations of sample water in some of the sample points illustrate the complex interactions between the various components of the aquatic environment. Some compounds or components may inhibit algal growth as well as the toxic activity of other components in high concentrations or undiluted samples. This may, however, be limited if sample water concentration decreases. These components, ordinarily repressed at high concentrations, subsequently exhibit their toxic effects on the algae, possibly resulting in an increased percentage growth inhibition at lower concentrations.

The Mann-Whitney U Test did not prove a difference in the winter percentage growth inhibition (median = 87.5658) and the summer percentage growth inhibition (median = 100.159), $U = 1.5$, $\alpha = 0.05$. This result suggests that although the wetland is inundated to a greater extent due to increased rainfall in the summer months, this does not change the toxicity of the wetland system to the test species. Notwithstanding the fact that the Mann-Whitney U Test shows no difference in overall growth inhibition, it is noteworthy that the change from the upstream point to the downstream point from winter to summer differs markedly (from a 36.97% increase in winter to a 54.85% increase in growth inhibition in summer). This may indicate that contaminants within the wetland, in summer and winter, impact the production (or lack thereof) of *S. capricornutum* to a greater extent than the input waters do. In this way, the wetland may be acting as a source of contaminants rather than a sink. In summer, when more water is in the system due to rainfall events (as discussed in Chapter 5) in the area, there is a possibility that the wetland is under greater pressure from upstream pollutants already (Estèbe *et al.,* 1998). As such, the wetland is less able to limit contamination from the inflow point to the outflow point.

Although this result was of consequence, upon examination of the median of the percentage growth inhibition from the respective seasons, a difference between the medians was noted ($Median_{SUMMER} - Median_{WINTER} = 100.159\% - 87.5658\% = 12.5932\%$). This may indicate a need for more samples to be included to ensure that the test accurately portrays the difference between seasons, if there are any.

Chapter 7: Birds as bioindicators

The use of birds as bioindicators is explored in this Chapter (Figure 18).

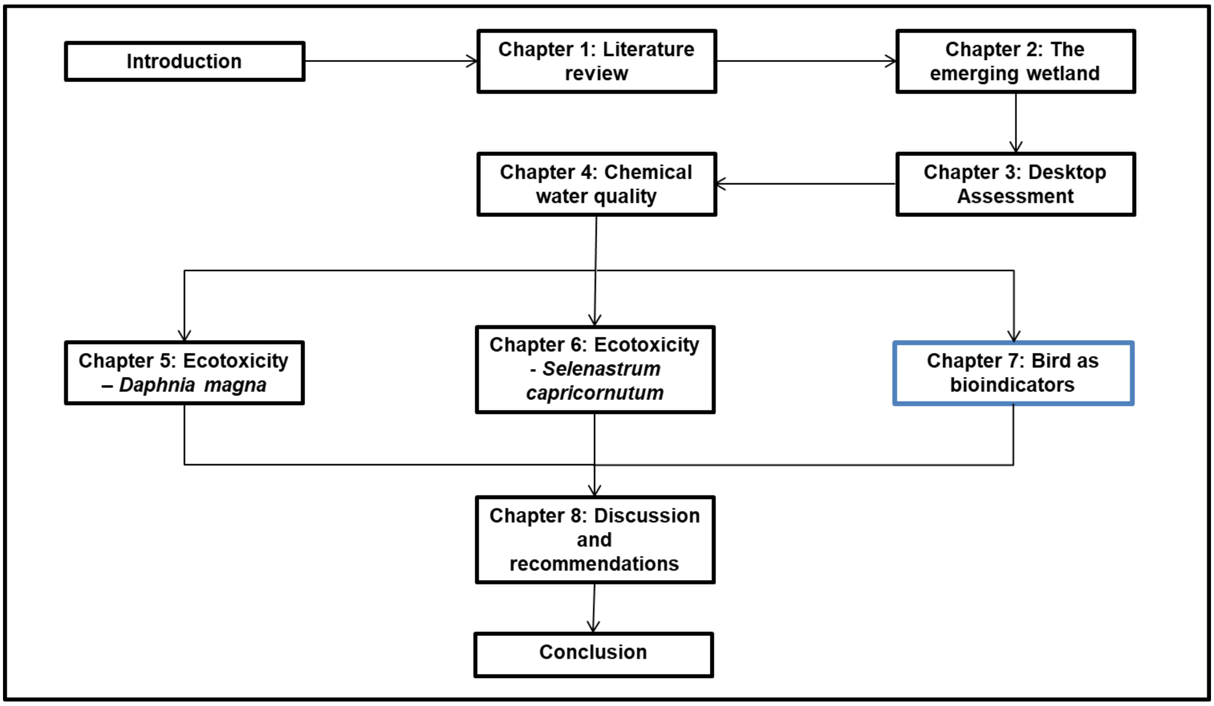

Figure 18: Structure of the dissertation - Chapter 7

Methodology

Bird surveys were conducted in conjunction with the Vaal Bird Club. The Vaal Bird Club makes use of the Southern African Bird Atlas Project (SABAP2) application (SABAP2, 2019).

SABAP2 is a citizen science project that allows for the recording of species and abundances, as well as the GPS location of each bird sighting. This allowed for real-time data collection, whilst contributing to a greater database of bird sightings in southern Africa (SABAP2, 2019).

The Vaal Bird Club conducted monthly bird surveys at the study site for the duration of the study. A total of six surveys were conducted from August 2018 to January 2019. The researcher participated in the surveys during the months of August and November 2018 and January 2019.

Data analysis

The data was inspected to ascertain the various bird species that were present that rely on the wetland for food sources and habitat. These species were subsequently investigated to determine what food sources they rely on, which can give an indication of the presence of these food sources in the wetland. Using this, inferences can be made as to the water quality needed to sustain these food sources.

Although all birds present at the wetland study site were recorded, only the birds that are directly dependent on wetlands and water sources were considered for use as birds as bioindicators. This is because the aim of the investigation is to ascertain what habitat and feeding requirements are provided by the wetland.

Birds directly associated with the wetland have been divided into seven categories, based on their foraging behaviours and preferences and habitat usage.

Results

The table below (Table 17) highlights the bird species associated with wetland and aquatic environments that were recorded at the wetland. As can be seen, 46 bird species that depend on the wetland were recorded. Appendix B provides a list of all birds that were identified at the wetland over the course of the six monthly surveys.

The Southern Red Bishop, the Egyptian and Spur-winged Goose, the Blacksmith Lapwing, and the Cape Wagtail were seen on every occasion during the six-month period. The Levaillant's Cisticola, Yellow-billed Duck, Three-banded Plover and Ruff were identified in most of the surveys (five out of the six). Birds that were only seen once, and can be considered rare at this wetland site during the study are the Black Crake, Great and Little Egret, Hamerkop, Little Grebe, Common Greenshank, Banded Martin, Black-winged Stilt, Woolly-necked Stork, Hottentot Teal, Caspian and Whiskered Terns, the African Reed Warbler as well as the Thick-billed Weaver. A number of species showed a degree of seasonality, with the Reed Cormorant, Grey-headed Gull, Common Moorhen and Cape Teal favouring the Winter to Spring months (August through to October), and the Yellow-crowned Bishop, African Sacred Ibis, Wood Sandpiper, Western Yellow Wagtail and Fan-tailed Widowbird appearing in Spring and towards Summer (October through to January). October experienced the most bird sightings (at 30 species associated with wetlands). The region is home to 118 inland water birds according to the bird distributions illustrated in Roberts Birds of Southern Africa (Hockey *et al.*, 2005). During the surveys, 46 of these inland birds were identified.

Table 18 describes the different habitat uses and feeding behaviours of the wetland birds. The first category describes birds that are often associated with wetland environments but not wholly dependent on them. These birds typically eat a variety of nectars, seeds and insects (aquatic or terrestrial). The second category describes species that are primarily herbivorous, and favour aquatic vegetation. The next two categories describe a variety of different omnivorous birds, with true omnivores having a highly diverse diet and birds that favour crustaceans and molluscs but with some plant matter in their diet. The remaining birds are primarily insectivorous or carnivorous, with one category favouring frogs and fish as well as some invertebrates, the other favouring the same food sources, however their feeding behaviour differs significantly in that they dive to catch food, and the last category being almost entirely insectivorous. The largest group are those that feed on insects, followed by those that eat frogs and fish as well as some invertebrates.

