
DEVELOPMENT OF DIATOM-BASED MONITORING TOOLS FOR ASSESSING DEPRESSIONAL WETLAND CONDITION IN THE MPUMALANGA HIGHVELD REGION, SOUTH AFRICA

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DECLARATION

I, Luisa Riato, declare that the thesis, which I hereby submit for the degree PhD 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.

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ABSTRACT

Diatoms have a successful history of use in assessments of wetland biological condition. In North America and across Europe, diatom assemblages are used for routine wetland condition assessments to meet the statutory requirements of the European Water Framework Directive and the National Aquatic Resource Survey by the US Environmental Protection Agency. In South Africa, the use of diatom assemblages as indicators of wetland condition may be a promising alternative to the traditional biotic assemblages employed, such as macroinvertebrates or macrophytes, which have proven to be ineffective. We present a preliminary investigation on the feasibility of diatoms in wetland biological assessments in South Africa by evaluating the use of diatoms as indicators of biological condition for depressional wetlands in the Mpumalanga Highveld region of South Africa.

Depressional wetlands typically found in this region are either temporary (seasonally inundated) or permanent depressions. Temporary depressional wetlands are expected to be affected by natural environmental disturbances (e.g., seasonal fluctuations in water-level which may cause changes in water chemistry) as compared to relatively stable permanent ones. Establishing whether diatoms are suitable indicators of natural environmental disturbances in temporary depressional wetlands in this region is necessary for further investigations of anthropogenic disturbances.

We sampled epiphytic diatoms from three least human-disturbed temporary depressional wetlands during various stages of inundation and showed that the species composition of epiphytic diatom communities were strong indicators of temporally changing environmental conditions. Using the same diatom and physical and chemical data, we also demonstrated that simplifying the taxonomy by using the functional composition (ecological guilds, life-forms) of the epiphytic diatom communities, can assess temporally changing environmental conditions as effectively as the species composition. Moreover, these functional groups provide valuable ecological information that is not available from the species data.

Acid mine drainage (AMD) is the predominant stressor in permanent depressional wetlands of the Mpumalanga Highveld region, where coal mines utilise these wetlands for storage of AMD, which has severe impacts on the structure and function of the ecosystem. In order to develop an approach for impact assessment and management of depressional wetlands in the region, we developed an epiphytic diatom multimetric index (MMI) for AMD impacted permanent depressional wetlands. This is also the first diatom index to quantify AMD impacts in wetland habitats. Data collected from 34 sites that represented a range of conditions along an AMD gradient within the Mpumalanga Highveld was used to select responsive diatom metrics which we combined into a multimetric index. We developed separate MMIs for classes of depressional wetland types in order to account for natural variation among diatom assemblages, and compared their performance with an MMI that did not account for natural variation. To account for natural variation, we classified reference sites based on diatom typologies and hypothesised that by using this approach, we would improve MMI performance. Overall, all MMIs performed considerably well, although grouping sites by diatom typology to account for natural variation improved MMI performance, especially the precision, responsiveness and sensitivity to disturbance.

We conclude that diatoms have strong potential for use in wetland ecological assessments in South Africa. The experimental and statistical approaches used in this study should expand our knowledge of diatom ecology and further advance the research and development of diatom bioassessment.

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Photograph by Dieter Kassier.

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MANUSCRIPTS AND CO-AUTHOR CONTRIBUTIONS

The following manuscripts were published during the review of this thesis. In each case, L.R. designed the study, took on the fieldwork, laboratory and statistical analysis and the writing of the manuscripts, with input from P.J.O., M.L. and V.D. who supervised the study. J.C.T, co-author on the Chapter 3 manuscript, oversaw the study design and analysis, and commented on the manuscript.

CHAPTER 2

Riato, L., Leira, M., Della Bella, V. & Oberholster, P.J. 2017. Diatom-based models for inferring water chemistry and hydrology in temporary depressional wetlands. *Hydrobiologia*, 1-17.

CHAPTER 3

Riato, L., Della Bella, V., Leira, M., Taylor, J.C. & Oberholster, P.J. 2017. A diatom functional-based approach to assess changing environmental conditions in temporary depressional wetlands. *Ecological Indicators*, 78: 205-213.

CHAPTER 1

GENERAL INTRODUCTION

Freshwater ecosystems are among the most altered systems on Earth (Gleick, 2014). Rivers, streams, lakes and wetlands have undergone chemical, physical and biological modifications as a result of large-scale hydrological changes, chemical-inputs, aquatic invasive species, overharvesting, and climate change (Carpenter et al., 2011). Freshwater resources are becoming increasingly scarce as a result and both the quantity and quality of our existing resources require special attention. Water resource management and monitoring have become an urgent need for Governments, and countries have been implementing national water monitoring programmes over several decades, mostly for rivers, streams and lakes (e.g., (Karr & Dudley, 1981; Pollard & Huxham, 1998; Birk et al., 2012).

Wetlands constitute a major aspect of freshwater resources. Yet, the importance of wetland monitoring has only been recognised recently (MacKay et al., 2009; Miller et al., 2016), despite the fact that half the global wetland area has been lost over the last century. Only 10% of the remaining wetlands are protected and many are in poor condition (Zedler & Kercher, 2005). The Ramsar Convention on Wetlands was accepted almost half a century ago as the first international regulation to protect wetlands at a global level. However, in Europe, only in the past decade has there been growing awareness of the ecological and functional importance of wetlands. In particular Mediterranean ponds, which have become a priority habitat under the EU Habitats Directive due to the ever-increasing loss and degradation of ponds and other small waterbodies as a result of urbanisation and agricultural development (Wood et al., 2003; Biggs et al., 2005; Della Bella & Mancini, 2009). In North America, the government has implemented the restoration of former wetlands or wetlands that have been severely degraded under the US watershed management programmes required by the US Clean Water Act (Section 404). In South Africa, wetland systems which face similar pressures have largely been ignored. A decade ago it was estimated that a large number (35-50%) of wetlands in South Africa have been lost or severely damaged due to drainage, reduced inflows, siltation, and encroachment and overharvesting (DWAF, 2005; Ewart-Smith et al., 2006). This decline has continued, making those wetlands that remain among South Africa’s most threatened natural areas.

Recognising the importance of wetland preservation, a National Wetlands Research Programme was initiated in 2003 by the Water Research Commission (WRC) in collaboration with other national government decision-makers such as Department of Water Affairs (DWA) and Department of Water and Environmental Affairs (DWEA), which was aimed at optimising wetland conservation in the context of management, protection, rehabilitation and sustainable use (Day & Malan, 2010). This research programme is largely concerned with wetland health and integrity. The assessment and sustainability of wetland health and integrity is an important component of the overall sustainability and resource assessment within wetlands. Considering this, one of the major initiatives of the programme is to develop biological assessment tools of wetland condition, some of which I review in the following section, while also pointing out knowledge gaps on the topic, which this thesis aims to address.

1.1 BACKGROUND

1.1.1 Biological assessment tools in wetlands

Barbour et al. (1999) defined a biological assessment as “an evaluation of the condition of a waterbody using biological surveys and other direct measurements of the resident biota in surface waters”. Water quality assessments using biota were first practised at the turn of the twentieth century. Kolkwitz & Marsson (1902, 1908, 1909) developed a system to monitor the effects of point source pollution from sewage discharges on aquatic fauna and flora (including diatoms) in Central Europe. This system, known as the Saprobic system, was based on observations of temporal and spatial changes in abundance and distribution of biological taxa in response to organic pollution. Subsequently, techniques using aquatic biota to assess pollution in rivers were developed and the approach of biological indicators was developed.

Biological indicators are taxa or functional groups which are sensitive to changes (biochemical, physiological, or behavioural) within their ecosystem and whose function, population or status provides an integrated record of the ecological integrity of the system (e.g., the cumulative impacts of various pollutants on the ecosystem and the timescale the system has been impacted) (Karr, 1981). Since the 1960’s, the use of multiple biological indicators in biological assessment and monitoring techniques of aquatic resources has become widespread (Gerhardt, 2002). Species composition of major taxonomic groups (from different trophic levels) incorporating a wide range of environmental tolerances can provide multiple data sources to evaluate the resource condition in a “biotic index” or “multimetric” approach (Karr & Chu, 1999). Karr (1981) proposed the index of biological integrity, or IBI, which was antecedent to the development of multimetric indices (MMIs).

MMIs are commonly used in biological assessments because they include a collection of individual community metrics that reflect the effects of human disturbance on biological condition (Karr, 1991). Fish, macroinvertebrates, macrophytes and diatom communities have been widely used as a tool for biologically monitoring aquatic ecosystems (Allen et al., 1999;

Hering et al., 2006a; Johnson & Hering, 2009) although Stevenson et al. (2013) highlighted the fact that diatoms are the only group used in all three freshwater habitats (rivers/streams, lakes and wetlands) of the National Aquatic Resource Surveys by the US Environmental Protection Agency (USEPA). The development of MMIs using diatom assemblages are well documented in North America as effective assessment tools for streams, lakes and wetlands at varying geographic scales, from states and ecoregions (e.g., Wang et al., 2005, 2006; Miller et al., 2016) to national-scale assessments (Stevenson et al., 2013).

In South Africa, biological assessment techniques for river health assessments are well established (e.g., Dickens & Graham, 2002; Taylor et al., 2005; Dallas et al., 2010). In the case of South African wetlands, however, there is currently no definitive, well developed method for assessing ecological condition (Rountree et al., 2013). This is fundamental to the effective management, monitoring and rehabilitation of wetlands, and is also a requirement of the South African National Water Act (NWA, 1998). The NWA describes the framework that allows for water resource protection and use in a sustainable manner. The ecological condition of an aquatic habitat needs to be ascertained for effective implementation of the NWA.

Wetlands vary widely due to local and regional-scale differences in climate, geology, soils, topography, hydrology, water chemistry, vegetation, and other factors, such as human disturbance (Tiner, 1996), and as such, wetland functions differ. For example, in nutrient and sediment retention, carbon sequestration, floodwater storage and biodiversity protection (Cohen et al., 2016). This underlines the need for clear criteria for wetland functions assessments and the use of field tested and established assessment methodologies.

There has been growing efforts to develop biological monitoring tools to assess the condition of wetlands in South Africa as part of the Wetland Health and Importance (WHI) Research Programme (an integral part of the National Wetlands Research Programme). However, various biological monitoring techniques using macroinvertebrates and macrophytes to determine the ecological integrity of wetlands (mainly depressional wetlands) in the Western Cape Province and the Mpumalanga Highveld region of South Africa revealed relatively poor results (Corry, in press; Bird & Day, 2010, both of whom attempted the MMI approach; Day and Malan, 2010; Ferreira et al., 2012). Studies in both temporary and permanent depressional wetlands showed that macroinvertebrate compositions were largely determined by environmental and spatio-temporal factors independent of human disturbances, whilst human disturbance played a relatively minor role (Bird et al., 2013; Ferreira et al., 2012). Thus, the use of macroinvertebrates to develop an index of depressional wetland condition in these regions was recognised as unsuitable (Ferreira et al., 2012; Bird et al., 2013). This may also apply to depressional wetlands in general throughout the country, given the results of other studies internationally which have found few associations between invertebrate diversity and the high degree of natural disturbances (e.g., fluctuating water and nutrient levels, salinity changes, seasonal drying) associated with these dynamic wetland types (Tangen et al., 2003; Wang et al., 2006; Cooper et al., 2007).

Additionally, problems have been encountered using macrophytes as biological indicators due to a lack of basic ecological information on wetland plant taxa in South Africa (Corry, in press). While amphibian richness in depressional wetlands have been shown to decrease with increasing urbanisation in the Highveld region (Kassier, 2011) sampling amphibians can be impractical and resource intensive (USEPA, 2003). Species richness, relative abundances and composition between different seasons can differ markedly, highlighting the importance of long-term sampling to sufficiently represent the amphibian community of an area (e.g., Gardner et al., 2007). This level of sampling intensity is, however, time-consuming and financially costly.

Diatom indicators of biological condition of wetlands have provided reliable methods for assessing depressional wetland condition and diagnosing potential stressors in systems outside of South Africa, mainly in North America (e.g., epiphytic and benthic multimetric indices for Florida isolated marshes and cypress dome condition: Lane, 2007; Lane & Brown, 2007; Reiss et al., 2010, benthic diatoms in forested wetlands along a hydrological gradient: Lane et al., 2009, and relationships between paleo-diatoms and hydrologic permanence in Carolina Bays by Gaiser et al., 1998, 2001, 2004) and a few studies in Mediterranean climate regions (e.g., indicator taxa along a gradient of anthropogenic pressure in ponds: Blanco et al., 2004; Della Bella et al., 2007; Della Bella & Mancini, 2009). Yet, little is known about the diatom communities of depressional wetlands in the Mpumalanga Highveld region, and furthermore, there are no diatom-based methods to assess their ecological quality in this region or throughout South Africa.

Diatoms are effective tools for assessing wetland condition for three fundamental reasons: their ecological importance in aquatic systems, their applicability as indicators of habitat quality, and their accessibility (Stevenson et al., 2010). Round (1993) lists a number of reasons as to why diatoms are valuable tools of biomonitoring. This will be discussed in the following section, with a focus on the advantages of using diatoms in wetland ecological assessments which are of particular relevance to this study.

1.1.2 Rationale for using diatoms in wetland assessments

Diatoms (class Bacillariophyceae) are an extremely diverse group of algae (approximately 100,000 taxa) (Mann & Vanormelingen, 2013) and are often the most common, species-rich type of phytoplankton or periphyton in aquatic ecosystems (Bennion et al., 2010). This diversity makes diatoms valuable biological indicators since each taxon has a unique microhabitat preference that determines its spatial and temporal distribution (Gaiser & Rühland, 2010). This, coupled with the fact that an increase in the number of taxa provides redundancies of information in the dataset and increases the confidence of environmental inferences (Ovaskainen & Soininen, 2011), highlights the advantage of using diatoms to assess the ecological integrity of an aquatic resource.

Diatoms are essentially mixotrophic organisms and therefore nutrients and light availability plays a key role in their growth rate (Stoermer & Smol, 1999). In small, shallow wetlands such as depressional wetlands, the littoral zone can often be the main contributor of total primary productivity (Bennion et al., 2010). The low level light attenuation in shallow waters combined with an elevated access to nutrients promotes the proliferation of diatom communities (Wetzel, 2006). Hence, the species composition of littoral diatoms can serve as an important biological indicator in shallow, standing waterbodies. On account of their significant contribution to wetland productivity, Gaiser & Rühland (2010) characterised diatoms as "ecosystem engineers": they can control nutrient and gas fluxes, influence soil structure and formation, limit light availability to plant species and form the basis of aquatic food webs (dominant primary producers).

Diatoms are eukaryotic, unicellular organisms that have shorter generation times than all other biological indicators, including fish and macroinvertebrates (Harding et al., 2005). Thus, it is generally accepted that they reproduce and respond rapidly to a wide range of pollutants, and in doing so provide early warning indicators of wetland degradation and habitat restoration success (van Dam et al., 1998). Several studies have shown the significant relationship between shallow-water epilithic diatom community structure (attached to hard substrates such as rocks and stones) in standing waters and water chemistry variables such as total phosphorus (TP), total nitrogen (TN), dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), pH, specific conductivity, calcium (Ca), chlorine (Cl) (e.g., Bennion, 1994; Schönfelder et al., 2002; DeNicola & Kelly, 2014). The TP and TN optima of diatom communities are routinely used to infer eutrophication in shallow wetlands given the prevalence of nutrient enrichment as the key pressure on shallow, lowland waters (Bennion et al., 2010). In addition, conductivity gradients used to reflect the impacts of acid mine drainage (AMD) have been strongly correlated with the distribution of epiphytic diatom communities (attached to plants) in wetlands (Pan & Stevenson, 1996).