Table 17: Sightings of birds associated with the wetland environment (surveys conducted by the Vaal Bird Club)

	Species	**August 2018**	**September 2018**	**October 2018**	**November 2018**	**December 2018**	**January 2019**
Euplectes orix	Bishop, Southern Red	X	X	X	X	X	X
Euplectes afer	Bishop, Yellow-crowned			X		X	X
Ixobrychus minutus	Bittern, Little	X					
Cisticola tinniens	Cisticola, Levaillant's	X	X	X		X	X
Microcarbo africanus	Cormorant, Reed	X	X	X			
Amaurornis flavirostra	Crake, Black		X				
Dendrocygna viduata	Duck, White-faced Whistling						X
Anas undulate	Duck, Yellow-billed	X	X	X		X	X
Ardea alba	Egret, Great			X			
Egretta garzetta	Egret, Little			X			
Alopochen aegyptiaca	Goose, Egyptian	X	X	X	X	X	X
Plectropterus gambensis	Goose, Spur-winged	X	X	X	X	X	X
Tachybaptus	Grebe, Little	X					

ruficollis							
Tringa nebularia	Greenshank, Common			X			
Chroicocephalus cirrocephalus	Gull, Grey-headed	X	X	X			
Scopus umbretta	Hamerkop			X			
Ardeola ralloides	Heron, Squacco				X		X
Threskiornis aethiopicus	Ibis, African Sacred			X		X	X
Plegadis falcinellus	Ibis, Glossy		X	X		X	X
Vanellus senegallus	Lapwing, African Wattled	X	X	X		X	
Vanellus armatus	Lapwing, Blacksmith	X	X	X	X	X	X
Riparia cincta	Martin, Banded					X	
Gallinula chloropus	Moorhen, Common	X	X				
Charadrius pecuarius	Plover, Kittlitz's	X	X			X	X
Charadrius tricollaris	Plover, Three-banded	X	X	X		X	X
Philomachus pugnax	Ruff	X	X	X		X	X
Calidris	Sandpiper, Curlew			X			

ferruginea							
Tringa glareola	Sandpiper, Wood			X		X	X
Tadorna cana	Shelduck, South African	X				X	
Gallinago nigripennis	Snipe, African	X	X	X		X	X
Himantopus himantopus	Stilt, Black-winged	X					
Calidris minuta	Stint, Little	X	X	X		X	X
Ciconia episcopus	Stork, Woolly-necked			X			
Anas capensis	Teal, Cape	X		X			
Anas hottentota	Teal, Hottentot	X					
Anas erythrorhyncha	Teal, Red-billed	X		X			X
Hydroprogne caspia	Tern, Caspian		X				
Chlidonias hybrid	Tern, Whiskered					X	
Motacilla capensis	Wagtail, Cape	X	X	X	X	X	X
Motacilla flava	Wagtail, Western Yellow					X	X
Acrocephalus	Warbler, African Reed			X			

baeticatus							
Acrocephalus gracilirostris	Warbler, Lesser Swamp	X	X	X		X	
Bradypterus baboecala	Warbler, Little Rush	X	X	X		X	
Amblyospiza albifrons	Weaver, Thick-billed	X					
Euplectes axillaris	Widowbird, Fan-tailed					X	X
Euplectus albonotatus	Widowbird, White-winged		X	X		X	

Table 18: Bird habitats and foraging behaviours (Hockey *et al.*, 2005)

Food type or Habitat dependence	Description
Birds associated with wetlands but not entirely dependent on wetlands • Southern Red Bishop • Yellow-crowned Bishop • African Wattled Lapwing • Red-billed Teal • Fan-tailed Widowbird • White-winged Widowbird • Banded Martin • Thick-billed Weaver	Typical foraging involves scything and stripping of plants, as well as ground foraging and catching insects in flight. The Southern Red Bishop forages mainly on seeds and plant matter, including creeping setaria (*Setaria flabellate*) Guinea grass (*Panicum maximum*), flowers from common reeds and wild dagga and *Phragmites australis*, with some insects such as beetles or beetle larvae, caterpillars, dragonflies and spiders, as well as small crustaceans (*Talorchestia sp.*). The Yellow-crowned Bishop feeds on creeping setaria, guinea grass and natal redtop, with some insects. The African Wattled Lapwing forages on a large proportion of insects, such as grasshoppers, termites and aquatic insects. In addition, some grass seeds and worms are eaten. The Red-billed Teal feeds on the seeds of water grass (*Eriochloa stapfiana*), swamp cut grass (*Leersia sp.*), ragweed, and pondweed amongst others. Insects include amphipoda, odonata larvae, hemiptera and coleopteran. The fan-tailed widowbird is known to eat the seeds of a number of different grasses, including jungle rice (*Echinochloa colona*), finger grass and *Paspalum dilatatum*, along with some termites and caterpillars. The White-winged Widowbird also favours grass seeds, such as *Hyparrhenia* and *Pennisetum sp.* It also drinks nectar from *Aloe marlothii* and feeds on some insects. The Banded Martin forages on aerial insects. However, it is known to burrow near aquatic environments. The Thick-billed Weaver feeds largely on plant matter, with some termite alates also eaten. Plant

	matter includes seeds of river thorn (*Acacia robusta*), and buffalo-thorn (*Ziziphus mucronata*). However, its primary association with wetlands is its roosting, where it roosts in reeds.
Birds that feed on plant matter • White-faced Whistling Duck • Egyptian Goose • Spur-winged Goose	All three of the White-faced Whistling Duck, Egyptian Goose and Spur-winged Goose frequent shallow water of seasonal and permanent water bodies. The Egyptian Goose probes into the shallow water for plant matter. It is mainly a grazer and seed stripper, passing seed heads through the bill with rapid champs of the mandible. The White-faced Whistling Duck and Spur-winged Goose typically rake the floor of the water body before submerging their heads and necks to forage. All three birds are known to eat shoreline sedges, such as *Cyperus* articulates, snake root (*Polygonum senegalense*), as well as sago pondweed (*Potamogetom pectinatus*) and wavyleaved pondweed (*P. crispus*). A number of grasses are also foraged, including signal grass (*Urochloa dactylon*), couch grass (*Cynodon dactylon*), goose grass (*Eleusine indica*) and couch panicum (*Panicum repens*). During moulting, the only food source relied upon by the Egyptian Goose is aquatic algae, pondweed and couch grass (*Cynodon dactylon*).
Birds that are omnivorous • Common Moorhen • South African Shelduck • Hottentot Teal • Yellow-billed Duck	Foraging varies given the varied diets of these omnivorous birds, however feeding typically involves head-dipping and submerging their bills in the water. They also all up-end occasionally whilst foraging. The South African Shelduck also scythes the water. The Yellow-billed duck is a filter feeder. As omnivores, these birds feed on wide variety of different food sources, largely related to wetland and aquatic environments. This includes water plants, molluscs, worms, insects, fish and tadpoles. The Common Moorhen feeds on filamentous algae, mosses etc. and berries, as

	well as some arachnids. The South African Shelduck also eats Phyllopoda (tadpole and shield shrimp), natostracan (*Apus numidicus*) and conchostracan (*Caenestheriella sp.*), crustacean and tendipedid larvae, and submerged plant matter such as algae (*Spirogyra* and *Lagarosiphon sp.*) and hydrophytes. The Hottentot Teal includes fluke snails (*Bulinus natalensis*) in its diet, as well as wavyleaved pondweed, ragweed (*Ambrosia artemisifolia*), swamp grass, and small frogs. The Yellow-billed Duck eats an assortment of mostly plant matter, such as sago pondweed (*P. pectinatus*), sedges (*Eleocharis sp.*), oxygen weed (*Lagorosiphon sp.*) with some animal matter, including chironomid larvae, mayflies, dytiscid larvae (water beetles), grasshoppers and snails (*Bulinus sp.*).
Birds that feed on worms, crustaceans and molluscs, and small fish • Black Crake • Common Greenshank • Blacksmith Lapwing • Curlew Sandpiper • Wood Sandpiper • African Snipe	Foraging on the short grass adjacent to shorelines, on shorelines or on floating vegetation is common, with food being pecked or probed from mud, water plants or the water surface. Scything of the water surface is seen in the Common Greenshank and Wood Sandpiper. Darting at small fish is also seen. The varied diet of these species includes earthworms, various annelids, molluscs, diptera, Lepidoptera, coleopteran, hemiptera and odontata, tadpoles, frogs, small fish. Some plant matter also consumed, such as duckweed (*Lemna sp.*) and seeds of water lilies (*Nymphaea sp.*). The Black Crake is known to also scavenge crabs, frogs, fish and small birds. The Common Greenshank feeds on fish such as tilapia (*Oreochromis spp.*), and also fish fry. The Curlew Sandpiper favours gastropods, crustaceans such as isopods and amphipods, as well as insect adults, pupae and larvae.
Birds that feed on frogs and fish, and invertebrates	Foraging typically includes walking or wading through shallow water, wing-flicking or foot-stirring

• Little Bittern • Great Egret • Little Egret • Grey-headed Gull • Hamerkop • Squacco Heron • African Sacred Ibis • Whiskered Tern • Little Stint	to disturb prey. The Little Egret and Hamerkop also hover above water and snatch prey from the surface. The Grey-headed Gull runs through shallow water, skimming the water, or plunge-diving to collect food. The Whiskered Tern also plunge-dives, or surface-dips whilst foraging. Amphibians and fish make up a large part of their diets; however reptiles and aquatic invertebrates are also eaten. The Grey-headed Gull is an opportunistic feeder and is known to scavenge scraps of food it encounters. The Hamerkop feeds specifically on *Xenopus spp.*, and there is suggestion that they are largely dependent on them as food (they do eat other food such as fish and other frogs, and invertebrates).
Birds that dive for frogs and fish • Reed Cormorant • Little Grebe • Caspian Tern	Feeding typically involves diving for fish and amphibians. The Reed Cormorant typically forages in water less than two meters deep, however it can go as far as 10 m. Slow moving fish are typically foraged; however the Reed Cormorant mainly feeds on frogs, especially in smaller water bodies. This includes *Xenopus laevis*, the common river frog *Afrana angolensis* and common caco *Cacosternum boetgeri*. The Little Grebe feeds on small fish and frogs, tadpoles as well as small crustaceans and molluscs it encounters. The Caspian Tern feeds almost exclusively on fish.
Birds that feed on insects • Levaillant's Cisticola • African Reed Warbler • Little Rush Warbler • Western Yellow Wagtail	Foraging typically occurs on shorelines, in soft mud, with birds probing bills into mud. Insects are also taken from the water surface. The Glossy Ibis and Ruff Ruff also swim or wade short distances in shallow water, with the Cape Wagtail occasionally wading to forage. The Black-winged Stilt is known to scythe water in search of insects. The Cape Teal occasionally dives for food in addition to wading and filter-feeding. The Lesser Swamp Warbler, African Reed Warbler

• Glossy Ibis • Black-winged Stilt • Ruff • Woolly-necked Stork • Cape Teal • Cape Wagtail • Lesser Swamp Warbler • Kittlitz's Plover • Three-banded Plover • Little Stint	and Levaillant's Cisticola forage on reeds and aquatic plants for insects. The Little Stint is also known to forage whilst walking on floating algal mats (as does the Cape Wagtail), or wade into shallow waters. Insects, many of which are aquatic insects make up the primary food source of these birds. This includes waterbugs, aquatic beetles, boatmen and dragonflies. The Ruff Ruff also feeds on brine shrimp (*Artemia spp.*) and seeds. The Black-winged Stilt and Woolly-necked Stork also feed on small gastropods, crustaceans, and small fish. The Cape Teal also feeds on *Xenopus spp.*, crustaceans and some plant matter (Sago pondweed etc.). Kittlitz's Plovers and Three-banded Plovers, and the Little Stint also forage on worms, crustaceans and molluscs in addition to aquatic insects.

Discussion

Birds are an important and valuable factor to examine when assessing water quality. The higher the diversity of bird species in an area, the richer the biodiversity (Loftie-Eaton, 2014). This is because birds rely on a variety of food sources and habitats whilst also providing a food source, and even sometimes habitats for other organisms. For example, the Hamerkop nests over or near water bodies. As such, the aquatic environment provides both food sources and nesting availability for the Hamerkop. However, nests are often parasitized by other birds, reptiles or even bees (Hockey *et al.*, 2005).

The results of the bird survey indicate that there is a variety of food sources made available by the presence of the wetland. This includes various aquatic plants, insects, invertebrates, amphibians and fish.

Most wetland birds were found to be insectivorous. They fed on various aquatic and terrestrial insects; however most were found to forage on the water surface, at the water edge, and on the shorelines in mud. The wetland environment provides all these opportunities throughout the system, such as open water in the ponds (east and west pond), and water edge and shorelines at the transitional area between the seasonal and permanent zones and at the free-flowing upstream and downstream divergence and convergence. Insects are important features of wetlands as they provide a vital energy pathway, linking autotrophs to higher trophic levels (Dodson and Hanazato, 1995).

The number of bird species that are reliant on frogs, fish and various aquatic invertebrates are second most prominent in the wetland system. This indicates that along with a habitat that caters for insectivorous birds, the wetland also provides food which includes small fish, fish fry and frogs. *Xenopus laevis*, or the Common Platanna, are highly likely to be present at the wetland. This is evidenced by the presence of the Hamerkop which is noted to be associated with platanna (Loftie-Eaton, 2014). In addition to this, other species such as Cape Teal are also known to feed on platanna. As can be seen in the table (Table 18), there are three bird species, the Reed Cormorant, Little Grebe and Caspian Tern, that exclusively feed on various fish and amphibians. However, their presence at the wetland may be due to the proximity of the wetland to the Taaibosspruit. Although the presence of fish and amphibians was not assessed, there is a greater likelihood that they would be present in the free flowing waters of the river rather than the wetland environment. This constraint may be present with all bird data collected as birds are highly mobile and their presence may be incidental (Furness, Greenwood and Jarvis, 1993). The presence of both the Little Grebe and Caspian Tern was also limited to one occasion each, which in turn limits the inferences that can be made as to their association with the wetland.

Numerous crustaceans, phyllopoda (tadpole and shield shrimp), gastropods and annelids are also likely to be found within the environment. This is demonstrated by a number of the birds throughout the six monthly surveys being identified as foragers of these species.

Plant material that may be present in the wetland include various pondweeds (such as the wavy-leaved or sago pondweed), and aquatic algae as well as numerous terrestrial grasses. This may be made possible by the emergent nature of the wetland. The wetland boundaries are more likely to have both terrestrial and aquatic species as the extent of the wetland increases over time (Chapter 3). The Environmental Sieve model is a model that describes possible succession in a wetland, and is related to the life histories and habitat preferences of different vegetation. As water levels, in this case, or other environmental factors such as salinity, or presence of pollutants, change in the wetland, plant species that are not adapted to the new environmental conditions are 'sieved' out and replaced by those that can utilise the environment (Middleton, 2018).

Birds make exceptionally good bioindicators, as there is often extensive bird data available over many years, and they are easily identifiable and well-studied. As such, there is a vast amount of information that can be used in addition to the actual presence of birds due to the insight already gained regarding bird behaviour, foraging, breeding etc. (Furness, Greenwood and Jarvis, 1993 and Ormerod and Tyler, 1993). Although this survey was only conducted over six months and there was no additional historical bird data for the wetland, the bird surveys offered insight into a number of different aspects, such as the various food sources over a number of trophic levels that are likely to be available in the wetland, as well as high-level seasonality of different bird species. However, the potential of using birds as bioindicators and biomonitors in different areas is remarkable. Historical data over a number of habitat types has been collected over the years by a large network of bird clubs and enthusiasts. More recently, with the addition of the SABAP Android and iPhone applications, recording and accessing sightings and surveys have been made easier throughout South Africa (SABAP2, 2019). Furthermore, birds are sensitive to changes to the environment (Koskimies, 1989; Loftie-Eaton, 2014).

The bird surveys provide evidence that suggest that the wetland supports a number of different trophic levels and food webs, from algae and plant matter, to invertebrates, gastropods, amphibians, reptiles, fish and many different birds. Seasonal differences were not clearly identifiable, and long term data could provide more insight into the changing nature of the wetland environment.

Chapter 8: Discussion and recommendations

This chapter discusses the synthesis of the results that were gathered during the study and the implications that they may have on the wetland and greater environment (Figure 19).

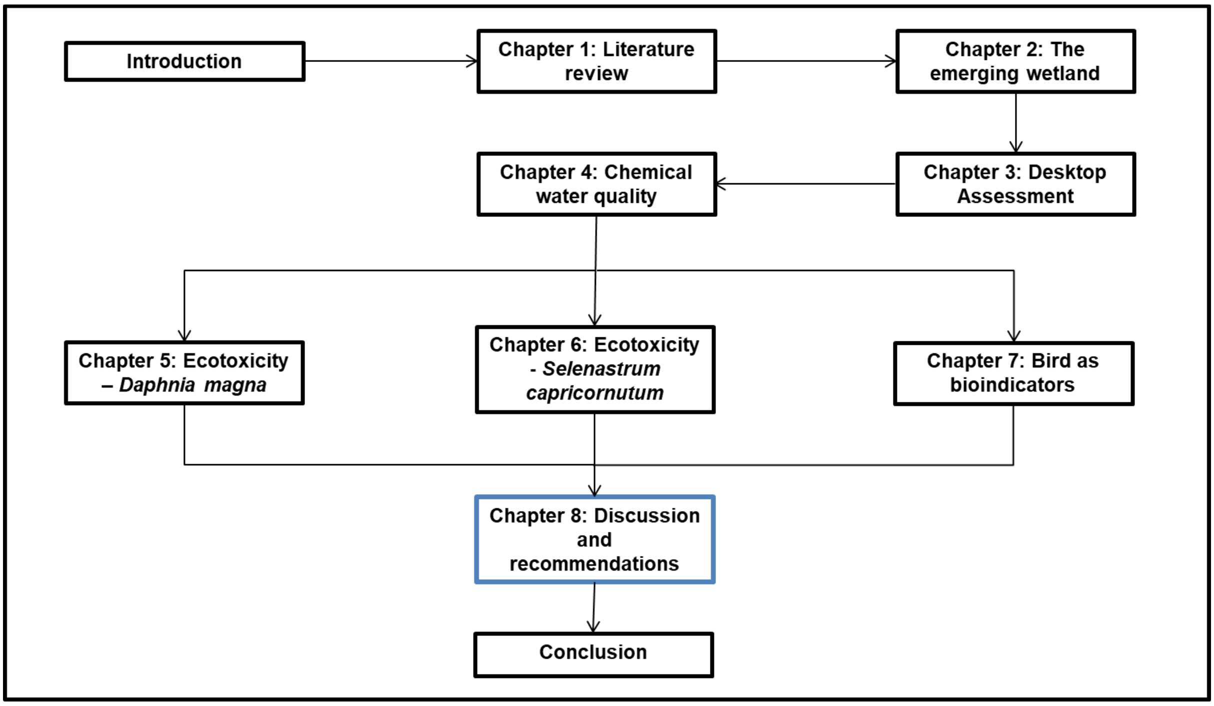

Figure 19: Structure of the dissertation - Chapter 8

The quality of the aquatic environment can be viewed as the concentrations of various water quality parameters, the composition and the fitness of the biota present in the system and the temporal and spatial changes experienced by the water body, both internally and externally (Meybeck *et al.*, 1996). Of particular concern to this wetland are the possible nutrient pathways present within the system that may influence water quality and ecosystem fitness within the wetland and the surrounding aquatic environments.