Drainage from mines, particularly abandoned mines, can cause extreme surface-water acidification through the accelerated oxidation of iron pyrite (FeS_2) and other sulphidic minerals to sulphuric acid (Ochieng et al., 2010). This arises from the exposure of these minerals to both oxygen and water, during and after the mining and processing of metal ores and coals (Johnson & Hallberg, 2005). AMD impacted waters typically have a low pH between 2 and 4 with a high concentration of sulphate and metals (e.g., iron, manganese and aluminium) (McKnight et al., 2001). These alterations to the chemical environment usually result in significant changes to the diatom community composition (Verb & Vis, 2000). Consequently, diatoms are commonly used to assess AMD impacted aquatic resources (mostly streams) to determine the effectiveness of remediation efforts (e.g., Young, 1976; Pinto & Taddei, 1977; Sabater et al., 2003; Oberholster et al., 2013; Hamilton et al., 2015).

Diatoms can tolerate highly acidic environments which other algae and other groups of organisms (fish, macroinvertebrates…) may find toxic (DeNicola & Stapleton, 2002; Kelly et al., 2012). Several studies have demonstrated the accumulation of metals in diatom communities when exposed to them in natural environments (e.g., Absil & Scheppingen, 1996; Levkov & Krstic, 2002; Lavoie et al., 2012; Tang et al., 2014; Leguay et al., 2016). These algal communities act as major sinks for many toxic inorganic chemicals and can therefore serve as a valuable tool for monitoring heavy metal contamination in aquatic resources.

Ecologically widespread, diatoms can occupy dry habitats to freshwater and marine systems, which make them valuable tools for comparison among ecosystems (e.g., streams, lakes, wetlands, estuaries) with organisms of the same group (Stevenson & Smol, 2003). Another advantage to examining diatoms is that they are uniquely characterised by resistant siliceous cell walls (frustules), which have a lasting permanence in sediments and record habitat history (Hall & Smol, 2010). This allows for the assessment of ephemeral aquatic habitats at any time of year even when these systems are dry (e.g., Lane et al., 2009).

Diatom-based information can be collected not only from sediments (epipelic habitats) but from the water column (planktonic) and as previously mentioned, from the epilithon and epiphytic habitat. However not all shallow wetlands, in particular depressional wetlands investigated in this study, possess hard substrates such as rocks and stones to sample from. King et al. (2006) suggested that, where epilithic habitats may be absent or restricted and do not represent a major component of the wetland benthic diatom community, epiphytic diatoms ideally from emergent macrophytes, should be sampled. Previous studies developing diatom-based evaluation tools for wetlands identified epiphytic diatoms as more responsive to anthropogenic disturbance than planktonic or benthic taxa, and in so doing, advocated their use in wetland assessments (e.g., Pan & Stevenson, 1996; Lane & Brown, 2007). For this reason, the current study will focus on the use of epiphytic diatom communities to develop diatom-based assessment tools for depressional wetland condition.

Diatoms are relatively easy to sample and sampling and analysis are cost effective compared to using other groups of organisms (Round, 1993), for example amphibians, as highlighted previously. Their taxonomy is reasonably well documented thereby samples can be identified to species-level more easily, which can then be archived for long-term records (Stevenson & Smol, 2003). Nevertheless, diatom taxonomy has been rapidly changing due to continual taxonomical changes, thus making diatom identification to species-level often challenging (Tapolczai et al., 2016).

The use of diatoms in biological assessments can complement other biological indicators, as well as physical and chemical data to provide information on whether the ecological integrity of an aquatic system has been modified and the cause of those modifications (Stevenson et al., 2010). For this reason, the study of diatoms has become a valuable component in a range of monitoring and assessment programmes; from paleoecological assessments to document climate-related changes in boreal and Arctic lakes (e.g., Gregory-Eaves et al., 1999; Rühland et al., 2008; Paul et al., 2010) to modern diatom assessments to detect natural and human-induced changes in wetlands (see review in Gaiser & Rühland, 2010).

1.1.3 Biotic diatom indices

In recent decades, modern diatom assemblages have been employed to assess the ecological status of aquatic resources based on the use of single diatom metrics, wherein only genera assessed as either specifically tolerant or sensitive to pollution are included (Stevenson et al., 2013). Diatom indices, for the most part, have been developed for assessing the trophic status of rivers in Europe (e.g., generic diatom index (GDI): Rumeau & Coste, 1988; the specific pollution-sensitivity index (SPI): CEMAGRAF, 1982; the biological diatom index (BDI): Lenoir & Coste, 1996; Prygiel, 2002; and the eutrophication pollution diatom index (EPI-D): Dell'Uomo, 1996). These indices have demonstrated their feasibility for use in some South African rivers (de la Rey et al., 2004; Taylor et al., 2007, 2009; Walsh & Wepener, 2009;

Harding & Taylor, 2011), even though they were originally developed in regions with contrasting climates.

Whilst some studies have proven the applicability of indices developed for rivers, in shallow, standing freshwaters in central Europe (Ács et al., 2005; Bolla et al., 2010) and the Mediterranean (Blanco et al., 2004; Della Bella et al., 2007; Cejudo-Figueiras et al., 2010), the suitability of these indices can be limited, even for rivers, since most diatom indices derive their calculations based on species distributions found in specific regions (Stenger-Kovács et al., 2007).

Diatom indices that have been developed specifically for wetlands are limited (Antón-Garrido et al., 2013), and in South Africa there has been no attempt to develop a diatom index of wetland condition to assess the ecological status of wetlands. Rountree et al. (2013) endorsed the use of the SPI (developed for rivers) to assess the water quality of various wetland types in South Africa, as part of the requirements for Wetland Reserve Determinations, a component of the Resource Directed Measures (RDM) to meet the demands of the NWA. Yet the use of the SPI was based on recommendations by Matlala et al. (2011) who tested a limited sample size of palustrine, coastal wetlands in the Western Cape. Rigorous testing of the SPI to assess its applicability on a more comprehensive coverage of wetlands throughout the country is essential. This is particularly vital for highly dynamic closed-basin wetlands, such as depressional wetlands, which may be subject to strong fluctuations in their physico-chemical environment, particularly salinity levels (Gasse et al., 1995; Saros & Fritz, 2000). Any attempts to use existing diatom indices such as the SPI to determine the biological integrity of depressional wetlands will possibly result in misleading conclusions. For example, depressions with naturally high salinities or organic content may be rated with a low SPI score (i.e. poor water quality). Existing diatom indices used to determine anthropogenic stress in freshwater systems relate to the abundances of stress-tolerant species, which may be equally tolerant to natural stressors (e.g., elevated nutrients, organics and salinity) as to anthropogenic ones, a salient issue which needs to be addressed.

1.1.4 Depressional wetlands and their importance, both ecologically and socio-economically.

Traditionally, limnological research has focused on large, deep lakes, but in the last two decades smaller, shallower waterbodies, including depressional wetlands, have received increasing research attention (Padisák & Reynolds, 2003; Scheffer, 2004; Bennion et al., 2010; Gaiser & Rühland, 2010). Depressional wetlands are physically different from other wetland types; they are defined as those encompassed by upland habitat that do not appear to have any connectivity to other wetlands or waters via distinct surface water connections (Tiner, 2003). Many of these depressional wetlands can, however, have some degree of connectivity to other wetlands and waterbodies via subsurface or groundwater flows or by temporary spillovers (Leibowitz, 2003).

There are several factors influencing the hydrochemistry of depressional wetlands such as the frequency of flushing, the periodicity of inundation and surface area to catchment area ratio, with systems having a low ratio being the most saline (Russell, 2008). It is because of these processes that depressional wetlands encompass a wide chemical gradient ranging from free-draining, temporary, freshwater systems to groundwater-fed, permanently inundated saline systems.

Since depressional wetlands are generally small, it might be assumed that their loss may have minor importance. Nevertheless, various studies have highlighted that the degree of functional loss is not relative to size (e.g., Gibbs, 1993; Semlitsch & Bodie, 1998). Much of the significance associated with depressional wetlands is attributed to biodiversity. A rich biota is often associated with these wetlands which studies have shown to be partly ascribed to the effects of hydroperiod regime (i.e. the duration of standing water) (Gaiser & Johansen, 2000; Dimitriou et al., 2009). Temporary depressional wetlands, those that are intermittently inundated and dry on some periodicity, are associated with shorter hydroperiods in comparison to permanent wetlands (Calhoun et al., 2016). Hydroperiod regime is a major factor that influences the structure and dynamics of floral and faunal communities, where shorter hydroperiods in temporary depressional wetlands allows for these wetlands to often support unique biotic communities (e.g., Williams et al., 2004; Foti et al., 2012; Boix & Batzer, 2016). Thus, loss of depressional wetlands may have a greater impact on regional biodiversity comparative to other wetland types (Leibowitz, 2003).

In addition to their ecological value, depressional wetlands also provide significant socio-economic benefits (Gala & Young, 2015; Calhoun et al., 2016). From prehistoric to present day, these wetlands have served as an important source of water and minerals in hot dryland environments (Shaw & Bryant, 2011). In the Mpumalanga Highveld region, depressional wetlands are a key piece of the rural economy, providing support to cattle farming, which is intrinsically linked to rural livelihoods and cultural heritage. Moreover, depressional wetlands of this region serve as an essential piece in the ecotourism mosaic for the area.

1.1.5 Depressional wetlands of the Mpumalanga Highveld region and current threats

In the Mpumalanga Highveld region, depressional wetlands are exceptionally numerous (± 2600 depressions) however limnological knowledge of these systems is extremely limited, which is surprising considering their uniqueness in terms of water chemistry, flora and fauna (Hutchinson et al., 1932).

Depressional wetlands in this region are naturally formed and characteristically circular or oval in shape (Goudie & Wells, 1995). A number of theories have been suggested to explain their formation; one which may be a major contributor to their formation and shape is their geological structure (Shaw & Bryant, 2011). Fractures, faults and dyke intersections common in this area are weak points in the rock which are more susceptible to weathering. Increased weathering exacerbates leaching of salts, which encourages further basin development (Marshall & Harmse, 1992).

Their basin-shaped morphometry, often increasing in depth from the perimeter to the centre allows for the accumulation of water. These systems are shallow, and even when fully inundated, are usually less than 3m deep (Allan et al., 1995). They typically receive most of their water via diffuse subsurface flows, with surface flows playing a minor role (McCarthy et al., 2007). Rain falls within the wetland catchment area, infiltrates the soil, and flows subsurface into the system (Fig. 1.1). It is this dependence on rainfall which makes many depressional wetlands temporary. Allan (1985) reported more than half of the depressional wetlands in the Mpumalanga Highveld region are temporary wetlands.

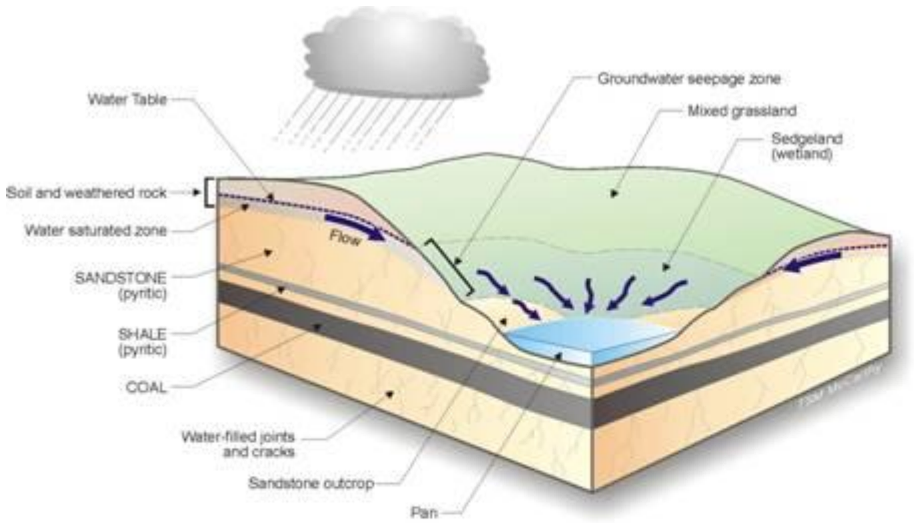

Figure 1.1 Diagram illustrating the geology and hydrology of a typical depressional wetland in the Mpumalanga Highveld region (from (McCarthy et al., 2007). Note the term ‘pan’ is commonly used in southern Africa to refer to depressional wetlands.

Depressional wetlands are one of the most valuable wetland types in the Mpumalanga Highveld region in terms of providing habitat for vulnerable species such as the Greater and Lesser Flamingo (*Phoenicopterus roseus*, *P.minor*), the Grass Owl (*Tyto capensis*), and the African Bullfrog (*Pyxicephalus adsperus, Pyxicephalus edulis*) (Lidwala Consulting Engineers (SA) (Pty) Ltd, 2012). Yet there is still little known about the biological communities of depressional wetlands in this region and considerably less information available on diatom distribution and diversity in these systems. The only comprehensive investigation of depressional wetlands in the Mpumalanga Highveld region, including the species composition of diatoms, is that by Hutchinson et al. (1932) who provided an ecological study of a variety of temporary and permanent depressional wetlands in the area. However, taxonomic identification of diatoms was mostly undetermined.

In a more recent study, Riato et al. (2014) investigated the diatom species composition within a variety of depressional wetland types in this region which encompass a broad range of size, vegetation and above all water chemistry. This study highlights the diversity and complexity of temporary depressional wetlands in terms of their biological and physico-chemical characteristics. It is therefore essential to examine the characteristics that define these highly variable systems in order to distinguish changes in biological communities caused by human disturbances from natural variations. This is particularly vital in an area such as the Mpumalanga Highveld where depressional wetlands are subject to extreme alterations in their physical and chemical environment as a result of extensive human disturbance. The major threat to these aquatic resources has been increasing mining development, although extensive agricultural practises have also contributed to wetland degradation and loss. The main stressors of concern are generally toxic heavy and/or trace metal contamination as a result of AMD slowly filtering into the wetlands or from direct storage of AMD; and nutrient enrichment as a result of return flows from fertilised lands or livestock waste (de Klerk et al., 2012). This, coupled with the ever-increasing demand for energy and food resources in South Africa, highlights the need to improve our understanding of how such impacts effect the ecological functioning of depressional wetlands in order to improve the management and protection of these wetlands.

1.2 OUTLINE OF THESIS

Remarkably, despite the growing interest in the topic, very few examples of diatoms as biological indicators in shallow waterbodies in Africa have been published and furthermore, no indices of biological condition of wetlands based on diatoms have been created anywhere in the continent. In this thesis, I aim to address this knowledge gap by providing a preliminary investigation of the feasibility of using diatoms for the biological assessment of depressional wetlands in South Africa (Fig. 1.2).

This study will provide insight into how changing environmental conditions, both natural and human-induced, influences diatom community structure and functioning in depressional wetlands in the Mpumalanga Highveld region. On a broader scale, this study offers an opportunity to expand our current knowledge of wetland diatom community organisation and the dynamics of populations in spatio-temporally variable environments.