In the testing of water quality, guidelines such as the World Health Organisations Water Quality Assessment guide encourage the use of a mixed approach that includes both the measurement of physical and chemical parameters, and the use of toxicity testing (WHO, 1996). Toxicity testing provides a useful understanding of the nature of the water in terms of its impact on different species, whilst physical and chemical parameters can be used to identify specific areas of concern.

The results of the study, gathered through chemical water quality analysis and the use of bioindicators, suggest that the water quality in the wetland is of concern. Chemical water analysis (Chapter 4) showed that the numerous water quality parameters, including the concentration of nitrates and nitrites, phosphates and sulphates, as well as TDS measures exceeded various national and/or international guidelines (DWAF, 1996a-e; WHO, 2017). Both *D. magna* and *S. capricornutum* toxicity tests suggested toxicity of the water throughout the wetland to the test organisms (USEPA, 2002; DWAF, 2006). The bird surveys, conducted by the Vaal Bird Club, gave insight into the various bird species present that the wetland supported, with approximately 40% of the possible inland water birds having appeared at the wetland during the six monthly surveys (Hockey *et al.*, 2005). The bird surveys also gave the opportunity to infer different food sources that may be made available by the wetland (Ormerod and Tyler, 1993).

Significant seasonal differences in water quality were not noted in the analysis. However, differences in the pattern of *D.* magna LC_{50} and *S. capricornutum* growth inhibition were examined. Greater differences were noted from the divergence (upstream) to the convergence (downstream) during the summer, and tended to lean towards decreased water quality through the wetland. Changes in the various aspects from the winter sampling to the summer sampling could be attributed to many different factors; however the changes may be related to rainfall. As discussed in Chapters 5 and 6, rainfall events can mobilise contaminants in the catchment, and transport them downstream (Estèbe *et al.*, 1998). This loading in the wetland may make it more difficult for the wetland to assimilate pollutants and compounds.

The results of the chemical water quality analysis indicate that the wetland, in both winter and summer, is exhibiting some (albeit small) degree of denitrification ability, as well as the ability to store phosphorous. In contrast, sulphate concentrations increase within the wetland. As both nitrogen and sulphur cycles in wetlands rely on the wetlands ability to maintain anoxic condition in order to transform the compounds and liberate the gas from the wetland, the results may indicate that the wetland has not established consistent anaerobia. Phosphorous, however, is stored in sediment (Mitsch and Gosselink, 2000). The emerging nature of the wetland may be the reason that the wetland is still able to assimilate the phosphorous. This functionality may not continue indefinitely as the wetland system matures. The increase of sulphate concentration as well as the limited assimilation of nitrogen and phosphorous may also be influenced by evapotranspiration, which can result in the concentration of these compounds in the wetland (Eary, 1998; Bauer-Gottwein *et al.*, 2007; Humphries *et al.*, 2011).

The reasons for poor water quality may be attributed to activities within the catchment. As discussed in Chapter 2, the wetland is situated downstream of both agricultural and industrial activities. These processes and practices, along with urban runoff, add a number of different chemical compounds and microbes to water before it reaches the wetland. As such, this can culminate in the degradation of water quality entering the wetland.

Wetland functions and ecosystem services

Although the wetland may not be considered functional in terms of the improvement of water quality, it remains a functioning system. Wetlands are dynamic systems which provide a host of ecosystem and cultural services. The mere fact that it fails to perform one or even a number of the tasks typically associated with wetlands does not detract from the other services it provides. In this case, the wetland supports various different organisms, from algal populations to vegetation, benthic invertebrates, as well as possible amphibians, fish, and numerous birds. It also has shown the possible ability to assimilate phosphates and to a lesser degree nitrates.

Notwithstanding the aforementioned functionality of the wetland system, poor water quality in wetlands may pose a threat to the various species that are present within wetland environments. This is especially true when degradation of surrounding habitats, largely due to land transformation for urbanisation, agriculture or industry development, make remaining wetlands more attractive to species. Although these environments may be favourable, if wetlands are highly degraded, the possibility of them being ecological traps increases (Sievers *et al.*, 2018). This can be applied to the Sasolburg emerging wetland system, as the wetland is subject to large amounts of complex wastewater throughput. As indicated by the results, the water quality in the wetland is poor. This highly altered system may place greater pressure on organisms which make use of the environment, compromising their fitness over time (Hale *et al.*, 2019).

Wetlands receiving wastewater often tend to be more eutrophic, and as such will appear more productive at some stage of eutrophication. This creates an attractive habitat for waterfowl which feed on plant matter and invertebrates (López-perea *et al.*, 2019). As discussed previously (Chapter 6) it is unlikely that at present the system will present as eutrophic as indications suggest that there are other contaminants that are limiting extreme increases in productivity.

However, it is important to note that in its current condition, the wetland may still provide specialised habitats for certain species. For example, the presence of the Cape Teal is unlikely in low salinity waters (Hockey *et al.*, 2005). Due to the high salinity, the wetland becomes a suitable habitat for the species through provision of appropriate food sources such as pondweed, invertebrates or Platanna. This, however, does not mean that the wetland is truly suitable or will remain a suitable habitat for the Cape Teal, as the system may be disguising underlying pollutants which can impact on the long-term fitness of the organism or the system may undergo changes which impact on water quality in a different manner. The wetland also certainly does not provide suitable refuge for all organisms and as such will not always act as an ecological trap, should it act as one at all.

The change of a wetland from sink to source

Reddy and DeLaune (2008) stress that wetland processes result in three different outcomes. Although wetlands are typically viewed as sinks for nutrients and contaminants, or 'nature's kidneys', wetlands are in fact sinks, sources and transformers of nutrients and contaminants. At any given time, wetlands may function in one, two or all three ways, depending on the aspect being investigated. A good way to illustrate this, as discussed before, is the apparent ability of the wetland of study to act as a sink for phosphates, but as a source of sulphates to the environment.

Chronic overloading

Wetlands typically prevent the direct transport of contaminants, generated within upland catchment areas, into aquatic ecosystems. This is due to their positioning in the landscape, with wetlands generally occurring in the riparian zone, between the terrestrial catchment area and receiving rivers or streams (Reddy and DeLaune, 2008). However, when wetlands are subject to chronic overloading by nutrients, toxicants and pollutants, they may lose their ability as sinks and instead become the source of pollution into these aquatic systems.

According to Dodds and Whiles (2010), there is evidence (on review of various wetland studies) to suggest that all wetlands serve to retain nitrogen whereas a number of wetlands may act as a source of phosphorous to the environment. If loading rates exceed the rate of assimilation, wetlands may not be able to process various chemicals, causing the wetland to expel these chemicals largely untransformed.

Water retention or residence time

As discussed in the literature review, nutrients are not generally regarded as pollutants (Furness, 1993). As essential components in the growth of plants, their presence in water bodies is accepted and predicted. Despite this, nutrients can become pollutants if found in excess such that the aquatic environment cannot process the nutrients to the best of their ability. In wetlands specifically, a contributing factor to this may be the water retention time in the wetland which prevents excess nutrients being removed from the system (Tong *et al.*, 2019).

Wetlands, and in fact all bodies of water, undergo self-purification. This process is largely dependent on time though. The longer an aquatic system has to process nutrients or pollutants, the greater its ability to self-purify (Okafor, 2011).

Much effort has been and is being put into researching ways of increasing water residence time in constructed wetlands for the purpose of water treatment as greater residence time allows for greater contaminant removal (Conn and Fiedler, 2006; Romain *et al.*, 2015; Luan and Burgos, 2019).

Recommendations

Underlying all scientific research, scientists acknowledge that patterns observed may be influenced by a variety of factors that are not considered in the direct interpretation of the results obtained when investigating a scientific theory. This includes instrumentation that may be biased, environments of study that are distinctive or even unusual samples which may be representative of outliers in the study (Montello and Sutton, 2006).

Sampling was conducted in two different ways as a result of constraints imposed by the environment. Although there have been studies that suggest the use of UAVs is practical and can yield accurate results, so too have there been studies that suggest that there is less control over sampling and as such may provide a limitation to analysis (Koparan *et al.*, 2018; Lally *et al.*, 2019).

It is recommended that more sampling be done in the future to ascertain seasonal variation, as well as shifts in the wetland functionality going forward. It would also be beneficial to collect more samples at every sampling occasion. Bird surveys could be conducted on an on-going basis, with valuable long term information on the changing wetland environment being gained. The way in which bird species interact with the wetland environment could also be more thoroughly studied.

Conclusion

At the outset of this study, the aim of the research was to assess the contribution of the emerging wetland system to nutrient assimilation and water quality improvement. Four objectives were identified. The first was to examine the extent of the wetland, as well as seasonal changes to the extent. The second objective was intended to assess the changes in water quality throughout the wetland. The third objective was to examine the ecological impacts of the water quality through ecotoxicity testing and inspection of chemical water quality. Lastly, water quality and assimilation of nutrients were to be examined for seasonal changes. This study encompassed all these features and it was determined that the buffering capacity of this emerging wetland is severely compromised due to a number of possible factors. Furthermore, there were no significant seasonal changes in either that of the chemical water quality or ecotoxicological studies. Although the wetland is limited in terms of nutrient assimilation, it still provides ecosystem services in the form of habitat provision and food sources, amongst many others that were not assessed.