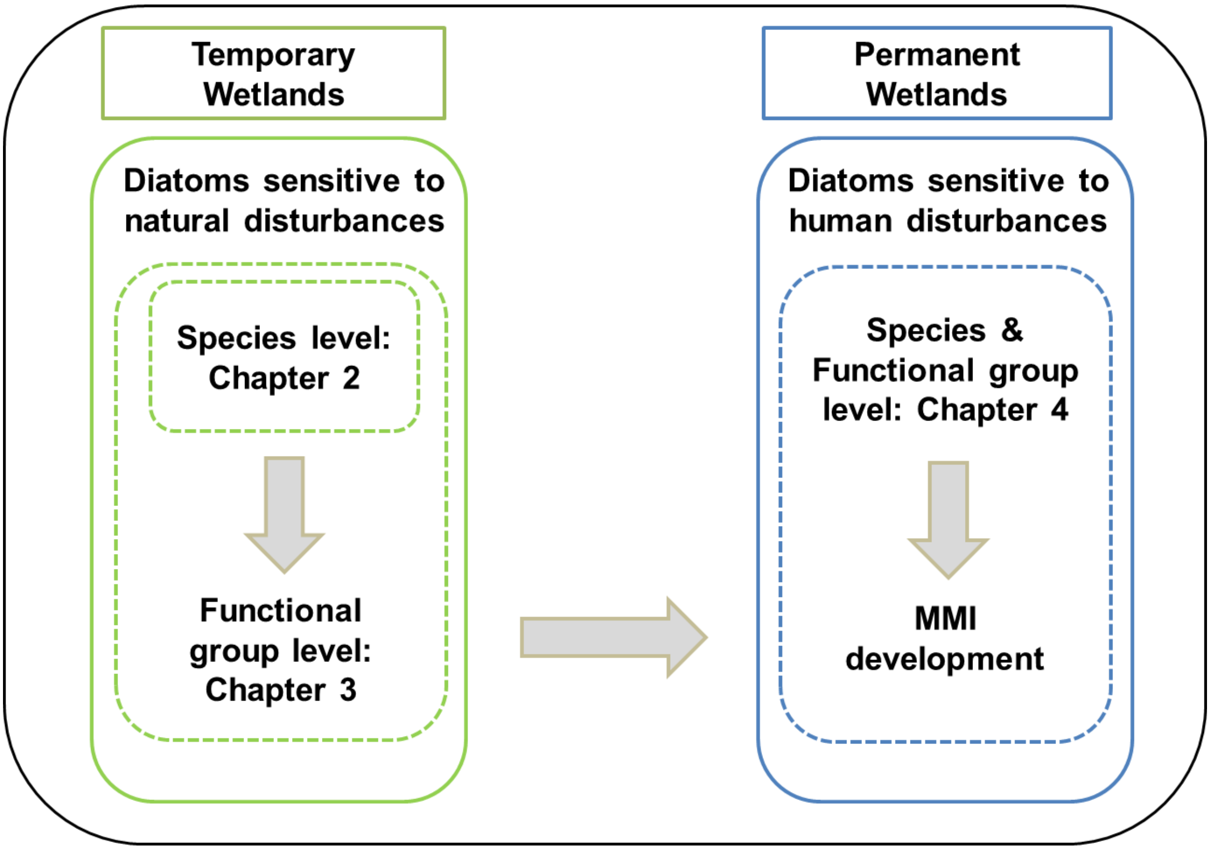

Figure 1.2 Thesis outline showing the input from each chapter. The depressional wetland type that is the focus of each chapter is represented by a separate colour. Dashed boxes specify the taxonomic level addressed.

1.2.1 Chapter Description

Diatoms have a successful history of use as biological indicators of depressional wetland condition and in diagnosing potential stressors, as previously discussed in Section 1.1.1. In South Africa, the use of diatoms as indicators of wetland functional integrity resulting from anthropogenic impacts is a promising alternative to the traditional methods employed (e.g., macroinvertebrate or macrophyte indices) which have proved ineffective. Establishing a reference condition of variation due to natural disturbances (e.g., seasonal fluctuations in both water-level and water chemistry) in temporary depressional wetlands using diatoms is necessary for further investigations of anthropogenic disturbances. In Chapter 2, we investigate whether epiphytic diatoms are suitable indicators of natural environmental disturbances in temporary depressional wetlands in the Mpumalanga Highveld region by assessing the strength of the relationships between species occurrence and environmental variables. We also determine if it is possible to establish a reference condition of this wetland type using diatoms as indicators.

Chapter 3 constitutes a very similar study, but instead focuses on using a broader taxonomic resolution to assess wetland condition. The diatom data and physical and chemical data from Chapter 2 are used to explore the feasibility of employing functional groups - life-forms and ecological guilds (discussed in more detail in Chapter 3) as simple, effective and reliable indicators of natural environmental change in temporary depressional wetlands in the region. No previous examples of diatom functional groups and their response to changing environmental conditions in temporary depressional wetlands have ever been published. Together these chapters allow us to expand our current knowledge of the natural effects of hydrological changes on the surface water chemistry in temporary depressional wetlands and its influence on diatom taxonomic and functional group composition.

In the Mpumalanga Highveld region, mines utilise wetlands, including depressional wetlands, for dirty water storage whereby mines are pumping AMD directly into the wetland (Ochieng et al., 2010). As a result the hydrologies of these wetlands used for mine wastewater storage are typically permanently inundated. For this reason, we focus on developing an index to quantify mining impacts using epiphytic diatoms in permanent depressional wetlands. This will be the first diatom MMI specifically designed to assess mining severity in wetlands. In Chapter 4, we use metrics which reflect different features of biological condition, such as taxonomic composition, diversity and functional groups (Davies & Jackson, 2006), to develop an MMI of permanent depressional wetland condition sensitive to mining impacts. Natural variation in biological metrics can reduce the accuracy of ecological assessments (Hawkins et al., 2010a). To account for natural variation among the permanent depressional wetlands in this study, we create separate MMIs for classes (discrete categories) of permanent wetlands and evaluate the efficacy of a wetland classification approach for improving MMI performance.

Finally, in Chapter 5, the results are synthesised and evaluated in the framework of diatom ecology, community structure and biological assessments of depressional wetlands in South Africa. This chapter discusses the limitations of the research and recommends possible directions and priorities for future research works on diatom biological assessments in depressional wetlands.

1.2.2 Chapter structure

Each chapter is compiled as a standalone paper to enable the publication of the research. Hence, each chapter contains the following sections - abstract, introduction, materials and methods, results, discussion and conclusion. There is some replication of ideas in the introduction sections and some repetition in the materials and methods sections. Repetition was omitted where feasible; however, in some instances replication was necessary for the intelligibility of each chapter. For those chapters that have been accepted for publication, the manuscript has been edited and arranged to conform to the rest of the thesis.

CHAPTER 2

DIATOM-BASED MODELS FOR INFERRING WATER CHEMISTRY AND HYDROLOGY IN TEMPORARY DEPRESSIONAL WETLANDS

Published: Hydrobiologia

ABSTRACT

Information on the response of temporary depressional wetland diatoms to human-induced disturbances is a limited and important component for the development of temporary wetland biological assessments in human-modified landscapes. Establishing a reference condition of variation due to natural disturbances in depressional wetlands using diatoms is necessary for further investigations of anthropogenic impacts. We examined the temporal and spatial responses of epiphytic diatom communities to natural environmental disturbances within three least disturbed wetlands in the Mpumalanga Province, South Africa. Alkalinity, Na^+ and Cl^-, water depth and total relative evapotranspiration (ETo) accounted for the highest proportion of temporal variation in composition of epiphytic diatoms, as revealed by canonical correspondence analysis (CCA). Alkalinity and Na^+, Cl^- explained a much higher proportion of species variation, using partial CCA. A simple weighted-averaging (WA) with inverse deshrinking produced reasonably robust models for Na^+ (r^2_{boot} 0.71), depth (r^2_{boot} 0.64) and alkalinity (r^2_{boot} 0.46), not for Cl^- and ETo. We determined species optima and tolerances for Na^+, depth and alkalinity which can facilitate identification of anthropogenic impacts based on changes of indicator taxa assemblages. Our study provides a basis for newly developed quantitative tools to be used in biomonitoring studies and evaluations of reference conditions for temporary wetland management.

2.1 INTRODUCTION

Despite the widespread recognition of diatoms as a valuable indicator tool in wetland biological assessments (Gaiser & Rühland, 2010), research on the use of diatoms to assess the biological condition of depressional wetlands is limited (Lane et al., 2009). Nevertheless, the usefulness of diatoms as a biological assessment tool in depressional wetlands has been demonstrated in several studies based mostly in the USA (e.g., epiphytic and benthic multimetric indices for Florida isolated marshes and cypress dome condition: Lane, 2007; Lane & Brown, 2007; Reiss et al., 2010; benthic diatoms in forested wetlands along a hydrological gradient: Lane et al., 2009; relationships between paleo-diatoms and hydrologic permanence in Carolina Bays by Gaiser et al. 1998, 2001, 2004) and a few studies in Mediterranean climate regions (e.g., indicator taxa along a gradient of anthropogenic pressure in ponds: Della Bella et al., 2007; Della Bella & Mancini, 2009). Wetland biological assessments using algae have focused on diatoms as they are often the most abundant, species-rich group of algae within the phytobenthos or periphyton (Bennion et al., 2010) and the taxonomy and autecology of freshwater diatoms are relatively well-established (USEPA, 2002).

Depressional wetlands, those encompassed by upland habitat (Tiner, 2003), have exceedingly dense occurrences in the Mpumalanga Highveld region of South Africa (Riato et al., 2014). The wetlands in this region are diverse in flora and fauna and can contain endemic species, the status of which is critical to compliance with the National Environmental Management: Biodiversity Act (2004). Much of the significance associated with depressional wetlands is attributed to biodiversity. A rich biota is often associated with these wetlands which studies have shown to be partly ascribed to the effects of hydroperiod duration (Gaiser & Johansen, 2000; Dimitriou et al., 2009). Consequently, loss of depressional wetlands may have a greater impact on regional biodiversity comparative to other wetland types (Leibowitz, 2003).

Increasing coal mining and agricultural operations in the Mpumalanga Highveld have contributed to substantial loss and degradation of depressional wetlands as a result of hydrological modifications, toxic heavy and/or trace metal contamination, salinisation and nutrient enrichment (CSIR, 2010; Ochieng et al., 2010). The physical and chemical properties of water in aquatic ecosystems (e.g., pH, ionic composition, nutrient availability and light) are not only influenced by anthropogenic impacts but also natural processes (Stenger-Kovács et al., 2013). In order to understand the anthropogenic impacts on wetland systems, their influences must be characterised as distinct from those due to natural environmental variability.

Depressional wetlands, specifically temporally hydrated systems, can undergo substantial natural hydrological variability and fluctuations in ionic concentration and composition related to seasonal or longer-term climatic fluctuations (Gasse et al., 1995). In closed-basin systems such as these depressional wetlands, seasonal variations of precipitation and evaporation successively concentrate and dilute the water, and in turn cause significant changes in ionic concentration and composition (Saros & Fritz, 2000; Leibowitz et al., 2016). Ionic concentration and composition have been found to be the most important factors influencing the structure of temporary wetland diatom communities as a result of seasonal and climate-driven changes (Gasse et al., 1995; Gell et al., 2002; Tibby et al., 2007).

Diatoms are widely used as indicators to monitor environmental changes due to their range of response to ionic content and composition (Potapova & Charles, 2003). Numerous diatom-based inference models for estimating ionic concentration have been developed using large deep-lake datasets (e.g., Fritz et al., 1993; Cumming et al., 1995). Some studies have developed similar models for inferring lake water alkalinity (e.g., Roux et al., 1991; Köster et al., 2004). Few studies have developed inference models using depressional wetlands, though existing studies have demonstrated strong model performances in measuring salinity and ionic composition of large spatial datasets from North and East Africa (Gasse et al., 1995), and on a smaller spatial scale, salinity estimates derived from several seasonally monitored sites in Australia (Tibby et al., 2007). In addition, Gaiser et al. (1998) examined the relationship of diatoms to hydrological properties, such as water level fluctuations and drying in temporary ponds, and developed robust hydroperiod inference models for intermittent ponds of the Atlantic Coastal Plain.

In South Africa, biological assessment techniques for river health assessments are well established (e.g., Dickens & Graham, 2002; Taylor et al., 2005). In the case of South African wetlands, however, there is currently no definitive, well-developed method for assessing ecological condition (Rountree et al., 2013). Such methods are fundamental to the effective management, monitoring and rehabilitation of wetlands, and are also a requirement of the South African National Water Act (NWA, 1998). Biological monitoring techniques using macroinvertebrates and macrophytes to determine the ecological integrity of wetlands (mainly depressional wetlands) in the Western Cape Province and the Mpumalanga Highveld region performed poorly (Bird and Day, 2010; Day and Malan, 2010; Ferreira et al., 2012). Studies in both temporary and permanent depressional wetlands showed that macroinvertebrate compositions were largely determined by environmental and spatio-temporal factors independent of human disturbances (Ferreira et al., 2012; Bird et al., 2013). Problems have been encountered using macrophytes as biological indicators due to a lack of basic ecological information on wetland plant taxa in South Africa. In addition, little is known about the composition and function of diatom communities in depressional wetlands in the Mpumalanga Highveld region, and there are no diatom-based methods to assess ecological quality in this region or throughout South Africa. In recent years, international research of diatoms has focused on tolerance limits of individual diatom species and in search of relationships between species occurrence and environmental variables (e.g., Stenger-Kovács et al., 2007; Potapova, 2011).

There is an urgent need to develop a biological assessment tool for assessing temporary depressional wetland conditions in the Mpumalanga Highveld region. The research presented here examines three least disturbed natural temporary depressional wetlands during various stages of inundation. The main goal was to investigate whether diatoms are suitable indicators of changing environmental conditions in these wetland types by assessing the strength of the relationships between species occurrence and environmental variables. It achieves this through the completion of three aims: (a) to determine the most important variables structuring diatom species composition between the three wetlands and within each wetland; (b) to develop quantitative inference models specific to the most important environmental variables between the three wetlands; and (c) to identify diatom indicator taxa for the most robust inference models.

2.2 MATERIALS AND METHODS

2.2.1 Study area

The study area is located in the Bapsfontein region of South Africa, which borders the Mpumalanga Highveld at an altitude of 1606m, with underlying geologies of weakly cemented sandstones and fissile shales and climate as those found in the Mpumalanga Highveld (Tooth & McCarthy, 2007). Three temporary depressional wetlands (average water depth < 2m) were selected based on their representativeness of this wetland type in the Mpumalanga Highveld (of which there are approximately 2600 depressional wetlands in the region), and having similar macrophytes, conductivity, surrounding land-use and close proximity to one another (radius of 1km) (Fig. 2.1). The study sites (S1, S2 and S3) were sampled once a month from March 2011 (period of high inundation) to September 2012 (drying out period).

Figure 2.1 Map of study area showing location of the depressional wetland sites (S1, S2 and S3); inset shows study area location in South Africa.

By the end of the sampling period in September 2012, S1 almost dried out completely and S2 and S3 were reduced to two shallow pools (July – Sept 2012). This was the limit for collecting data as shortly after S1 was dry, and even though we could still collect water at S2 and S3, we required even sampling for a balanced dataset. When full, S1 and S3 had a surface area of 59,300m^2 and 57,900m^2, with a maximum depth of 84.3 and 99.8cm, and a mean depth of 46.8cm and 66.6cm, respectively, while S2 was the largest of the sites which had a surface area of 202,600m^2, a maximum depth of 114.4cm and a mean depth of 70.7cm. S3 was similar in surface area to S1 but as its basin profile was more concave, it had a greater depth than S1 and therefore remained inundated throughout the study. S2 also remained inundated throughout the study period as it had a similar depth to S3 with a large surface area.

The study area is situated within the Highveld climate region of South Africa. Here monthly means of daily maximum temperature range from 28 to 30°C in the summer months (December to March), while minimum temperatures in the same months are between 12 and 16°C (Schulze, 1997). More than half of the precipitation occurs in the summer period between October and March (with a maximum in January) (Dyson, 2009). Depressional wetlands in this area are typically ephemeral and tend to be fully inundated during summer and dry out in winter, where water loss is mostly attributable to evaporation (Ferreira, 2012). From January to March 2011, the late summer rainfall amounted to 447mm (Agricultural Research Council, Pretoria) which exceeded the average 309mm over the same period (Dyson, 2009). Random periods of above-average rainfall can affect the duration of inundation in ephemeral wetlands (Henry et al., 2016). This phenomenon was observed at all three study sites which remained inundated throughout winter (May-August) in 2011. The land-use in the catchment is mostly dryland cultivation (between 69-74%) and natural grassland (18-19%). The seepage band around each depression site acts as a wetland buffer in the sense that it absorbs high surface flow and any nutrients, contaminants and sediment inputs during high rainfall events. Human-induced disturbance to wildlife was low as suggested by the presence of African bullfrog *Pyxicephalus adspersus* and fairy shrimp Anostraca sp.