References

Aalto, S.L., Kaski, O., Salonen, K. and Pulkkinen, K. (2013) 'Responses of algae, bacteria, Daphnia and natural parasite fauna of Daphnia to nutrient enrichment in mesocosms', *Hydrobiologia*, 715, pp. 5–18. doi: 10.1007/s10750-012-1261-3.

Anderson, N.J. and Stedmon, C.A. (2007) 'The effect of evapoconcentration on dissolved organic carbon concentration and quality in lakes of SW', *Freshwater Biology*, 52, pp. 280–289. doi: 10.1111/j.1365-2427.2006.01688.x.

Bauer-Gottwein, P., Langer, T., Prommer, H., Wolski, P. and Kinzelbach, W. (2007) 'Okavango Delta Islands: Interaction between density-driven flow and geochemical reactions under evapo-concentration', *Journal of Hydrology*, 335, pp. 389-405.

Bori, J., Vallès, B., Navarro, A. and Riva, M.C. (2016) 'Geochemistry and environmental threats of soils surrounding an abandoned mercury mine', *Environmental Science and Pollution Research*, 23, pp. 12941–12953. doi: 10.1007/s11356-016-6463-1.

Boyer, J.N., Kelble, C.R., Ortner, P.B. and Rudnick, D.T. (2009) 'Phytoplankton bloom status: Chlorophyll a biomass as an indicator of water quality condition in the southern estuaries of Florida, USA', *Ecological Indicators*, 9(6 SUPPL.), pp. 56–67. doi: 10.1016/j.ecolind.2008.11.013.

Cairns Jr., J., McCormick, P.V. and Niederlehner, B.R. (1993) 'A proposed framework for developing indicators of ecosystem health', *Hydrobiologia*, 263, pp. 1-44.

Chislock, M.F., Doster, E., Zitomer, R.A. and Wilson, A.E. (2013) 'Eutrophication: Causes, consequences, and controls in aquatic ecosystems', *Nature Education Knowledge,* 4(4).

Chua, A. (2019) *Testing Water Quality with a Colorimeter - Konica Minolta Color, Light, and Display Measuring Instruments,* Konica Minolta, Available at: https://sensing.konicaminolta.asia/testing-water-quality-with-a-colorimeter/.

CLIMATE-DATA.ORG (2019) *Climate Sasolburg*, Available at: https://en.climate-data.org/africa/south-africa/free-state/sasolburg-27320/.

Collins, N.B. (2005) 'Wetlands: The basics and some more', Free State Department of Tourism, Environmental and Economic Affairs.

Conn, R.M. and Fiedler, F.R. (2006) 'Increasing hydraulic residence time in constructed stormwater treatment wetlands with designed bottom topography', *Water Environment Research*, 78, pp. 2514–2523.

Cunha, P. de S.L., Lanna, E.A.T., Bastos, R.K.X., Quadros, M., Rezende, F.P. and de Freitas, L.M. (2010) 'Fertilizers for *Daphnia* sp. (Crustacea, Cladocera) production in experimental tanks', *Revista Brasileira de Zootecnia*, 39, pp. 955–960.

D'Agostino Sr., R.B., Sullivan, L.M. and Beiser, A.S. (2006) *Introductory applied biostatistics,* Belmont: Brooks/Cole Cengage Learning.

Daigger, G.T. (2004) 'Nutrient Removal in Fixed-Film Processes: Current Design Practices' in Surampalli, R.Y., Tyagi, R.D. (eds.) *Advances in water and wastewater treatment*. Reston, Virginia: American Society of Civil Engineers.

Davis, L. (1995) *A handbook of constructed wetlands - a guide to creating wetlands for: agricultural wastewater, domestic wastewater, coal mine drainage, stormwater in the Mid-Atlantic Region*, Edited by L. Davis, Washington, D.C.: Government Publishers.

Dodds, W.K. and Whiles, M.R. (2010) *Freshwater ecology: concepts and environmental applications of limnology,* Second edn, Amsterdam: Academic Press, an imprint of Elsevier (Aquatic Ecology Series).

Dodson, S. and Hanazato, T. (1995) 'Commentary on effects of anthropogenic and natural organic chemicals on development, swimming behavior, and reproduction of Daphnia, a key member of aquatic ecosystems', *Environmental Health Perspectives*, 103, pp. 7–11.

Dokulil, M.T. (2007) 'Chapter 9 Algae as ecological bio-indicators', *Trace Metals and other Contaminants in the Environment,* 6, pp. 285-327.

Donnenfeld, Z., Crookes, C. and Hedden, S. (2018) *A delicate balance: Water scarcity in South Africa*, Available at: https://issafrica.org/research/southern-africa-report/a-delicate-balance-water-scarcity-in-south-africa.

Driesen, K. (2015) *Acute aquatic toxicity test of caffeine with Daphnia magna Straus and biomonitoring of PRB and Intensive Green Filter wastewater treatment systems*, Ghent University.

DWAF (Department of Water Affairs and Forestry) (1996a) *South African water quality guidelines: Volume 1 - Domestic use*, Pretoria.

DWAF (Department of Water Affairs and Forestry) (1996b*) South African water quality guidelines: Volume 3 - Industrial use*, Pretoria.

DWAF (Department of Water Affairs and Forestry) (1996c) *South African water quality guidelines - Agricultural use: Volume 4 – Irrigation*, Pretoria.

DWAF (Department of Water Affairs and Forestry) (1996d) *South African water quality guidelines - Agricultural use: Volume 5 - Livestock watering,* Pretoria.

DWAF (Department of Water Affairs and Forestry) (1996e) *South African water quality guidelines: Volume 7 - Aquatic ecosystems,* Pretoria.

DWAF (Department of Water Affairs and Forestry) (2003) *The management of complex industrial wastewater discharges: introducing the Direct Estimation of Ecological Effect Potential (DEEEP) approach; a discussion document,* Pretoria.

DWAF (Department of Water Affairs and Forestry) (2005) *A practical field procedure for identification and delineation of wetlands and riparian areas,* Pretoria. Available at: http://biodiversityadvisor.sanbi.org/wp-content/uploads/2016/07/DWS-wetland-delineation-manual.pdf.

DWAF (Department of Water Affairs and Forestry) (2006) 'Method 3003 *Selenastrum capricornutum* Growth Inhibition Test Microplate Assay', *Methods Manual*, Biology Laboratory, Resource Quality Services, Pretoria.

Eary, L.E. (1998) 'Predicting the effects of evapoconcentration on water quality in mine pit lakes', *Journal of Geochemical Exploration*, 64, pp. 223–236.

Elnabarawy, M.T., Welter, A.N. and Robideau, R.R. (1986) 'Relative sensitivity of three Daphnid species to selected organic and inorganic chemicals', *Environmental Toxicology and Chemistry*, 5, pp. 393–398.

Estèbe, A., Mouchel, J.-M. and Thévenot, D.R. (1998) 'Urban runoff impacts on particulate metal concentrations in River Seine'*, Water, Air, and Soil Pollution: An International Journal of Environmental Pollution*, 108, pp. 83–105.

Fairchild, J.F., Ruessler, D.S. and Carlson, A.R. (1998) 'Comparative sensitivity of five species of macrophytes and six species of algae to atrazine, metribuzin, alachlor, and metolachlor', *Environmental Toxicology and Chemistry*, 17(9), pp. 1830–1834.

Fargašová, A. and Kizlink, J. (1996) 'Effect of organotin compounds on the growth of the freshwater alga Scenedesmus quadricauda', *Ecotoxicology and Environmental Safety*, 34(2), pp. 156–159. doi: 10.1006/eesa.1996.0057.

Faulkner, S.P. and Richardson, C.J. (1989) 'Physical and chemical characteristics of freshwater wetland soils', in Hammer, D.A (ed.) *Constructed wetlands for wastewater treatment: Municipal, industrial and agricultural*, Michigan: Lewis Publishers, pp. 41-72.

Flügel, W.A. (1995) 'River salination due to dryland agriculture in the Western Cape Province, Republic of South Africa', *Environment International*, 21(5), pp. 679–686. doi: 10.1016/0160-4120(95)00073-T.

Ford, E.D. (2000) *Scientific method for ecological research*, Cambridge, UK: Cambridge University Press.

Furness, R.W. (1993) 'Birds as monitors of pollutants' in Furness, R.W. and Greenwood, J.J.D. (eds.) *Birds as monitors of environmental change*. London: Chapman & Hall, pp. 86-143.

Furness, R.W., Greenwood, J.J.D and Jarvis, P.J. (1993) 'Can birds be used to monitor the environment?' in Furness, R.W. and Greenwood, J.J.D. (eds.) *Birds as monitors of environmental change*. London: Chapman & Hall, pp. 1-41.

Gambrell, R.P., Wiesepape, J.B., Patrick Jr., W.H. and Duff, M.C. (1991) 'The effects of pH, redox, and salinity on metal release from a contaminated sediment', *Water, Air, and Soil Pollution,* 57, pp. 359-367.