2.2.2 Sampling and laboratory methods

We collected one sample of epiphytic diatoms from the dominant macrophyte species, *Leersia hexandra,* (approximately 80% of the wetland) at the deepest point in the basin centre at each site to ensure the comparability of diatom community samples between water bodies as recommended by King et al. (2006). Five submersed macrophyte stem sections were snipped at 5-20cm below the water surface and placed into a zip lock bag with a small amount of distilled water. Epiphytes were dislodged from all stems by shaking the samples for 2 min following Zimba & Hopson (1997) which allows for the collection of >90% of diatoms attached to stems. We collected a total of 63 samples; 21 samples from each site. Samples were returned to the laboratory where they were acid cleaned and mounted on microscope slides (Battarbee et al., 2001). We counted 400 valves along transects and identified to the lowest feasible taxonomic level using standard European diatom floras (such as Krammer & Lange-Bertalot, 1986-1991; Lange-Bertalot, 2000-2002; Lange-Bertalot et al., 2001), several papers on the Southern African flora by Cholnoky, Schoeman and Archibald (e.g., Schoeman & Archibald, 1976-1980), and books and recent papers on *Gomphonema* taxonomy (e.g., Reichardt & Lange-Bertalot, 1999; Rose & Cox, 2014; Reichardt, 2015).

In this thesis, we decided to use an open nomenclature taxonomic terminology, so we have named a taxon as affinis (abbreviated: spec. aff.) when the specified diatom belongs to a species close to but different from it; and we named a taxon as cf. when the specified diatom belongs probably or possibly to this species but with some doubts.

As we have doubts whether this could indicate either a new variant or a taxon not yet described, there is need of further studies to elucidate the actual taxonomic location of the material.

We sampled chemical and physical variables at the same location and time as the diatom sampling. Water samples (1 L) were collected in an acid-cleaned, high-density polyethylene bottle at a preferable depth of approximately 10cm, and then kept on ice during transportation to the laboratory. Samples were analysed for biological oxygen demand (BOD), NH_4^+, total Kjeldahl nitrogen (TKN), NO_3^-, NO_2^-, P, PO_4^{3-}, total phosphorus (TP), Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, SO_4^{2-}, dissolved organic carbon (DOC), turbidity, total suspended solids (TSS), alkalinity and conductivity. Analysis was conducted by Waterlab (Pty) Ltd. laboratory in Pretoria following standard procedures (APHA, 1998). Temperature, pH, dissolved oxygen (DO) and water depth were measured in the field using a Hach HQ40D Dual Input Multi-Parameter meter.

We included total relative evapotranspiration (ETo) data in the analysis as a proxy for the seasonal (summer–winter) gradient, in order to determine if ETo had an influence on the distribution of epiphytic diatoms. ETo data, calculated from hourly air temperature and wind speed data using the FAO-56 Penman-Monteith method (Zotarelli et al., 2010), was collected from the Agricultural Research Council meteorological station located 6km from the study sites. For each sampling month, we used the monthly ETo, calculated as the mean of the daily ETo values for the sampling month.

2.2.3 Statistical analysis

We used PRIMER 6's (Clarke & Gorley, 2006) analysis of similarity (one-way ANOSIM, Bray-Curtis distance measure, 999 permutations) to compare community compositions among the three study sites. We used the R statistic value produced by ANOSIM to assess whether the species composition of each site were significantly different from those of other sites. The R value ranges from −1 (where similarities across different sites are higher than within sites) to 1 (where similarities within sites are higher than between sites) (Clarke & Gorley, 2001).We identified the taxa most responsible for dissimilarity between and similarity within sites using similarity percentage analysis (SIMPER) (Clarke, 1993). We employed non-metric multidimensional scaling (NMDS) to visualise differences in composition among sites using PC-ORD 5.10 (McCune & Mefford, 2006).

We conducted canonical correspondence analysis (CCA) using CANOCO 4.5 (ter Braak and Šmilauer, 2002) to examine relations between diatom species composition and physical and chemical variables between the three wetland sites (n= 63). Inclusion of physico-chemical parameters in the final analysis was based on eliminating collinear variables with a high variance inflation factor (VIF>10) (ter Braak & Šmilauer, 1998). We applied step-wise forward selection to select a final set of physico-chemical parameters that related best to the diatom species composition (ter Braak & Šmilauer, 1998). We used partial CCA to separate and investigate the relative importance for the species data of sets of explanatory variables on the diatom community (Borcard et al., 1992). We used step-wise forward selection and Monte Carlo permutation tests (999 permutations) to test the significance (cut-off point of P = 0.05) of the forward selected variables and the CCA and partial CCA axes. The P-values were adjusted for multiple comparisons using the Bonferroni correction. Ordination methods based on gradient analysis were unsuitable for exploring environment-species relationships within each wetland site because of the reduced number of samples (n=21). For this reason, we examined differences in environmental variables pair-wise between sites using the Tukey honestly significant difference (HSD) post-hoc test, following an analysis of variance (ANOVA).

To estimate the width of ecological niches of selected common taxa, we developed and validated weighted-averaging (WA) regression and calibration models using C2 1.7.2. (Juggins, 2003). WA values using common methods (WA with inverse deshrinking (WA-inv), WA with classical deshrinking (WA-class), and WA with tolerance down weighting (WAT)) were calculated for the physical and chemical variables identified as most highly correlated with the CCA axes. The performance of the models were evaluated by comparing the coefficient of determination (r^2) between diatom-inferred and observed values for physical and chemical variables of interest and the apparent root mean squared error (RMSE). WA models were tested using the bootstrapping cross validation method which corrects for overly optimistic estimates of model performance (Birks et al., 1990). We selected the best performance models as those with the lowest RMSEP and the highest bootstrapped r^2 value (Lotter et al., 1997). We applied outlier analysis with a cut-off of three standard deviations in PC-ORD. We used WA regression to estimate the ecological optima and tolerances along the variable gradient of interest for each taxon.

A taxon can be classified as a good indicator if it has a strong correlation to the environmental variable of importance, a narrow tolerance, a well-defined optimum and a high abundance (Stevenson et al., 1991). Taxa with a high N2 are likely to have well defined optima, where N2 is Hill's (Hill, 1973) diversity measure. We calculated Pearson correlation coefficients to investigate the strength of the relationships between the most common taxa and the most robust inference models in order to identify strong indicators of environmental change.

We square root transformed taxa abundance in all analyses to reduce the effect of highly variable population densities on ordination scores. Only those species that reached relative abundance of at least 1% in at least four samples per dataset were included in the analyses to reduce the influence of rare taxa. Relative abundance of 1% is widely used in diatom assessments as the cutting line between rare and common taxa (Potapova & Charles, 2002, 2003a; Lavoie et al., 2009a). Excluding taxa based on their frequency of occurrences can vary among studies e.g. 10 or less samples (Potapova & Charles, 2004), or 5 or less samples (Gillett et al., 2011). In our study, we selected at least 4 samples which, together with removing at least 1% relative abundance, was a suitable cut-off for the removal of rare taxa from our dataset.

We used distribution plots and skewness testing to evaluate whether the physical and chemical variables should be transformed to normalise the data distributions. All variables, except depth, were log transformed to reduce skewness and normalise distributions.

2.3 RESULTS

2.3.1 Physical and chemical characteristics of sites

There was a clear seasonal pattern in ETo and rainfall during the study period (Fig. 2.2). ETo and rainfall decreased at the end of summer 2011 and remained relatively low (mean ETo 2.18mm, rainfall range 0 - 0.54mm), up until the end of winter/beginning of spring 2011 when there was a significant increase in ETo and rainfall, both reaching maximum values in mid-summer 2011(mean 4.76mm and 5.24mm, respectively). This was followed by a steady decline in ETo until mid-winter 2012 (mean 1.9mm) after which there was a rapid increase in ETo until the end of sampling (mean 3.66mm). During mid-summer 2011 until autumn/winter 2012 there was a substantial decrease in rainfall (from mean 5.24 to 0.3mm); thereafter there was very little rainfall until the end of sampling. At all three sites, water levels gradually declined from winter 2011 (range 84.3 - 114.4cm) until the end of sampling (range 2.1 - 27.8mm), even during the heaviest rain events in mid-summer 2011.

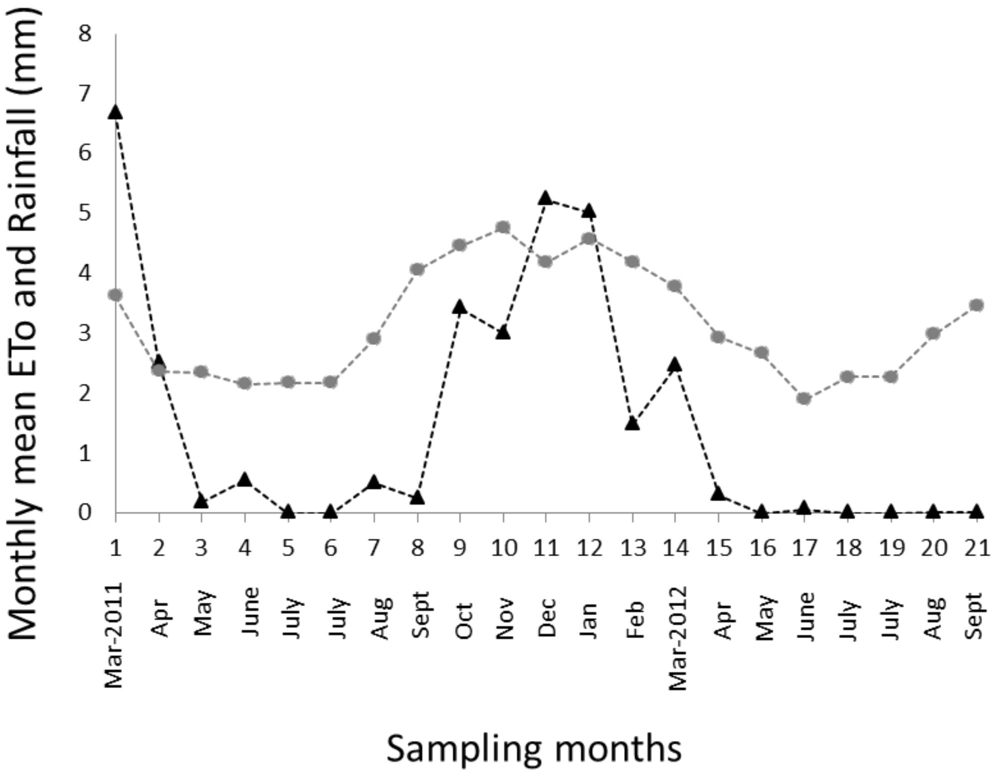

Figure 2.2 Monthly changes of total relative evapotranspiration (ETo) and rainfall during the sampling period. For each sampling month, we used the monthly ETo (circle) and rainfall (triangle), calculated as the mean of the daily ETo and rainfall values for the sampling month. Month 1: end of summer 2011; 2 - 7: autumn-winter 2011; 8 - 14: spring-summer 2011-2012; 15 - 20: autumn-winter 2012; 21: spring 2012. Note: there were two sampling events at the start and end of both July 2011 and July 2012**.**

A relatively narrow range of physical and chemical variables were observed among the three sites (Table 2.1). The sites were characterised by a reasonably low ionic content where mean pH ranged from 7.2 to 7.5 and mean conductivity from 147 to 155.8µScm^{-1}. The highest conductivities were measured at the end of the sampling period when the sites were drying out. Maximum conductivities at S1 and S2 were 460 and 438µScm^{-1}, respectively which was relatively higher than at S3 (313µScm^{-1}). Similarly, indicators of organic matter concentrations (e.g., TSS and TKN) were highest at all sites during the drying out phase (Table 2.1) where concentrations of organic matter were substantially higher at S1 and S2 (e.g., Max TSS S1: 3136, S2: 3322, and S3: 296mgL^{-1}). Nutrient concentrations were low at all sites where mean TP ranged from 0.4 to 1.2mgL^{-1} and mean NO_3 and NO_2 from 0.2 to 0.5mgL^{-1}.

Table 2.1 Mean values, ± standard deviation and ranges of the physical and chemical variables of the study sites used in the analyses, where n= 21 for each site. P is the significance level associated with analysis of variance (ANOVA, $P < 0.05$) for testing differences of each variable among sites. (-) represents no significant difference.

	S1			S2			S3			P
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	
ETo (mm)	3.2 ± 0.9	1.9	4.8	3.2 ± 0.9	1.9	4.8	3.2 ± 0.9	1.9	4.8	-
Depth (cm)	46.8 ± 24.5	2.1	84.3	70.7 ± 28.6	27.8	114.4	66.6 ± 21.9	23	99.8	<0.01
Temperature (°C)	16 ± 6.3	6.9	27.6	19.5 ± 6.2	11.6	30.1	19.8 ± 7	11	32.1	-
DO (ppm)	2.4 ± 2.4	0	9.3	5.2 ± 8.6	0	42.2	5.2 ± 5.6	1	28.5	<0.01
BOD^a	21 ± 31.5	5	126	22.3 ± 49.7	5	228	8.9 ± 11.5	5	56	-
DOC^a	23.5 ± 18.5	4.3	79	13 ± 16.4	2.8	78	14.3 ± 6.9	4.9	30	<0.01
pH	7.2 ± 0.6	6.3	8.6	7.5 ± 0.5	6.9	8.6	7.4 ± 0.6	6.7	8.7	-
Conductivity (µScm^{-1})	154.5 ± 98.2	59	460	155.8 ± 95.8	50	438	147 ± 59.9	76	313	-
Alkalinity^a	68.2 ± 37.5	28	188	85.9 ± 56.4	44	284	69.5 ± 27.1	40	152	-
Ca^{2+a}	12.3 ± 6.1	6	35	20.9 ± 15.7	9	82	12.8 ± 5	7	30	<0.01
Mg^{2+a}	6.8 ± 3.2	2	18	7.4 ± 5.7	3	28	7.5 ± 3.5	3	14	-
Na^{+a}	12.6 ± 7.2	1	36	2.8 ± 2.2	1	10	7.7 ± 4.8	2	20	<0.0001
K^{+a}	5.9 ± 10.1	0.5	35	6.9 ± 6.6	3.3	31	5.1 ± 3.5	1.1	15.3	< 0.05
SO$_4^{2-a}$	2.5 ± 0.1	2.5	3	2.4 ± 0.3	1	2.5	2.9 ± 2.1	1	12	-
Cl^{-a}	7.6 ± 5.3	2.5	22	3.6 ± 2.8	2.5	13	6 ± 2.7	2.5	11	< 0.001
Turbidity (NTU)	154.4 ± 554.7	3.9	2565	98.4 ± 369.1	1.8	1707	19.8 ± 23.3	6.8	115	-
TSS^a	310.5 ± 767.6	0.5	3136	205.9 ± 717.2	0.5	3322	45.5 ± 67.3	0.5	296	-
N-NO$_3^-$ and N-NO$_2^{-a}$	0.5 ± 0.9	0.1	3.3	0.2 ± 0.1	0.1	0.5	0.3 ± 0.4	0.1	1.6	-
TKN^a	8.7 ± 16.7	0.1	67	7 ± 19.7	0.1	92	2.9 ± 3.1	0.3	15	-
Total phosphorus^a	1.2 ± 2.3	0.1	9.6	0.9 ± 1.9	0.1	8.5	0.4 ± 0.6	0.1	2.8	-
Ortho-phosphate^a	0.1 ± 0	0.1	0.1	0.1 ± 0	0.1	0.2	0.1 ± 0.2	0.1	1	-
Phosphate^a	0.2 ± 0.4	0.01	1.8	0.1 ± 0.3	0.01	1.6	0.1 ± 0.01	0.01	0.4	-
NH$_4^{+a}$	0.3 ± 0.4	0.1	1.5	0.2 ± 0.3	0.1	1.6	0.2 ± 0.1	0.1	0.4	-

^{a} mgL^{-1}. Alkalinity as CaCO$_3$.

ANOVA indicated significant differences in mean depth, Na$^+$, Cl$^-$, Ca^{2+}, K$^+$, DOC and DO among the three sites (Table 2.1, Fig. 2.3). A post hoc Tukey test ($P < 0.05$) showed that site S1 differed significantly from S2 and S3 in depth and DO; all sites differed significantly in Na$^+$; S2 differed significantly from S1 and S3 in Cl$^-$ and Ca^{2+}; and S1 differed significantly from S2 in K$^+$ and DOC but both sites were not significantly different from S3 (Fig. 2.3).

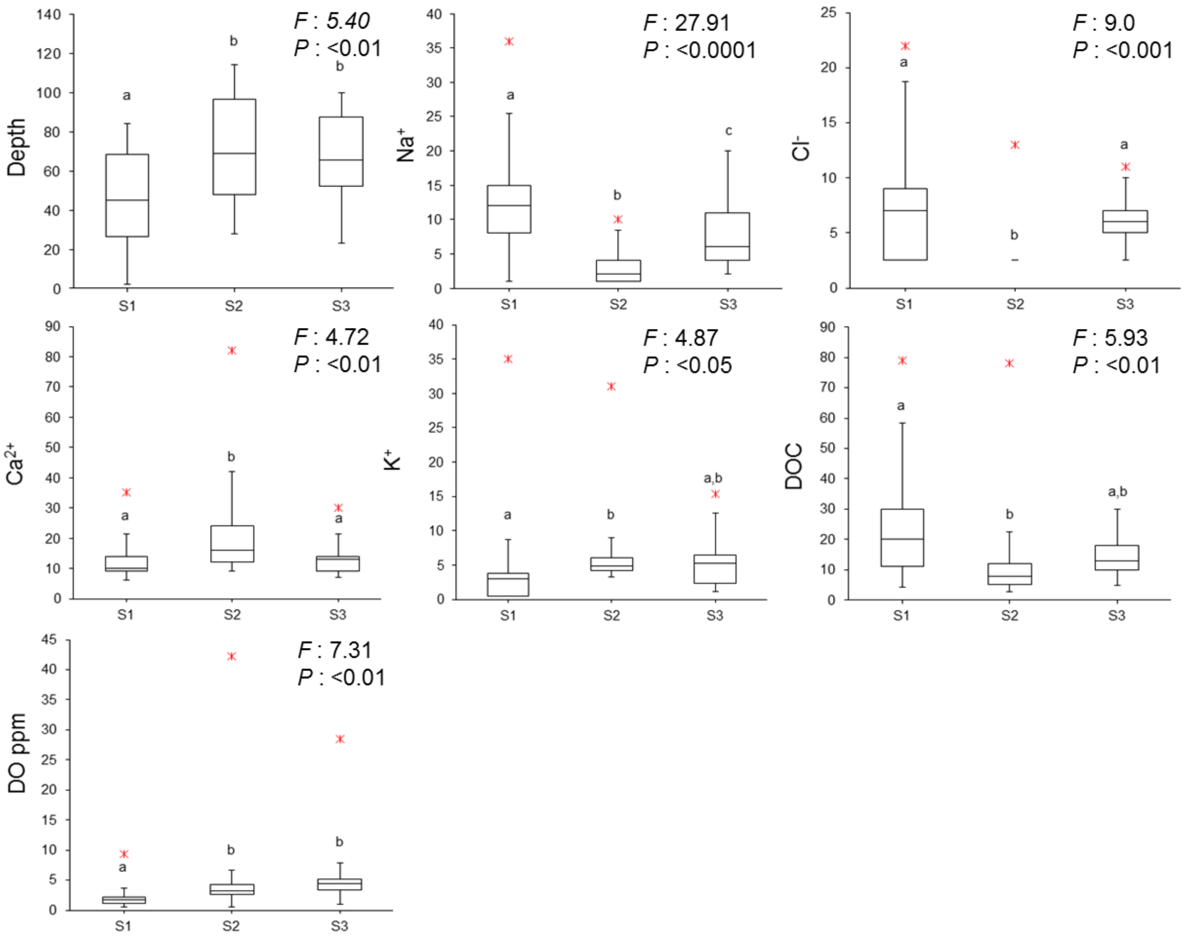

Figure 2.3 Box plots and Tukey HSD post-hoc test showing the physical and chemical differences between the three study sites in paired comparisons. Within each graph, sites with the same letter code are not significantly different. Fisher value (F) and the probabilities associated with ANOVA ($P < 0.05$) are displayed for each variable. For each variable, degrees of freedom (df) between groups = 2 and within groups = 60. Lines represent the medians, boxes represent the interquartile ranges (25–75%), whiskers represent 1.5 interquartile ranges and crosses represent outliers.

2.3.2 Composition

A total of sixty-three diatom samples were analysed and 83 taxa were identified. The most frequently observed taxa at each site was somewhat variable (S1, *Nitzschia acidoclinata* Lange-Bertalot: (27% of the identified valves), *Encyonema mesianum* (Cholnoky) Mann: (23%), *Eunotia bilunaris* (Ehrenberg) Schaarschmidt: (14%); S2, *Nitzschia acidoclinata*: (43%), *Nitzschia gracilis* Hantzsch: (18%); S3, *Nitzschia acidoclinata*: (17%), *Achnanthidium minutissimum* (Kützing) Czarnecki: (14%), morphologically similar *Gomphonema* spec. aff. *angustatum* (Kützing) Rabenhorst: (11%), *Encyonema mesianum*:

(9%)). ANOSIM indicated significant differences (global R = 0.43, P < 0.001) in diatom taxa composition between the three study sites. The analysis revealed that variation in diatom taxa composition was greater among sites than within each site, as significant differences were identified between S1 and S2 (R value 0.50, P < 0.001), S1 and S3 (R value 0.34, P < 0.001), and S2 and S3 (R value 0.45, P < 0.001). *Nitzschia acidoclinata* was significantly more widespread at S2 than in S1 and S3, where it contributed to over 30% of the similarity as revealed by SIMPER (Supplementary Table S2.1). Only two taxa contributed up to 50% of the average similarity at S2 (*Nitzschia gracilis* being the second top contributor). For this reason, S2 diatom communities varied less during the sampling period, as indicated by the highest average similarity (S2: 58.4%, S1: 54.3%, S3: 51%). NMDS ordination illustrated this pattern where S2 diatom communities varied the least through time in S2 (the closer the numbered symbols the more similar the diatom community between sampling months) (Fig. 2.4). The strong separation of S3 monthly samples indicated that S3 communities were the least similar relative to S1 and S2 communities (Fig. 2.4). S3 displayed the most variation in taxa composition, as reflected by the lowest average similarity and was characterised by the presence of *Gomphonema* spec. aff. *angustatum* and *Achnanthidium minutissimum* which were not common at any other site. S1 communities varied throughout the study, but to a lesser extent than communities at S3 (Fig. 2.4). Taxa which contributed the most to the similarity within S1 were *N. acidoclinata*, *Encyonema mesianum* and *Eunotia bilunaris* (Supplementary Table S2.1).

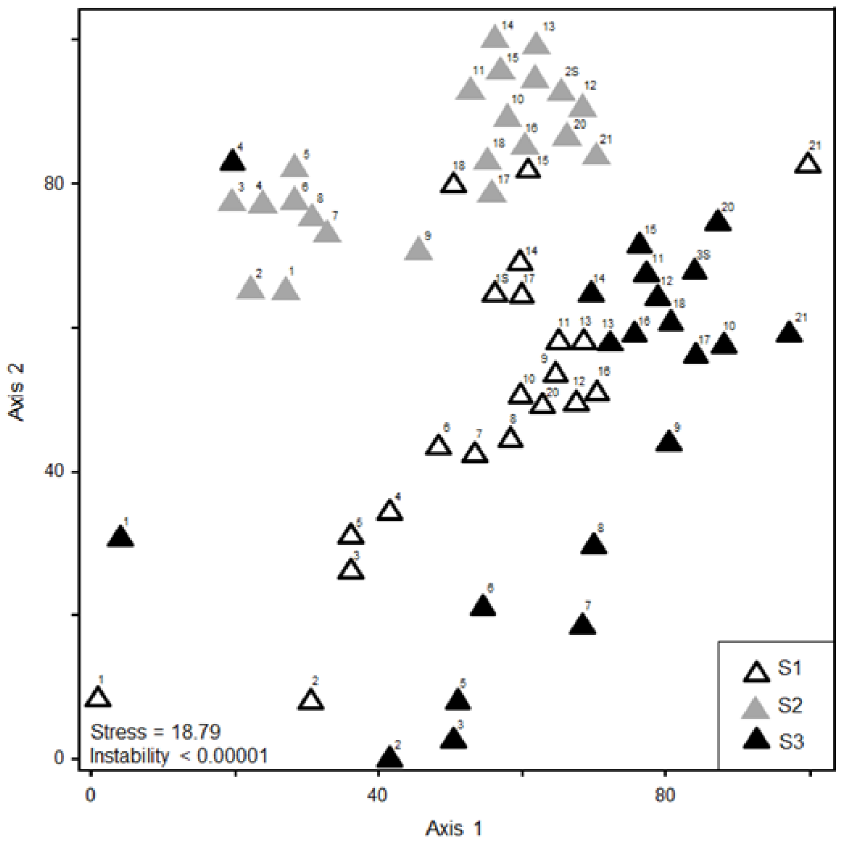

Figure 2.4 Non-metric multidimensional scaling (NMDS) ordinations of diatom communities from the three study sites during each month of sampling (n = 21 months/site). Numbers refer to each consecutive sampling month from March 2011 to September 2012. The stress value, 18.79, for our NMDS with 2 dimensions falls under 'Usable' (Clarke, 1993).

2.3.3 Environmental predictors

CCA was performed on 50 diatom taxa (after removal of rare taxa) in order to assess which were the most structuring environmental variables. Alkalinity, Na$^+$, Cl$^-$, depth and ETo were the most important variables that contributed the most to structuring the diatom communities; these variables significantly correlated with all four axes ($P \leq 0.01$, 999 Monte Carlo permutations, Bonferroni corrected) and represented 55.5% of the total explained variation in diatom species distributions. Alkalinity and Na$^+$ accounted for the largest portion of the total unconstrained variance (9.4% each). The first two axes explained the largest portion of variance in the species data. The first axis explained 14.5% of the variation in diatom species composition and was associated with alkalinity, depth and ETo, variables determined by local climate to fluctuations in both water-level and water chemistry in response to seasonal changes (Fig. 2.5, Table 2.2). The second axis explained 8.9% of the variation in the species data and was related to Na$^+$ and Cl$^-$ which reflect the marine origins of the bedrocks.

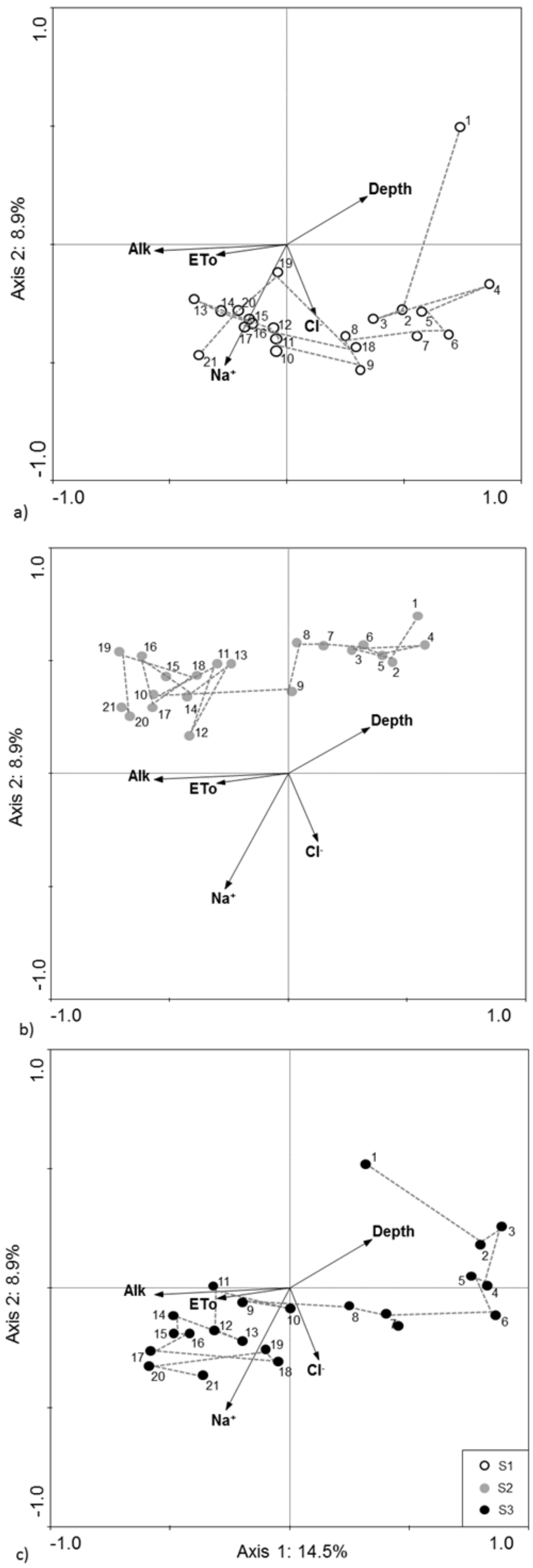

Figure 2.5 Canonical correspondence analysis (CCA) ordination plots for (a) S1, (b) S2 and (c) S3 diatom communities and significantly correlated physical and chemical variables. The dotted line shows the trajectory of successive sampling months in the ordination space. The number

refers to each sampling month. Percentage of explained variance for axis 1 and 2 is related to the variation in diatom species composition.

Table 2.2 Correlations of selected variables with the first three canonical correspondence analysis axes (999 Monte Carlo permutations). The variables listed explained significant (Bonferroni corrected; P≤ 0.01) variation in diatom species data.

	Axis 1	Axis 2	Axis 3
Explained Variation (%)	14.5	8.9	4.5
Alkalinity	-0.68	-0.10	-0.01
Na^+	-0.29	-0.82	0.01
Cl^-	0.19	-0.46	-0.05
Depth	-0.59	0.46	-0.33
ETo	-0.32	-0.06	-0.11

At the beginning of sampling in March 2011, the diatom communities in all three sites were located in the upper right quadrant of the plot which corresponded to the high water-levels recorded at each site in the first month of sampling (Fig. 2.5). Over the course of sampling, S1, S2 and S3 communities responded initially to water-level fluctuations, followed by changes in ionic concentration (alkalinity) and/or composition (Na^+, $Cl^−$) but the shifts in community structure for all three sites did not occur simultaneously for the most part of the study. In all three sites, we observed a distinct diatom species composition change in early summer (month 10), this coincided with a steady decline in ETo (Fig. 2.2). The S2 and S3 diatom communities were gathered along the depth gradient during the first 8 to 9 months of sampling. In month 10, paralleled with Max ETo, S2 communities shifted to the upper left quadrant where they were dispersed along an alkalinity gradient throughout the second half period of sampling, whilst S3 communities shifted to the lower left quadrant where they responded to fluctuations in alkalinity, and Na^+ and $Cl^−$ ions.

In contrast, S1 communities exhibited a significant change in community structure immediately after the first sampling month at the onset of autumn (the start of the dry season). Fig. 2.5 (a) illustrates a notable shift in S1 communities in month 2 to the bottom lower quadrant associated with $Cl^−$. S1 communities were gathered around the $Cl^−$ gradient until month 10 (Max ETo reached) where communities shifted between Na^+ and $Cl^−$ until the end of the study.