Gheorghe, S., Stoica, C., Vasile, G.G., Nita-Lazar, M., Stanescu, E. and Lucaciu, I.E. (2017) 'Metals toxic effects in aquatic ecosystems: Modulators of water quality' in Tutu, H. (ed.) *Water Quality*, InTechOpen Open Access Publisher. doi: 10.5772/65744.

Ghermandi, A., van den Bergh, J.C.J.M, Brander, L.M., de Groot, H.L.F. and Nunes, P.A.L.D. (2010) 'Values of natural and human - made wetlands : A meta-analysis', *Water Resources Research*, 46. doi: 10.1029/2010WR009071.

Groffen, T., Wepener, V., Malherbe, W. and Bervoets, L. (2018) 'Distribution of perfluorinated compounds (PFASs) in the aquatic environment of the industrially polluted Vaal River, South Africa', *Science of the Total Environment*. Elsevier B.V., 627, pp. 1334–1344. doi: 10.1016/j.scitotenv.2018.02.023.

Hammer, D.A. and Bastian, R.K. (1989) 'Wetlands ecosystems: natural water purifiers?' in Hammer, D.A. (ed.) *Constructed wetlands for wastewater treatment: municipal, industrial and agricultural*, Michigan: Lewis Publishers, Inc., pp. 5-19.

Hach (2006) 'Hydrolab DS5X, DS5, and MS5 water quality multiprobes', United States of America.

Hach (2013) 'Hach DR7890 Procedures Manual', United States of America.

Hale, R., Swearer, S.E., Sievers, M. and Coleman, R. (2019) 'Balancing biodiversity outcomes and pollution management in urban stormwater treatment wetlands', *Journal of Environmental Management*, Elsevier, 233, pp. 302–307. doi: 10.1016/j.jenvman.2018.12.064.

Hockey, P.A., Dean, W.R. and Ryan, P.G. (eds) (2005) *Roberts' Birds of Southern Africa*. 7th edition. The Trustees of John Voeckler Bird Book Fund, Cape Town.

Holt, E.A. & Miller, S.W. (2010) Bioindicators: using organisms to measure environmental impacts, *Nature Education Knowledge* 3(10).

Humphries, M.S., Kindness, A., Ellery, W.N. and Hughes, J.C. (2011) 'Water chemistry and effect of evapotranspiration on chemical sedimentation on the Mkuze River floodplain, South Africa', *Journal of Arid Environments*, 75, pp. 555-565.

Integrated Water Resource Studies (2014) 'The National State of Water Resources: quarterly report for July to September 2014'.

Ireland Environmental Protection Agency (2001) *Parameters of water quality - Interpretation and standards*, Wexford.

Jankowski, T., Livingstone, D.M., Bührer, H., Forster, R. and Niederhauser, P. (2006) 'Consequences of the 2003 European heat wave for lake temperature profiles, thermal stability, and hypolimnetic oxygen depletion: Implications for a warmer world', *Limnology and Oceanography*, 51, pp. 815-819.

Jeppesen, E. *et al.* (2015) 'Ecological impacts of global warming and water abstraction on lakes and reservoirs due to changes in water level and related changes in salinity', *Hydrobiologia*, 750(1), pp. 201–227. doi: 10.1007/s10750-014-2169-x.

Jury, M.R. (2016) 'Climate influences on Vaal River flow', *Water SA*, 42, pp. 232–242.

Kalff, J. (2002). *Major Ions, Carbonate System, Alkalinity, pH*, Available at: http://web.pdx.edu/~sytsmam/limno/Limno09.6.IonsandANC.pdf.

Koparan, C., Koc, A.B., Privette, C.V., Sawyer, C.B. and Sharp, J.L. (2018) 'Evaluation of a UAV-assisted autonomous water sampling', *Water*, 10(655). doi: 10.3390/w10050655.

Koskimies, P. (1989) 'Birds as a tool in environmental monitoring', *Annales Zoologici Fennici*, 26, pp. 153-166.

Kremser, U. and Schnug, E. (2002): 'Impact of fertilizers on aquatic ecosystems and protection of water bodies from mineral nutrients', *Landbauforschung Völkenrode*, 52, pp. 81-90.

Krienitz, L., Bock, K., Nozaki, H. and Wolf, M. (2011) 'SSU rRNA gene phylogeny of morphospecies affiliated to the bioassay alga "'*selenastrum capricornutum*" recovered the polyphyletic origin of crescent-shaped chlorophyta', *Journal of Phycology*, 47, pp. 880–893. doi: 10.1111/j.1529-8817.2011.01010.x.

Lally, H.T., O'Connor, I., Jensen, O.P. and Graham, C.T. (2019) 'Can drones be used to conduct water sampling in aquatic environments? A review', *Science of the Total Environment*, Elsevier B.V., 670, pp. 569–575. doi: 10.1016/j.scitotenv.2019.03.252.

Loftie-Eaton, M. (2014) *Geographic range dynamics of South Africa's bird species*, University of Cape Town.

López-perea, J.J., Laguna, C., Jiménez-Moreno, M., Martín-Doimeadios, R.C.R., Feliu, J. and Mateo, R. (2019) 'Metals and metalloids in blood and feathers of common moorhens (*Gallinula chloropus*) from wetlands that receive treated wastewater', *Science of the Total Environment,* Elsevier B.V., 646, pp. 84–92. doi: 10.1016/j.scitotenv.2018.07.265.

Luan, F. and Burgos, W.D. (2019) 'Effects of solid-phase organic carbon and hydraulic residence time on manganese (II) removal in a passive coal mine drainage treatment system', *Mine Water and the Environment,* Springer Berlin Heidelberg, 38, pp. 130–135. doi: 10.1007/s10230-018-0548-7.

Ma, J., Wang, S., Wang. P., Ma, L., Chen, X. and Xu, R. (2006) 'Toxicity assessment of 40 herbicides to the green alga *Raphidocelis subcapitata'*, *Ecotoxicology and Environmental Safety*, 63(3), pp. 456–462. doi: 10.1016/j.ecoenv.2004.12.001.

Maciorowski, A. and Sims, J. (1981) 'Bioassays: Procedures and results', *Water Pollution Control Federation*, 54(6), pp. 830–848.

Marzullo, R. de C.M., Matai, P.H.L. dos S. and Morita, D. M. (2018) 'New method to calculate water ecotoxicity footprint of products: A contribution to the decision-making process toward sustainability', *Journal of Cleaner Production*, 188, pp. 888–899. doi: 10.1016/j.jclepro.2018.03.307.

McCormick, P.V and Cairns Jr., J. (1994) 'Algae as indicators of environmental change', *Journal of Applied Phycology*, 6, pp. 509–526.

Mellink, E., Luévano, J. and Riojas-López, M.E. (2018) 'Half a century of changes in waterbird populations in a semiarid wetland system', *Wetlands Ecology and Management*, 26, pp. 1047–1060. doi: 10.1007/s11273-018-9630-y.

Meybeck, M., Kimstach, V. and Helmer, R. (1996) 'Chapter 2 - Strategies for water quality assessment', in Chapman, D. (ed.) *Water quality assessments - A guide to use of biota, sediments and water in environmental monitoring*, 2nd edn, E&FN Spon.

Michaud, J.P. (1991) *A citizens' guide to understanding and monitoring lakes and streams*, Washington State Department of Ecology.

Middleton, B.A. (2018) 'Succession in Wetlands', in Finlayson, C.M. et al. (eds) *The wetland book: Structure and function, management, and methods*, Springer Nature, pp. 17–34. doi: 10.1007/978-90-481-9659-3.

Mitsch, W.J., Gosselink, J.G., Anderson, C.J. and Zhang, J. (2009), *Wetland ecosystems*, Hoboken: John Wiley & Sons.

Mitsch, W.J. and Gosselink, J.G. (2000) *Wetlands*, Third edition, Canada: John Wiley & Sons.

Moiseenko, T.I. (2008) 'Aquatic ecotoxicology: Theoretical principles and practical application', *Water Resources*, 35(5), pp. 530–541. doi: 10.1134/S0097807808050047.

Montello, D.R. and Sutton, P.C. (2006) *An introduction to scientific research methods in geography*, Thousand Oaks, California: Sage Publications.

Moore, R., Richards, G. and Story, A. (2008) 'Electrical conductivity as an indicator of water chemistry and hydrologic process', *Streamline Watershed Management Bulletin*, 11(2), pp. 25–29. doi: 10.1016/j.vetmic.2013.02.008.

National Research Council (U.S.) and Committee on Characterization of Wetlands (1995) *Wetlands: Characteristics and boundaries*, Washington, D.C.: National Academy Press. doi: 10.17226/4766.

Nicholson, S.E., Funk, C. and Fink, A.H. (2018) 'Rainfall over the African continent from the 19th through the 21st century', *Global and Planetary Change,* Elsevier, 165(May 2017), pp. 114–127. doi: 10.1016/j.gloplacha.2017.12.014.

Nickson, R.T., McArthur, J.M., Shrestha, B., Kyaw-Myint, T.O. and Lowry, D. (2005) 'Arsenic and other drinking water quality issues, Muzaffargarh District , Pakistan', *Applied Geochemistry*, 20, pp. 55–68. doi: 10.1016/j.apgeochem.2004.06.004.