2.3.4 Effects of ionic composition and concentration vs. hydrological factor

CCA showed that 31.1% of the total variation in the species composition could be explained by all five major variables: alkalinity, depth and ETo, Na^+ and Cl^-. The variance explained by the interactions between the two sets of variables, which included alkalinity, depth and ETo on CCA axis 1, and Na^+ and Cl^- on CCA axis 2 was 24.3% and 17.1%, respectively using partial CCA.

Alkalinity is not only related to depth and total relative evapotranspiration, but it is also related to ionic concentration. In order to determine how much of the influence on the variance in species composition was a result of hydrological factors, depth and ETo, and how much was a consequence of the effect of ionic concentration (alkalinity) and composition (Na^+, Cl^-), we tested the variance explained by the hydrological factors after partitioning out the effects of the other three variables (alkalinity, Na^+, Cl^-). Ionic concentration (alkalinity) and composition (Na^+, Cl^-) independently explained a much higher proportion (25.3%) of species variation than the hydrological factors, while the hydrological factors accounted for 10.8% of the variance.

2.3.5 Diatom Inference models

We developed diatom-based inference models for the five most important variables, alkalinity, Na^+, Cl^-, depth and ETo, using the 63-sample dataset. A simple weighted averaging model with inverse deshrinking (WA-inv) provided the highest predictive powers. Only WA-inv results are reported here (Table 2.3). Na^+ and depth produced the best performing models where diatom-inferred Na^+ was correlated with measured values with r^2 of 0.79 and predicted r^2 boot of 0.71, and diatom-inferred depth with an r^2 of 0.70 and r^2 boot of 0.64 (Fig. 2.6, Table 2.3). In comparison to the Na^+ and depth models, alkalinity provided moderate performance with an r^2 of 0.57 and r^2 boot of 0.46 between measured and predicted alkalinity. The Cl^- and ETo models displayed little robustness judged by bootstrapping of the dataset which produced an extremely low r^2 boot of 0.22 and 0.11, respectively.

Outlier analysis identified two outlier samples; one sample had an extremely low depth value and the other sample contained a high relative abundance of *Eunotia bilunaris*. The two samples were excluded from the analysis; no taxa were removed from the dataset as a result. The 61-sample dataset only marginally improved the model performance statistics (Table 2.3).

Table 2.3 Performance of weighted-averaging (WA) diatom-based inference models for the three study sites using inverse deshrinking. The apparent correlation between diatom-inferred and observed (r^2) and the root mean squared error (RMSE) are presented. A more conservative measure of WA model performance was obtained using the bootstrapping cross-validation method to derive estimates of RMSE of Prediction (RMSEP) and bootstrapped r^2 (r^2 boot).

	63-sample data set				61-sample data set			
	Inferred-observed		Predicted		Inferred-observed		Predicted	
	r^2	RMSE	r^2 boot	RMSEP	r^2	RMSE	r^2 boot	RMSEP
Alkalinity	0.57	0.28	0.46	0.32	0.62	0.25	0.52	0.28
Na^+	0.79	0.43	0.71	0.56	0.82	0.39	0.76	0.49
Cl^-	0.41	0.48	0.22	0.58	0.42	0.45	0.19	0.57
Depth	0.70	14.60	0.64	16.90	0.70	14.31	0.63	16.50
ETo	0.29	0.24	0.11	0.29	0.31	0.24	0.11	0.29

*RMSE and RMSEP are log transformed values except for those reported for Depth.

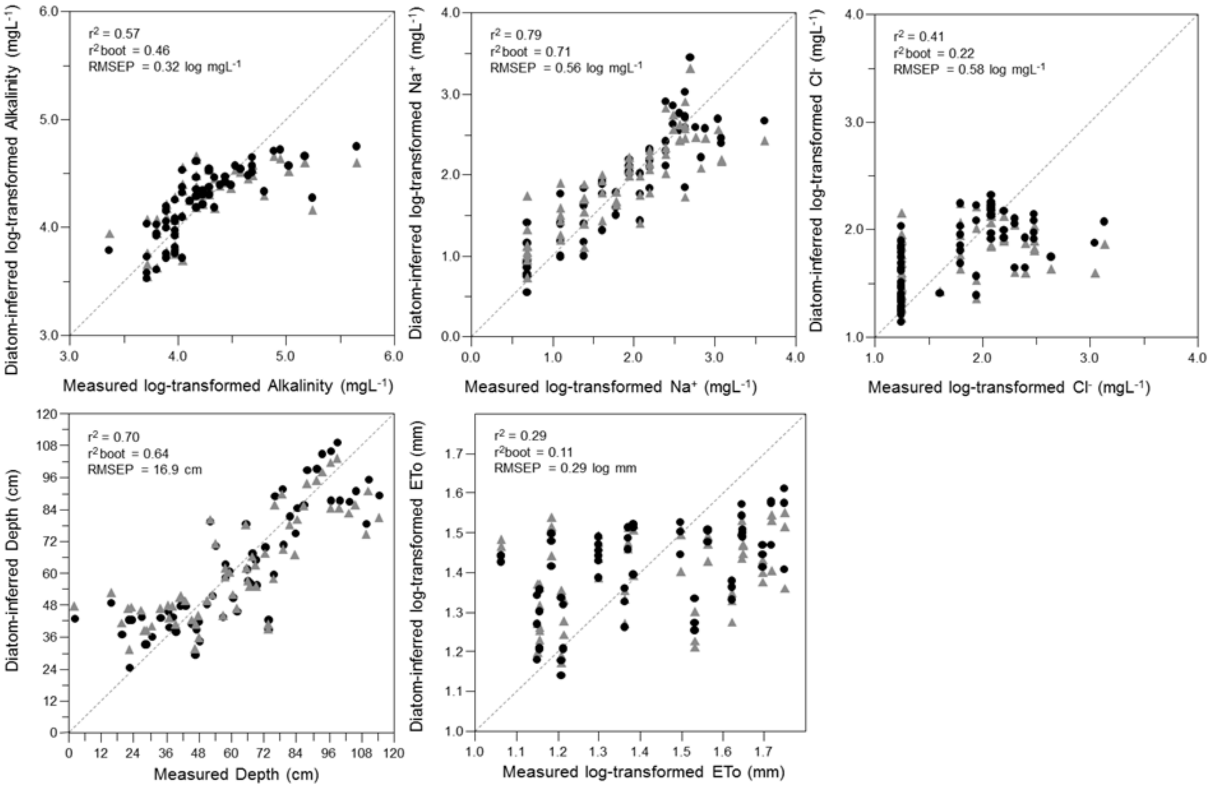

Figure 2.6 Weighted averaging (WA) calibration models with bootstrapping cross validation method for alkalinity, Na^+, Cl^-, depth and monthly mean total relative evapotranspiration (ETo). Relationships between measured and diatom-inferred values are plotted. Scatter plots display apparent (circle) and bootstrapped (triangle) estimates. Performance of models indicated by r^2 values (apparent and bootstrapped r^2 values (r^2 boot) and RMSEP (Root Mean Square Error of Prediction). Dashed line represents 1:1 line of 100 % correlation.

2.3.6 Indicator taxa

WA optima and tolerances of 50 taxa determined for Na^+, depth and alkalinity are shown in Supplementary Table S2.2. Despite the relatively narrow range of Na^+ in all three sites, Na^+ influenced diatom species composition. For example, low Na^+ sites were characterised by high relative abundances of *Gomphonema* spec. aff. *angustatum*, *Gomphonema exilissimum* (Grunow) Lange-Bertalot & Reichardt and *Nitzschia palea* var. *debilis* (Kützing) Grunow (optima range 3.3 – 3.7 mgL^{-1}). As Na^+ increased, *Achnanthidium minutissimum*, *Encyonema mesianum* and *Gomphonema parvulum* (Kützing) Kützing sensu lato became dominant (optima range 7.6 – 9.5 mgL^{-1}). Changes were also observed in diatom species composition along the alkalinity and depth gradient (Supplementary Table S2.2). Diatom assemblages were dominated by *Achnanthidium minutissimum* and *Nitzschia fruticosa* Hustedt in sites where depth was low and alkalinity was high (optima depth range: 47 - 47.4 cm; alkalinity: 82.7 – 83.6 mgL^{-1}), indicating the drying out period. Conversely, as depth increased and alkalinity decreased, *Nitzschia palea* var. *debilis* and *Gomphonema auritum* Braun ex Kützing became dominant (optima depth: 76 – 71.8 cm; alkalinity: 49.9 – 57.6 mgL^{-1}), indicating high inundation.

Of the most common taxa (Hills’ N2 >25, Reavie & Smol, 2001), *Nitzschia palea* var. *debilis*, *Gomphonema exilissimum*, *Gomphonema* spec. aff. *angustatum* and *Gomphonema spiculoides* Gandhi and showed significant correlations and a narrow tolerance to both Na^+ and alkalinity (Supplementary Table S2.2, Table S2.3). *Encyonema mesianum*, *Achnanthidium minutissimum*, *Nitzschia fruticosa*, *Nitzschia palea* var. *debilis* and *Gomphonema auritum* displayed a relatively narrow range and strong correlation with depth.

2.4 DISCUSSION

2.4.1 Diatom species composition

Comparing species composition among the three study sites, the assemblage structures of the sites were different and different species dominated. Site S2 was the most species homogenous of the three sites in which motile diatoms (fast moving species) *Nitzschia acidoclinata* and *Nitzschia gracilis* generally dominated the biofilm throughout the study. S2 was significantly Ca^{2+} richer and Na^+ poorer (Fig. 2.3) compared to the other two sites. Studies have demonstrated the importance of Ca^{2+} in diatom assemblage structure (e.g., Patrick & Reimer, 1966a; Blinn, 1993; Potapova & Charles, 2003). Ca^{2+} is important for growth and motility where motile taxa such as the *Nitzschia* genus utilise this ion for regulation of locomotion (Cohn & Disparti, 1994). Nevertheless, the demand for Ca^{2+} is widely variable among individual diatom taxa (Vymazal, 1995) and undefined for this species dataset. Moreover, Potapova & Charles (2003) found that many diatoms with relatively high optima for base cations (such as Ca^{+}) had low optima for Na^{+}; from which they concluded that the ratio of monovalent to divalent cations was a major factor influencing diatom species composition (see also, Gasse et al.,1983). It remains unclear, however, as to what extent the composition of major ions influenced the diatom assemblage structure at S2.

In contrast, site S3 displayed the most variation in species composition of all three sites. It was also associated with more stable water levels, lower turbulence and suspended solids throughout the study (Table 2.1). Water level fluctuations can directly affect sedimentation, resuspension, and the biogeochemical cycle in shallow depressions (Niemistö et al., 2008). This may result in large changes in the composition of epiphytic assemblages, and in extreme cases, where water depth and/or water clarity start to restrict growth, certain taxa may be removed completely (Leira et al., 2015). It is, therefore, conceivable that the stable water level and low turbid environment found at S3 may have been accountable for the comparatively large species variability.

2.4.2 Environmental predictors

Alkalinity and ionic composition, (essentially Na^{+} and Cl^{-}) were the most important explanatory variables of epiphytic diatom community composition in temporary depressional wetlands, as revealed by CCA. Patterns of diatom distribution could be attributed not only to hydrochemical variation, but also to hydrological processes. Hydrological factors, depth and total relative evapotranspiration were the second most important variables structuring diatom assemblages. As already demonstrated by several investigators (e.g., Blinn, 1993; Yang & Duthie, 1995; Gregory-Eaves et al., 1999; Köster et al., 2004), the concentration and composition of major ions, including Na^{+} and Cl^{-}, as well as water depth, are important predictors of the diatom distribution in lentic systems (deep lakes).

At sites S2 and S3, the deepest sites, the seasonal successions of species were largely explained by the depth gradient. In S1, which was significantly shallower than the other two sites (Fig. 2.3), ionic composition explained the largest portion of the variance in communities (Fig. 2.5). S1 displayed the largest variability in Na^{+} and Cl^{-} in comparison to S2 and S3 (Fig. 2.3). With respect to temporal variability of the chemical composition in S1, changes were more rapid than in S2 and S3. The variation in major ions is more acute in shallower wetlands than in deeper wetlands because shallower wetlands are more affected by changes in the hydrologic budget that can strongly influence water level and water chemistry (Caramujo & Boavida, 2010).

At the start of sampling, all sites were similar in that all communities correlated to depth. As sampling continued, the ionic content of each wetland evolved differently and in turn, differences in community composition were observed among the sites. Despite these differences, all sites showed a strong seasonal succession in diatom communities in early summer 2011, which coincided with a steep elevation in ETo, reaching maximum ETo value (Fig. 2.2). This demonstrates how wetlands are intricately tied to the climate system whereby climatic changes have the potential to significantly alter wetland hydrology and chemistry, and in turn, affect the species composition of the wetland's biota (Fritz et al., 2010).

Throughout the study, the species composition at the three sites shifted according to differences in alkalinity and Na^+ and Cl^-. The strength and composition of major ions may affect nutrient availability to diatoms (Tuchman et al., 1984; Saros & Fritz, 2000; Hagerthey et al., 2011). For example, an increase in Na^+ can promote the transport of silicate, a nutrient across the cell membrane (Bhattacharyya & Volcani, 1980). The amount of bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) (alkalinity) in the water influences the availability of Ca^{2+} and Mg^{2+} which has implications on the growth of diatoms (Potapova & Charles, 2003).

2.4.3 Diatom inference models

The diatom inference model (WA-inv) for Na^+ developed from our depressional wetland dataset had the highest performance (Fig. 2.6, Table 2.3). Our bootstrapped statistics for Na^+ (r^2 0.71) indicated that our Na^+ model was robust and quantifiable. Our models of alkalinity had moderate performance statistics (Fig. 2.6, Table 2.3). There is no comparable statistical information on Na^+, alkalinity and depth models derived from wetlands. The Cl^- and ETo inference models developed in our study had very poor predictive power. Poor performance of Cl^- and ETo models was much more evident after bootstrapping, and model performances did not improve significantly upon deleting outlier samples. Our models were developed from intensely monitored sites as opposed to snap shot measurements. To further strengthen our inference models we could increase the sample size of our calibration set (e.g., Wilson et al., 1996) by sampling more sites in the region over a longer period.

2.4.4 Indicator taxa

Common taxa well-correlated with Na^+, depth and alkalinity, and with narrow tolerance ranges may facilitate identification of anthropogenic impacts in these wetlands and possibly similar wetlands in the region, based on their presence vs. absence and/or changes in abundance. Strong indicators include *Gomphonema exilissimum*, *Gomphonema spiculoides* and *Nitzschia palea* var. *debilis* which had the lowest Na^+ and alkaline optimas; studies show that these taxa only appear in oligotrophic, slightly acid waters with low electrolyte content (Krammer et al., 1988; Karthick et al., 2011; Hofmann et al., 2013; Jüttner et al., 2013). *Nitzschia fruticosa* was affiliated with higher alkaline optimas and has also been described as favouring alkaline habitats in the Lake Victoria basin, East Africa (Triest et al., 2012). Rare taxon *Hantzschia amphioxys* (Ehrenberg) Grunow had the highest alkalinity optima and one of the lowest depth optima, indicating its ability to tolerate drier habitats. This aerophilic taxon is a soil diatom favouring low-moisture environments and has been correlated with low hydroperiod optima in temporary depressions elsewhere (Gaiser et al., 1998).

2.5 CONCLUSIONS

Results generated from this study demonstrate the utility of epiphytic diatoms as indicators of changing environmental conditions in temporary depressional wetlands in the Mpumalanga Highveld. Based on the strong correlations between the diatom species composition and a number of environmental variables, we were able to establish a robust model of reference condition of this wetland type in the region. For this reason, monitoring of epiphytic diatom assemblages may present a possible alternative in the environmental assessment of depressional wetlands in the Mpumalanga Highveld and possibly other regions of South Africa, where the use of other types of bioindicators, such as macroinvertebrates and macrophytes have proven to be ineffective. Future research on species-specific responses to anthropogenic impacts in the region (e.g., mining, agriculture) is recommended. Our standardised sampling methods allows for further work to develop this training set by investigating a variety of depressional wetlands in the region that encompass a wide range of ionic content, both natural and human-induced, over longer periods, thereby providing a more comprehensive assessment of ecological inference models.

CHAPTER 3

A DIATOM FUNCTIONAL-BASED APPROACH TO ASSESS CHANGING ENVIRONMENTAL CONDITIONS IN TEMPORARY DEPRESSIONAL WETLANDS

Published: Ecological Indicators

ABSTRACT

Functional-based assessments to identify the effects of human-induced disturbances on diatom communities are increasingly used. However, information on the response of functional groups to natural disturbances in temporary depressional wetlands is limited although important for the development of temporary wetland biological assessments. We assessed how diatom life-form and ecological guilds responded to a seasonal hydrological and hydrochemical gradient in three least human-disturbed, temporary depressional wetlands. We assigned species to their respective life-form and ecological guild groups and compared metric composition along the gradient. Overall, temporal variability in alkalinity and ionic composition, essentially Na^+, as well as hydrological factors, wetland depth and total relative evapotranspiration (ETo), were good predictors of diatom species and functional group composition. Low profile guilds dominated by pioneer life-forms showed the strongest relationship with higher disturbance levels (i.e. increasing Na^+, alkalinity with a decrease in depth). Similarly, the planktonic guild and tube-living, rosette and adnate life-forms dominated at higher disturbance levels whereas the high profile diatoms displayed the reverse trend. Our study shows the effectiveness of functional-based assessments beyond traditional species-based approaches for understanding and predicting community responses to temporal changes in environmental conditions. We also highlight the benefit of using both life-forms and ecological guilds where a broad set of metrics can enhance our understanding of the mechanisms relating diatom composition to environmental stressors and provide signs of underlying ecological processes.

3.1 INTRODUCTION

Over the past decade there have been increasing efforts to develop biological monitoring tools to assess the condition of wetlands in human-modified landscapes (e.g.,USEPA, 2016). In South Africa, although biological assessment techniques for river health assessments are well established (e.g., Dickens & Graham, 2002; Taylor et al., 2005), in the case of wetlands there is currently no definitive, well-developed method for assessing ecological condition. The need to develop wetland condition assessment methods is urgent, particularly in wetland rich areas such as the Mpumalanga Province where mining has contributed to substantial loss and degradation of depressional wetlands (Riato et al., 2014). Such activities have modified the surface water hydrology and hydrochemistry of temporary depressional wetlands to varying degrees, and in some cases has resulted in extreme alkalization, salinisation and permanent inundation in what was previously a fresh, ephemeral system.

Numerous studies have demonstrated the robustness of diatoms as a tool to assess the ecological status of shallow lakes and wetlands (reviews in Bennion et al., 2010, Gaiser & Rühland, 2010). Diatom-based indices have been developed to assess pollution in shallow systems, particularly in Europe and North America (e.g., Kelly et al., 2007; Lane & Brown, 2007; Stenger-Kovács et al., 2007). However, diatom-indices require species level identification which can be challenging due to the large diversity of species included in these indices and the continual taxonomical changes (Gottschalk & Kahlert, 2012). The presence of species complexes that are difficult to identify using standard taxonomic techniques constitutes an additional problem particularly when the finest taxonomic accuracy is required by the quality indices (B-Béres et al., 2014). Moreover, the suitability of these indices can be limited, since most diatom indices derive their calculations based on species distributions found in specific regions and thus are not broadly applicable (Stenger-Kovács et al., 2007; Lavoie et al., 2009b).

Rimet & Bouchez, (2012a) demonstrated the robustness of using a broader taxonomic resolution in biological assessments. For this reason, the use of trait-derived functional groups may be useful for wetland assessments, particularly in South Africa, where wetland diatom flora is poorly described, thus, taxonomic information required for species-level identifications is lacking. Moreover, the use of functional groups is likely to provide a greater understanding of the role by which important environmental drivers influence diatom community structure (Lange et al., 2016).

The application of diatom functional groups (life-forms and ecological guilds), have become increasingly popular in ecological assessments over the past decade (Tapolczai et al., 2016), mostly in response to nutrient and pesticide contamination in lotic systems (e.g., Passy, 2007a; Rimet and Bouchez, 2011) and mesocosm experiments (e.g., Morin et al., 2009; Bayona et al., 2014). However, few studies have applied functional groups as a community measure for the ecological status in lakes (e.g., Gottschalk & Kahlert, 2012; Leira et al., 2015) and only one other study has proven their applicability in wetlands (nutrient rich ditches) (Goldenberg Vilar et al., 2014).

Given the urgent need to develop a biological assessment tool for assessing temporary depressional wetland conditions in the Mpumalanga Province, the main goal of this research was to determine whether diatom functional groups can be used as simple, effective and reliable indicators sensitive to changing environmental conditions in temporary depressional wetlands in the region. Establishing a reference condition of variation due to natural disturbances (e.g., seasonal fluctuations in both water-level and water chemistry) in temporary depressional wetlands using diatoms is necessary for further investigations of anthropogenic impacts. Thus, we examined three least human-disturbed temporary depressional wetlands during various stages of inundation in order to understand the natural effects of hydrological changes on the water chemical composition and its influence on functional groups. We focused on four aims: (a) to identify the most important physico-chemical parameters influencing the structure of diatom species communities; (b) to compare those with the physico-chemical parameters structuring the functional groups; (c) to examine temporal dynamics of functional groups along the hydrological and hydrochemical gradient; and (d) to identify the groups most responsive to changes along the gradient.

3.2 MATERIALS AND METHODS

3.2.1 Study area

The study area is located in the Bapsfontein region of the Gauteng Province of South Africa which borders the Mpumalanga Highveld region at an altitude of 1606m asl., with underlying geologies of weakly cemented sandstones and fissile shales and climate as those found in the Mpumalanga Highveld. Three homogenous, temporary depressional wetlands (average depth < 2m) were selected in this area based on vegetation, conductivity, surrounding land-use and close proximity to one another (radius of 1km) (refer to Chapter 2; Fig. 2.1). The study sites (S1, S2 and S3) were sampled once a month from March 2011 (period of high inundation) to September 2012 (drying out period). In Chapter 2, Table 2.1 summarises the physico-chemical parameters distinguishing the sites S1, S2 and S3 and Section 2.2.1 provides a detailed description of the local climatology.

3.2.2 Sampling and laboratory methods

We collected one sample of epiphytic diatoms from at least five submersed macrophyte stems of the same vegetation type (*Leersia hexandra*) at the deepest point in the basin centre (pelagic zone) at each site to ensure the comparability between water bodies as recommended by King et al. (2006). Macrophyte stems were snipped and placed into a zip lock bag with a small amount of distilled water. Epiphytes were dislodged from all stems by shaking the samples for 2 min following Zimba & Hopson (1997).

Samples were returned to the laboratory where they were acid cleaned and mounted on microscope slides (Battarbee et al., 2001). We counted and identified 400 valves to the lowest feasible taxonomic level using standard European diatom floras (e.g., such as Krammer & Lange-Bertalot, 1986-1991; Lange-Bertalot, 2000-2002; Lange-Bertalot et al., 2001), several papers on the Southern African flora by Cholnoky, Schoeman and Archibald (e.g., Schoeman & Archibald, 1976-1980), and books and recent papers on *Gomphonema* taxonomy (e.g., Reichardt & Lange-Bertalot, 1999; Rose & Cox, 2014; Reichardt, 2015).

Diatom taxa were assigned to two types of functional groups a), life-forms and b), ecological guilds, in accordance with Passy (Passy, 2007a, 2007b) and Rimet and Bouchez (2012b). The taxa assigned to the various life-form and ecological guild groups are listed in Table 3.1. A taxon can be assigned to more than one life-form group since certain taxa can have multiple life-forms throughout its life-cycle (Rimet and Bouchez, 2012b). We adapted the functional groups to accommodate taxa identified in our study which were not mentioned in Rimet and Bouchez (2012b). A total of sixty-three diatom samples were analysed and 83 taxa were identified. We examined the seasonal patterns of the different functional groups based on their relative abundances in order to develop a more comprehensive understanding of temporal changes in biofilm structure and provide insight into the major parameters structuring group composition.

We sampled chemical and physical parameters at the same location and time as the diatom sampling. Water samples (1 L) were collected in an acid-cleaned, high-density polyethylene bottle and kept on ice during transportation to the laboratory. Samples were analysed for concentrations of biological oxygen demand (BOD), NH_4^+, total Kjeldahl nitrogen (TKN), NO_3^-, NO_2^-, P, PO_4^{2-}, total phosphate, Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, SO_4^{2-}, dissolved organic carbon (DOC), turbidity, total suspended solids (TSS), alkalinity and conductivity. Analysis was conducted by Waterlab (Pty.) Ltd. laboratory in Pretoria following standard procedures (APHA, 1998). Temperature, pH, dissolved oxygen (DO) and water depth were measured in the field.

We included total relative evapotranspiration (ETo) data in the analysis to represent the seasonal (summer–winter) gradient. ETo data, calculated from hourly data using the FAO-56 Penman-Monteith method (Zotarelli et al., 2010), was collected from a weather station owned by the Agricultural Research Council located 6km from the study sites. For each sampling month, we used the monthly ETo which was calculated as the mean of the daily ETo values for the sampling month.

Table 3.1 Assignment of taxa to life-forms and ecological guilds following Passy (2007a,b) and Rimet and Bouchez (2012b).

Functional groups	Definition of functional group classification	Taxa Assigned
Life-forms		
Mobile	Free moving e.g. some species vertically migrate into the sediments to acquire nutrients	*Achnanthes*, *Achnanthidium*, *Brachysira*, *Caloneis*, *Diadesmis*, *Encyonema*, *Eolimna*, *Eunotia*, *Frustulia*, *Gomphonema*, *Luticola*, *Mayamaea*, *Navicula*, *Nitzschia*, *Rhopalodia*, *Sellaphora*, *Stauroneis*
Pioneer	Species colonise bare substrates faster than other species	*Achnanthidium minutissima* var. *minutissima*, *A. minutissima* var. *affinis*, *A. saprophilum*
Tube-living	Species live in mucous substance within which they can move freely	*Frustulia*, *Encyonema mesianum*
Rosette colony	Species attached to substrate by a short stalk at one pole; colonies look fan-shaped	*Ulnaria acus*
Ribbon colony	Species attached to one another either by interlocking spines or by a layer of mucous on their valve face, forming long, ribbon-like colonies	*Eunotia bilunaris*, *Eunotia minor*, *Eunotia pectinalis*
Pedunculate	Species grows upright to substrate, attached either by a mucilage pad or by a stalk	*Achnanthes*, *Achnanthidium*, *Fragilaria*, *Ulnaria*, *Gomphonema*
Adnate	Species grows parallel to substrate, attached by their valve face	*Rhopalodia gibba*
Ecological guilds		
High profile	Species of tall stature, including erect, filamentous, branched, chain-forming, tube-forming, pedunculate, and colonial centrics	*Diadesmis*, *Encyonema mesianum*, *Eunotia*, *Fragilaria*, *Ulnaria Gomphonema*
Low profile	Species of short stature, including prostrate, adnate, small erect, solitary centrics, slow-moving species	*Achnanthes*, *Achnanthidium*, *Brachysira*
Motile	Fast-moving species	*Caloneis*, *Eolimna*, *Luticola*, *Mayamaea*, *Navicula*, *Nitzschia*, *Rhopalodia*, *Sellaphora*, *Stauroneis*,
Planktonic	Solitary or colonial centrics, pennates	*Cyclotella meneghiniana*, *Fragilaria tenera*, *Ulnaria acus*, *Nitzschia acicularis*

3.2.3 Data Analysis

We used CANOCO 4.5's (ter Braak & Šmilauer, 2002) redundancy analyses (RDA) (gradient length < 2 standard deviation units) to examine relations of physico-chemical parameters to diatom compositional structure at species and functional group level. Inclusion of physico-chemical parameters in the final analysis was based on eliminating collinear variables with a high variance inflation factor (VIF>10) (ter Braak & Šmilauer, 1998). We applied step-wise forward selection to select a final set of physico-chemical parameters that related best to the diatom species and functional group composition (ter Braak & Šmilauer, 1998). We calculated Spearman's rank correlations using SAS 9.4 (SAS Institute, Cary NC) and performed a Bonferroni-Holm correction for multiple comparisons (Holm, 1979) to determine which functional groups were sensitive to the most important parameters structuring the diatom communities.

Diatom species and functional group abundance was square root transformed and physico-chemical parameters not meeting normality assumptions were log transformed in order to normalise distributions and reduce skewness.

3.3 RESULTS

3.3.1 Functional group composition

The most common functional groups which contributed up to 50% of the counts belonged to the mobile life-form (37%), the high profile (17%) and motile guild (17%), and the pedunculate life-form (13%). Mobile diatoms were comprised of *Nitzschia acidoclinata* (31% of all mobile valves counted), followed by *Encyonema mesianum* (13%), *Nitzschia gracilis* (9%), *Eunotia bilunaris* (8%) and *Achnanthidium minutissimum* (7%). Motile diatoms were dominated by *Nitzschia acidoclinata* (67%), *Nitzschia gracilis* (19%) and *Nitzschia fruiticosa* (7%). High profile diatoms were mostly comprised of *Encyonema mesianum* (28%), *Eunotia bilunaris* (17%), *Gomphonema* aff. *angustatum* (14%), *Gomphonema parvulum* (10%) and *Gomphonema auritum* (9%). The most abundant pedunculates were *A. minutissimum* (20%), *G.* aff. *angustatum* (18%), *G. parvulum* (12%) and *G. auritum* (11%).

3.3.2 Seasonal patterns in functional group composition

Fig. 3.1 displays the seasonal patterns of the different life-forms and ecological guilds in the three study sites. There was a distinct succession between the high profile guild and the low and motile profile guild, particularly at sites S1 and S3. At the end of summer 2011, the high profile guild abundance steadily declined and was gradually replaced by the low profile and motile guild until the end of sampling. This was not the case for site S2, where the motile guild dominated the biomass throughout the study. The pioneer life-form followed similar seasonal patterns to that of the low profile guild for the reason that both groups were largely dominated by *Achnanthidium minutissimum.* Site S3 was dominated by *Gomphonema* taxa which were assigned to both the high profile guild and the pedunculate life-form. For this reason, seasonal patterns were comparable for these two groups at this site.

Albeit fairly uncommon, the rosette life-form comprised of *Ulnaria acus* at site S2 only, and the adnate life-form, represented by *Rhopalodia gibba* at S3, displayed similar abundance patterns, where growth started in summer 2011 and reached peak biomass during the summer season. The ribbon life-form, consisting of *Eunotia* taxa, rapidly declined at the end of summer 2011 and thereafter occurred infrequently throughout the study. The mobile life-forms mostly dominated the biomass throughout the study at all sites. However, an important decline in mobile abundance observed during winter 2011 for all sites, summer 2012 for S2 and autumn 2012 for S3, coincided with an increase in planktonic diatoms mostly dominated by *Fragilaria tenera*.

During autumn and winter 2011 until mid-summer 2012, the abundance of tube-living life-form diatoms, dominated by *Encyonema mesianum*, showed an important increase in abundance at site S1. *E. mesianum* then rapidly decreased until mid-winter 2012, when this taxon started to re-establish. The proportion of tube-living life-forms was comparatively lower at sites S2 and S3.