Nikolov, G., Gieva, E., Nikolova, B. and Marinov, M. (2016) ‘Virtual Colorimeter for Water Analysis’, in *2016 XXV International Scientific Conference Electronics (ET). IEEE*, pp. 1–4. doi: 10.1109/ET.2016.7753502.

Nordberg, G.F., Fowler, B.A., Nordberg, M. and Friberg, L.T. (2007) ‘Chapter 1: Introduction – general considerations and international perspectives’, in Nordberg, G.F (ed.) *Handbook on the toxicology of metals*, Third edition, Amsterdam: Academic Press, pp. 1-9.

Okafor, N. (2011) ‘Pollution of aquatic systems: pollution through eutrophication, fecal materials, and oil spills’, in Okafor, N. (ed.) *Environmental microbiology of aquatic and waste systems*, Springer, pp. 151-187.

Olson, J.R. and Hawkins, C.P. (2017) ‘Effects of total dissolved solids on growth and mortality predict distributions of stream macroinvertebrates’, *Freshwater Biology*, 62(4), pp. 779–791. doi: 10.1111/fwb.12901.

Ormerod, S.J. and Tyler, S.J. (1993) ‘Birds as indicators of changes in water quality’ in Furness, R.W. and Greenwood, J.J.D. (eds.) *Birds as monitors of environmental change*. London: Chapman & Hall, pp. 179-216.

Pal, M., Samal, N.R., Roy, P.K. and Roy, M.B. (2015) ‘Electrical Conductivity of Lake Water as Environmental Monitoring – A Case Study of Rudrasagar Lake’, *IOSR Journal of Environmental Science Ver. I*, 9(3), pp. 2319–2399. doi: 10.9790/2402-09316671.

Palmer, C.G., Muller, W.J., Gordon, A.K., Scherman, P-A., Davies-Coleman, H.D., Pakhomova, L. and de Kock, E. (2004) ‘The development of a toxicity database using freshwater macroinvertebrates, and its application to the protection of South African water resources’*, South African Journal of Science*, 100(11–12), pp. 643–650.

Persoone, G., Baudo, R., Cotman, M., Blaise, C., Thompson, K.Cl., Moreira-Santos, M., Vollat, B., Törökne, A. and Han, T. (2009) ‘Review on the acute *Daphnia magna* toxicity test – Evaluation of the sensitivity and the precision of assays performed with organisms from laboratory cultures or hatched from dormant eggs’, *Knowledge and Management of Aquatic Ecosystems*, (393), p. 01. doi: 10.1051/kmae/2009012.

Ramsar Convention Secretariat (2016) ‘An Introduction to the Convention on Wetlands (previously The Ramsar Convention Manual), Ramsar Convention Secretariat, Gland, Switzerland. Design’.

Reddy, K.R. and DeLaune, R.D. (2008) *Biogeochemistry of wetlands: science and applications*, Boca Raton: CRC Press.

RSA (Republic of South Africa) (2013) Department of Water Affairs and Forestry, Government Notice 665 - Government Gazette 36820. Pretoria, Available at: https://cer.org.za/wp-content/uploads/2014/02/Revision-of-General-Authorisations-2013.pdf.

Rojíčková, R. and Maršálek, B. (1999) ‘Selection and sensitivity comparisons of algal species for testing’, *Chemosphere*, 38(14), pp. 3329–3338.

Romain, V., Sylvie, D. and David, B. (2015) ‘Water residence time and pesticide removal in pilot-scale wetlands’, *Ecological Engineering,* Elsevier B.V., 85, pp. 76–84. doi: 10.1016/j.ecoleng.2015.09.040.

Roos, J.C. and Pieterse, A.J.H. (1995) ‘Salinity and dissolved substances in the Vaal River at Balkfontein, South Africa’, *Hydrobiologia,* 306(1), pp. 41–51. doi: 10.1007/BF00007857.

SABAP2 (2019) The Southern African Bird Atlas Project - Monitoring into the future, Available at: http://sabap2.adu.org.za/index.php.

SANBI (South African National Biodiversity Institute) (2011) *National Biodiversity Assessment 2011: synthesis report - an assessment of South Africa's biodiversity and ecosystems*, Available at: http://biodiversityadvisor.sanbi.org/wp-content/uploads/2016/07/NBA-2011-Synthesis-Report-low-resolution.pdf.

Scherman, P.A., Muller, W J. and Palmer, C.G. (2003) 'Links between ecotoxicology, biomonitoring and water chemistry in the integration of water quality into environmental flow assessments', *River Research and Applications,* 19(5–6), pp. 483–493. doi: 10.1002/rra.751.

Scholz, M. (2006a) 'Chapter 16 - Constructed wetlands', in Scholz, M. (ed.) *Wetland systems to control urban runoff,* Amsterdam: Elsevier, pp. 91–110. doi: 10.1016/B978-0-444-52734-9.50019-0.

Scholz, M. (2006b) 'Chapter 1 - Water quality standards', in Scholz, M. (ed.) *Wetland systems to control urban runoff,* Amsterdam: Elsevier, pp. 1–5. doi: 10.1016/B978-0-444-52734-9.50004-9.

Shubert, L.E. (1984) *Algae as ecological indicators*, London: Academic Press.

Sharma, R.C., Singh, N. and Chauhan, A. (2016) 'The influence of physico-chemical parameters on phytoplankton distribution in a head water stream of Garhwal Himalayas: A case study', *Egyptian Journal of Aquatic Research*, National Institute of Oceanography and Fisheries, 42(1), pp. 11–21. doi: 10.1016/j.ejar.2015.11.004.

Sievers, M., Hale, R., Parris, K.M. and Swearer, S.E. (2018) 'Impacts of human-induced environmental change in wetlands on aquatic animals', *Biological Reviews*, 93, pp. 529–554. doi: 10.1111/brv.12358.

Skulberg, O.M. (1964) 'Algal problems related to the eutrophication of European water supplies, and a bioassay method to assess fertilization influence of pollution on inland waters', in Jackson, D.F. (ed.) *Algae and Man*, New York: Plenum Press, pp. 262–99.

Slabbert, L. (2004) 'Methods for direct estimation of ecological effect potential (DEEEP)', South Africa: Water Research Commission (WRC report, no. 1313/1/04).

Smith, V.H., Tilman, G.D. and Nekola, J.C. (1999) 'Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems', *Environmental Pollution*, 100, pp. 179–196. doi: 10.1016/S0269-7491(99)00091-3.

Taylor, N.S. (2010) *Novel approaches to toxicity testing in Daphnia magna*, The University of Birmingham.

Tolotti, M., Thies, H., Nickus, U. and Psenner, R. (2012) 'Temperature modulated effects of nutrients on phytoplankton changes in a mountain lake', *Hydrobiologia*, 698(1), pp. 61–75. doi: 10.1007/s10750-012-1146-5.

Tong, Y. *et al.* (2019) 'Impacts of water residence time on nitrogen budget of lakes and reservoirs', *Science of the Total Environment*, 646, pp. 75–83.

Tonkopii, V. and Iofina, I. (2008) 'The usage of *Daphnia magna* as alternative bioobject in ecotoxicology', *Japanese Society for Alternatives to Animal Experiments*, pp. 565–567.

Tozer, D.C., Steele, O. and Gloutney, M. (2018) 'Multispecies benefits of wetland conservation for marsh birds, frogs, and species at risk', *Journal of Environmental Management*, Elsevier Ltd, 212, pp. 160–168. doi: 10.1016/j.jenvman.2018.01.055.

Tyagi, V.K., Chopra, A.K., Durgapal, N.C. and Kumar, A. (2009) 'Evaluation of *Daphnia magna* as an indicator of toxicity and treatment efficacy of municipal sewage treatment plant', *Journal of Applied Sciences and Environmental Management*, 11(1). doi: 10.4314/jasem.v11i1.46835.

USEPA (2002) *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms*, Washington, D.C.

USGS (2005) *National field manual for the collection of water-quality data*, Reston.

Valavanidis, A. and Vlachogianni, T. (2015) 'Ecotoxicity test methods and ecological risk assessment: Aquatic and terrestrial ecotoxicology tests under the guidelines of international organizations', Available at: https://www.researchgate.net/publication/281061444_Ecotoxicity_Test_Methods_and_Ecological_Risk_Assessment_Aquatic_and_Terrestrial_Ecotoxicology_Tests_under_the_Guidelines_of_International_Organizations.

Van Coillie, R., Couture, P. and Visser, S.A. (1983) 'Use of algae in aquatic ecotoxicology' in Nriagu, J. (ed.) *Aquatic toxicology*, New York: Wiley, pp. 487-502.

Wang, J., Fu, Z., Qiao, H. and Liu, F. (2019) 'Assessment of eutrophication and water quality in the estuarine area of Lake Wuli, Lake Taihu, China', *Science of the Total Environment*, Elsevier B.V., 650, pp. 1392–1402. doi: 10.1016/j.scitotenv.2018.09.137.

Water Environment Federation: Nutrient Removal Task Force (2011) *Nutrient removal*, New York: McGraw-Hill.

Whewell, W. (1989) *Theory of the scientific method*, Cambridge: Hackett Publishing Company.

WHO (World Health Organization) (1996) *Water quality assessments - a guide to use of biota, sediments and water in environmental monitoring*, Second edition, Cambridge: University Press.

WHO (World Health Organization) (2017) *Guidelines for drinking-water quality: Fourth edition incorporating the first addendum,* Geneva.

Zedler, J.B. and Kercher, S. (2005) 'Wetland resources: status , trends , ecosystem services, and restorability', *Annual Review of Environmental Resources*, 30, pp. 39–74. doi: 10.1146/annurev.energy.30.050504.144248.

Zhuang, Q., Zhu, X., He, Y., Prigent, C., Melillo, J.M., McGuire, A.D., Prinn, R.G. and Kicklighter, D.W. (2015) 'Influence of changes in wetland inundation extent on net fluxes of carbon dioxide and methane in northern high latitudes from 1993 to 2004', *Environmental Research Letters*, 10.

Appendix A

Table 19: Physico-chemical readings before the start to the winter *D. magna* acute toxicity test.

Winter sampling	Dissolved oxygen (mg/L)	Electrical conductivity (µS/cm)	pH (pH units)	Temperature (°C)
Control	7,11	238	7,5	21,1
		Upstream		
3.125%				
6.25%				
12.5%				
25%				
50%				
Undiluted	8,02	6340	7,33	20,1
		West pond		
3.125%	6,53	358	7,8	21,2
6.25%	6,57	504	7,62	21,1
12.5%	6,4	821	7,57	21,2
25%	6,23	1393	7,47	21,2
50%	5,75	2450	7,4	21,3
Undiluted	6,6	4990	7,5	21,4
		East pond		
3.125%	6,75	401	7,67	21,2
6.25%	6,83	592	7,58	21,2
12.5%	7,09	915	7,5	21,3
25%	7,3	1510	7,4	21,4
50%	7,25	2490	7,35	21,5
Undiluted	7,97	5220	7,2	21,6
		Downstream		
3.125%	7,57	428	7,67	20,6
6.25%	7,57	599	7,64	20,7
12.5%	7,51	923	7,59	20,1
25%	7,43	1551	7,49	19,7
50%	7,19	2530	7,49	19
Undiluted	7,88	4650	7,4	19

Table 20: Physico-chemical readings before the start to the summer *D. magna* acute toxicity test

Summer sampling	Dissolved oxygen (mg/L)	Electrical conductivity (µS/cm)	pH (pH units)	Temperature (°C)
Control	9,56	226	7,73	19,2
		Upstream		
3.125%	7,02	447	7,42	19,9
6.25%	7,14	658	7,45	19,9
12.5%	7,08	1033	7,39	19,8
25%	7,19	1793	7,36	19,9
50%	7,36	3200	7,25	20,2
Undiluted	7,49	6080	6,92	20,2
		West pond		
3.125%	6,97	605	7,61	19,9
6.25%	7,18	877	7,55	19,8
12.5%	7,01	1468	7,55	19,9
25%	7,03	2440	7,52	19,9
50%	6,99	3910	7,54	20,4
Undiluted	6,2	7610	7,52	20,7
		East pond		
3.125%	7	548	7,6	19,7
6.25%	7,07	837	7,6	19,6
12.5%	7,04	1380	7,62	19,7
25%	7,09	2260	7,64	19,8
50%	7,02	3790	7,64	20,4
Undiluted	6,54	7070	7,53	20,4
		Downstream		
3.125%	6,96	533	7,58	19,5
6.25%	7,08	792	7,64	19,4
12.5%	7,05	1307	7,7	19,5
25%	7,08	2230	7,77	19,9
50%	7,13	4090	7,71	20,1
Undiluted	7,01	6880	7,68	20,6

Appendix B

Common name	Scientific name
Bishop, Southern Red	*Euplectes orix*
Bishop, Yellow-crowned	*Euplectes afer*
Bittern, Little	*Ixobrychus minutus*
Buzzard, Common	*Buteo buteo*
Canary, Black-throated	*Serinus atrogularis*
Canary, Yellow	*Crithagra flaviventris*
Chat, Anteating	*Myrmecocichla formicivora*
Cisticola, Cloud	*Cisticola textrix*
Cisticola, Levaillant's	*Cisticola tinniens*
Cisticola, Wing-snapping	*Cisticola ayresii*
Cisticola, Zitting	*Cisticola juncidis*
Cormorant, Reed	*Microcarbo africanus*
Crake, Black	*Amaurornis flavirostra*
Cuckoo, Diederick	*Chrysococcyx caprius*
Cuckoo, Red-chested	*Cuculus solitarius*
Dove, Cape Turtle	*Streptopelia capicola*
Dove, Laughing	*Spilopelia senegalensis*
Dove, Red-eyed	*Streptopelia semitorquata*
Duck, Yellow-billed	*Anas undulata*
Egret, Great	*Ardea alba*
Egret, Little	*Egretta garzetta*
Egret, Western Cattle	*Bubulcus ibis*
Fiscal, Common (Southern)	*Lanius collaris*
Flycatcher, Fiscal	*Melaenornis silens*
Francolin, Orange River	*Scleroptila gutturalis*
Goose, Egyptian	*Alopochen aegyptiaca*
Goose, Spur-winged	*Plectropterus* gambensis
Grebe, Little	*Tachybaptus ruficollis*
Greenshank, Common	*Tringa nebularia*
Guineafowl, Helmeted	*Numida meleagris*
Gull, Grey-headed	*Chroicocephalus cirrocephalus*
Hamerkop	*Scopus umbretta*
Heron, Black-headed	*Ardea melanocephala*
Heron, Squacco	*Ardeola ralloides*
Ibis, African Sacred	*Threskiornis aethiopicus*
Ibis, Glossy	*Plegadis falcinellus*
Ibis, Hadeda	*Bostrychia hagedash*
Kite, Black-winged	*Elanus caeruleus*
Kite, Yellow-billed	*Milvus aegyptius*
Korhaan, Blue	*Eupodotis caerulescens*
Korhaan, Northern Black	*Afrotis afraoides*
Lapwing, African Wattled	*Vanellus senegallus*
Lapwing, Blacksmith	*Vanellus armatus*
Lapwing, Crowned	*Vanellus coronatus*
Lark, Red-capped	*Calandrella cinerea*
Longclaw, Cape	*Macronyx capensis*
Martin, Banded	*Riparia cincta*
Martin, Brown-throated	*Riparia paludicola*
Moorhen, Common	*Gallinula chloropus*
Myna, Common	*Acridotheres tristis*

Neddicky	*Cisticola fulvicapilla*
Ostrich, Common	*Struthio* camelus
Owl, Marsh	*Asio capensis*
Palm-swift, African	*Cypsiurus parvus*
Pigeon, Speckled	*Columba guinea*
Pipit, African	*Anthus cinnamomeus*
Pipit, Buffy	*Anthus vaalensis*
Plover, Kittlitz's	*Charadrius pecuarius*
Plover, Three-banded	*Charadrius tricollaris*
Prinia, Black-chested	*Prinia* flavicans
Quailfinch, African	*Ortygospiza atricollis*
Quelea, Red-billed	*Quelea quelea*
Reed Warbler, African	*Acrocephalus baeticatus*
Robin-chat, Cape	*Cossypha caffra*
Ruff	*Calidris pugnax*
Sandpiper, Curlew	*Calidris ferruginea*
Sandpiper, Wood	*Tringa glareola*
Shelduck, South African	*Tadorna cana*
Shrike, Red-backed	*Lanius collurio*
Snipe, African	*Gallinago nigripennis*
Sparrow, Cape	*Passer melanurus*
Sparrow, House	*Passer domesticus*
Spurfowl, Swainson's	*Pternistis swainsonii*
Starling, Cape Glossy	*Lamprotornis nitens*
Starling, Wattled	*Creatophora cinerea*
Stilt, Black-winged	*Himantopus himantopus*
Stint, Little	*Calidris minuta*
Stonechat, African	*Saxicola torquatus*
Stork, Woolly-necked	*Ciconia episcopus*
Swallow, Barn	*Hirundo rustica*
Swallow, Greater Striped	*Cecropis cucullata*
Swallow, South African Cliff	*Petrochelidon spilodera*
Swallow, White-throated	*Hirundo albigularis*
Swift, Horus	*Apus horus*
Swift, Little	*Apus affinis*
Swift, White-rumped	*Apus caffer*
Teal, Cape	*Anas capensis*
Teal, Hottentot	*Spatula hottentota*
Teal, Red-billed	*Anas erythrorhyncha*
Tern, Caspian	*Hydroprogne caspia*
Tern, Whiskered	*Chlidonias hybrida*
Thick-knee, Spotted	*Burhinus capensis*
Thrush, Karoo	*Turdus smithi*
Wagtail, Cape	*Motacilla capensis*
Wagtail, Yellow	*Motacilla flava*
Warbler, Lesser Swamp	*Acrocephalus gracilirostris*
Warbler, Little Rush	*Bradypterus baboecala*
Waxbill, Common	*Estrilda astrild*
Weaver, Southern Masked	*Ploceus velatus*
Weaver, Thick-billed	*Amblyospiza albifrons*
Wheatear, Capped	*Oenanthe pileata*
Whydah, Pin-tailed	*Vidua macroura*
Widowbird, Fan-tailed	*Euplectes axillaris*

Widowbird, Long-tailed	*Euplectes progne*
Widowbird, Red-collared	*Euplectes ardens*
Widowbird, White-winged	*Euplectes albonotatus*