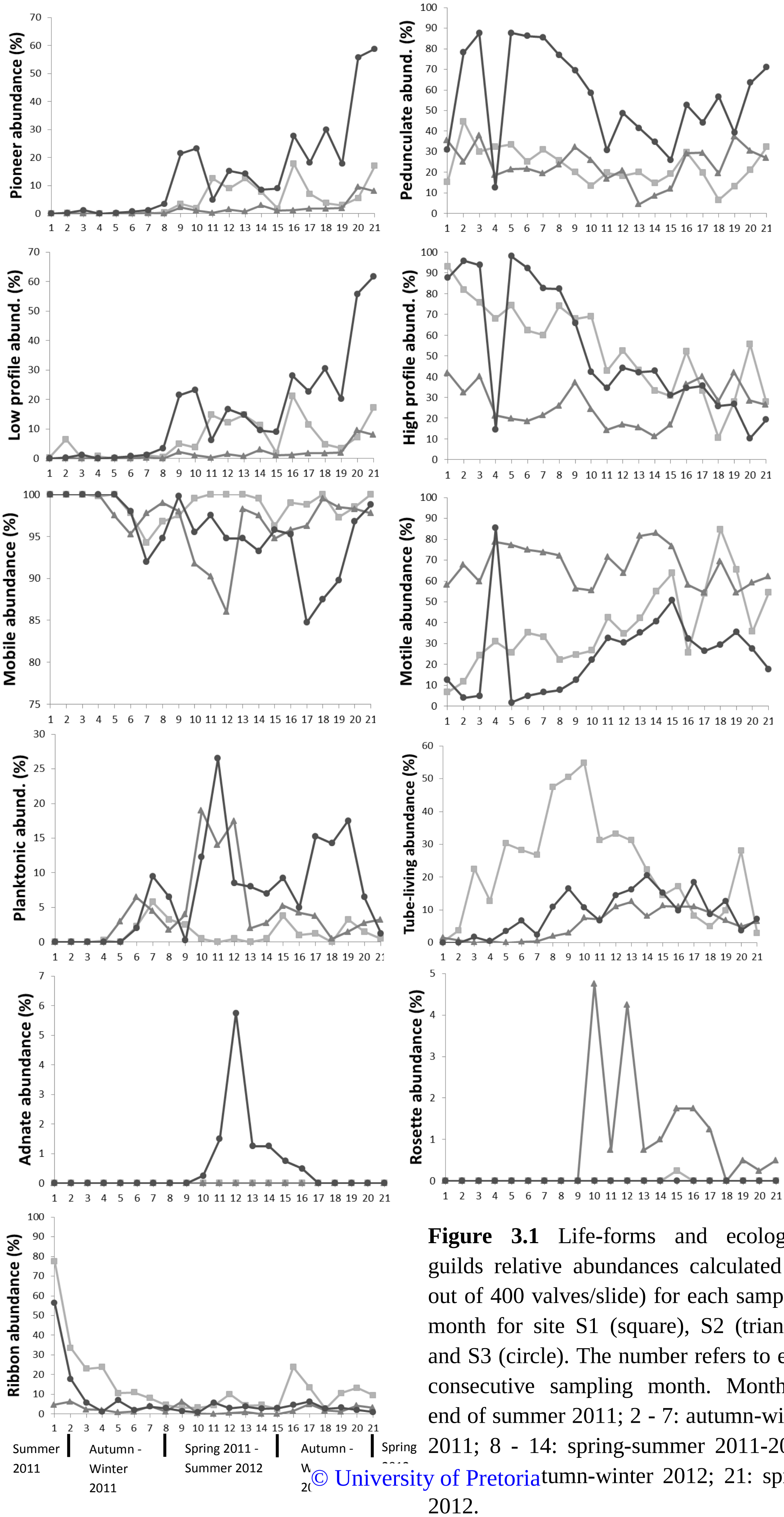

Figure 3.1 Life-forms and ecological guilds relative abundances calculated (% out of 400 valves/slide) for each sampling month for site S1 (square), S2 (triangle) and S3 (circle). The number refers to each consecutive sampling month. Month 1: end of summer 2011; 2 - 7: autumn-winter 2011; 8 - 14: spring-summer 2011-2012; [illegible]tumn-winter 2012; 21: spring 2012.

3.3.3 Environmental predictors

The relative importance of the physico-chemical parameters on the structure of the diatom communities were assessed using RDA for the species and functional group composition.

Fig. 3.2 shows the parameters representing the highest correlation with the first two axes of the RDA. The most important structuring parameters for both diatom species and functional group composition were Na^+ and alkalinity. Likewise, Cl^-, depth and ETo were the secondary most prominent drivers structuring diatom communities for both species and functional groups (Fig. 3.2). RDA using Na^+, alkalinity, Cl^-, depth and ETo explained 52% and 83.9% of the total explained variation for species and functional groups, respectively; all axes were significant ($P < 0.01$, 999 Monte Carlo permutations). The first two axes explained 20.5% of the variation in diatom species composition (Fig. 3.2a), and 60.9% of the variation in functional group composition (Fig. 3.2b). Redundancy coefficients for species and functional groups respectively indicated that the first axis was associated with alkalinity (0.64, -0.57) and ETo (0.30, -0.29), attributed to seasonal changes. The second axis was affiliated with Na^+ (-0.86, 0.56) and Cl^- (-0.38, 0.46) which reflect the marine origins of the bedrocks. In the species RDA, the second axis was influenced by depth (0.57) whilst in the functional group RDA, the first axis was correlated with depth (0.52).

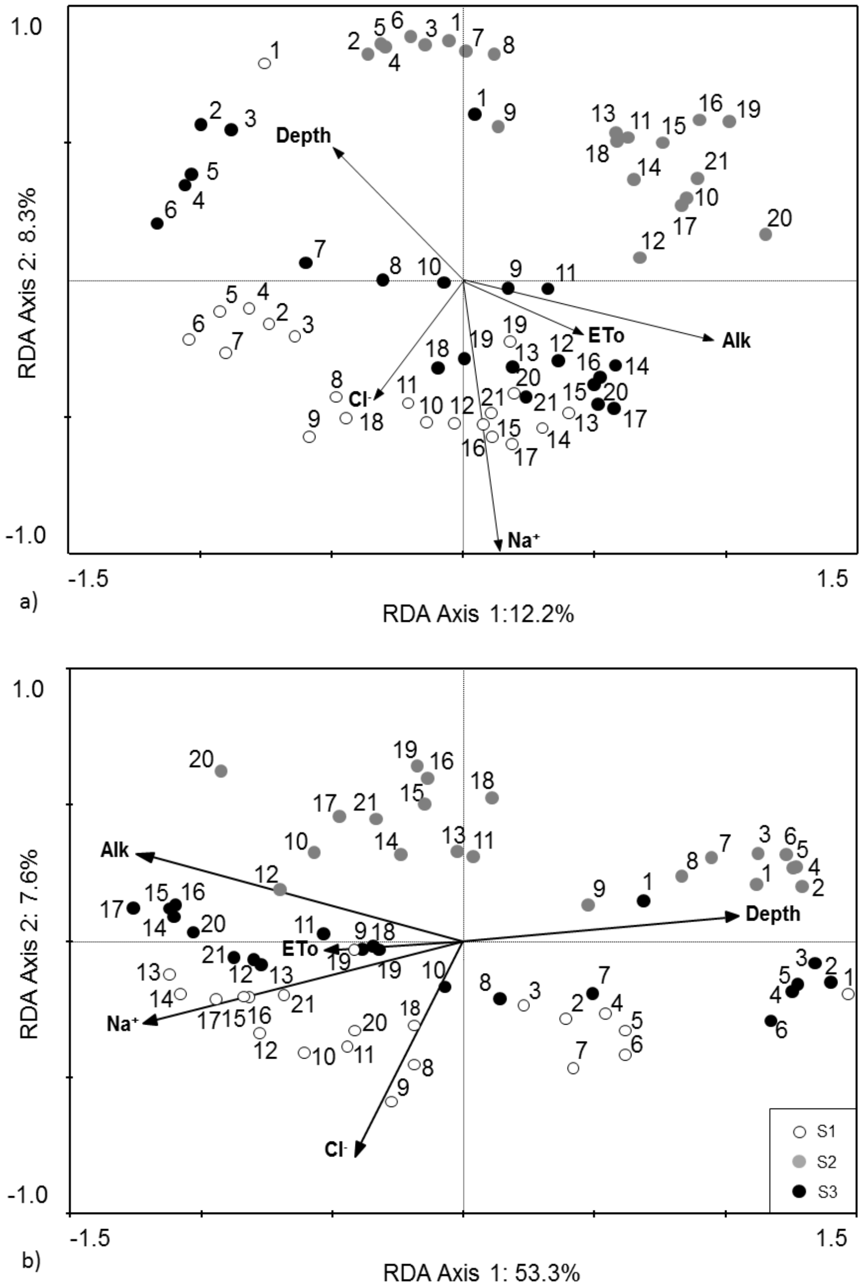

Figure 3.2 Redundancy analyses (RDA) ordination plots for (a) species composition and (b) functional group composition (life-forms and ecological guilds) and significantly correlated physico-chemical parameters. ETo represents the monthly mean total relative evapotranspiration. Noted on the axis is the percent variance explained by each axis. The circles represent the 3 study sites; S1 (open circle), S2 (grey circle) and S3 (black circle). The number refers to each consecutive sampling month.

At the beginning of sampling in March 2011, the diatom communities for both species and functional groups in all three sites were located in the upper quadrant of the plot which corresponded to the high water-levels recorded at each site in the first month of sampling (Fig. 3.2a,b). Over the course of sampling, S1, S2 and S3 species and functional group assemblages responded initially to water-level changes, followed by fluctuations in ionic concentration (alkalinity) and/or composition (Na^+, Cl^-).

Table 3.2 Spearman's rank correlation coefficients between diatom life-forms and ecological guilds and the five important physico-chemical parameters. Significance of each comparison is indicated by *P ≤ 0.05 and ** P ≤ 0.01. Significant correlations following the sequential Bonferroni-Holm method at a cut-off point of P = 0.05 are printed in bold.

	Depth		ETo		Alk		Na^+		Cl^-	
Mobile	0.12		-0.20		-0.35	**	-0.12		0.17	
Pioneer	**-0.63**		0.37	**	**0.68**		**0.70**		0.30	*
Tube-living	-0.39	**	0.34	**	0.21		**0.62**		0.25	*
Rossette	0.15		**0.41**		**0.52**		0.13		0.11	
Ribbon	-0.12		-0.17		-0.28	*	0.26	*	0.23	
Pedunculate	0.22		-0.09		-0.04		0.03		0.31	*
Adnate	0.37	**	**0.51**		**0.45**		**0.42**		0.36	**
High Profile	0.17		0.03		**-0.47**		0.02		0.28	*
Low Profile	**-0.64**		0.34	**	**0.63**		**0.75**		0.33	**
Motile	-0.03		-0.09		0.25	*	-0.24		-0.33	**
Planktonic	-0.11		0.30	*	**0.43**		0.15		-0.02	

Table 3.2 shows the significant relationships between the diatom functional groups and the five important environmental parameters based on Spearman's rank correlation and the Bonferroni-Holm correction. The pioneer life-form and low profile guild correlated significantly with three physico-chemical parameters and showed the strongest correlations compared to other functional groups (Table 3.2). They responded to higher concentrations of alkalinity and Na^+, in accordance with a decrease in depth. Similarly, Na^+ had a highly positive effect on the abundance of tube-living life-forms with a decrease in depth. However, the tube-living diatoms did not show significant correlations with depth after the Bonferroni-Holm correction.

The adnate life-form correlated significantly with three parameters, alkalinity, Na^+ and ETo although this group was not as sensitive to alkalinity and Na^+ as the pioneer life-form (Table 3.2). Likewise, the rosette life-form showed a significant and similar sensitivity to fluctuations in alkalinity and ETo. Alkalinity also had a significant positive effect on the abundance of planktonic guild diatoms whereas the high profile diatoms displayed the reverse trend. No functional group was significantly correlated to chloride after sequential Bonferroni correction. The mobile life-form and motile guild groups did not show any significant correlations with the five parameters after correction.

3.4 DISCUSSION

3.4.1 Taxonomical challenges and benefits of using diatom functional groups

A prominent feature of this wetland dataset is the abundance and variety of *Gomphonema* taxa, some of which belong to the species complexes of *Gomphonema parvulum*, *Gomphonema exilissimum* and *Gomphonema angustatum* or in many cases, were unidentifiable. Species complexes can be a salient issue when using classical species-based diatom biological assessment tools which require identification to species or subspecies taxonomic level for correct implementation (Lane, 2007). Blanco et al. (2004) highlighted such limitations when applying indices such as the BDI (Biological Diatom Index) in a Mediterranean shallow lake where *Gomphonema* species complexes dominated. Alternatively, using life-forms and ecological guilds where genus level identification is sufficient for the allocation of these metrics (Rimet and Bouchez, 2012b), would overcome such taxonomical challenges. Moreover, the use of a higher taxonomical group would reduce the effort in taxonomic identification, thus providing a more time and cost effective method; an important feature of environmental assessment for resource managers.

Contrary to Rimet and Bouchez (2012b), a few investigators (e.g., Passy, 2007a; Lavoie et al., 2009a; Lange et al., 2016) have highlighted the potential loss of some ecological information when using genus level taxonomy to assign taxa to functional groups. An ecological guild is not based on a single trait but combines multiple traits which include “life-form, attachment mechanism and motility” (Lange et al., 2016). Species which belong to the same genus group can have different traits and therefore classification of taxa into guilds may require species level identification. However, these limitations did not appear to affect our results most likely because many of the unidentifiable taxa in our dataset were of the *Gomphonema* genus, in which all taxa of this genus are classified as high profile diatoms with pedunculate, mobile life-forms.

3.4.2 Most sensitive functional groups to environmental changes in temporal wetlands

A strong correlation was observed between patterns of community composition using species and functional groups in this temporary depressional wetland dataset. Consistent with other studies (e.g., Goldenberg Vilar et al., 2014), the functional rather than species-based approach explained more of the variation in diatom community composition, most likely because diatom functional groups incorporate species that have similar response to environmental conditions and available resources. It could also be in part due to the dataset that is less noisy (83 taxa vs. 11 functional groups used in the ordinations). Diatom functional groups have been reported as effective indicators of temporally changing stressors and disturbances in streams (Stenger-Kovács et al., 2013), as was observed in our study. Temporal changes in the concentration and composition of major ions, essentially Na^+, as well as water depth, not only gave rise to pronounced taxonomic shifts in diatom assemblages but also produced distinct shifts in the composition of the diatoms' ecological guilds and life-form groups. Major ion dominance and water depth have already been identified as important drivers structuring diatom communities in lentic systems (e.g., Blinn, 1993; Yang & Duthie, 1995; Gregory-Eaves et al., 1999; Köster et al., 2004).

The low profile guild, mostly comprised of the pioneer life-form species, *Achnanthidium minutissimum*, showed a significant positive relationship with alkalinity and Na^+, while depth had a negative effect. A decrease in water-level can cause sediment resuspension in shallow lakes which can decrease the total nitrogen to total phosphorus (TN:TP) ratio in the water, since the ratio is typically lower in the surface sediment than in the water (Niemistö et al., 2008). Sediment resuspension and redistribution can also reduce the light conditions of the water column and as a result cause considerable depletion in colonisable substrate (Leira et al., 2015). Moreover, in closed-basin systems such as these temporary depressional wetlands, a decrease in depth as a result of seasonal variations in the balance between precipitation inputs and potential evaporation losses can give rise to the concentration of wetland water, and in turn may cause a significant increase in ionic concentration and composition (Saros & Fritz, 2000).

Low profile *A. minutissimum* has been widely documented as an early coloniser resistant to various disturbances including extreme fluctuations in lake water-level (Leira et al., 2015), low light exposure and low nutrient conditions in lakes (Gottschalk & Kahlert, 2012) and shear stress (Peterson & Stevenson, 1992). In addition, *A. minutissimum* has been reported as an alkaliphilous species with a high affinity for Na^+-dominated lakes (Gasse, 1986; Vázquez & Caballero, 2013), conditions which other species may find intolerable.

Bhattacharyya & Volcani (1980) demonstrated an increase in silicate (nutrient) uptake by a marine diatom species when a Na^+ gradient was imposed across the cell membrane. Our results verify that the influence of Na^+ on diatom community structure is undoubtedly different from the effect of other ions. However, mechanisms accountable for the higher or lower affinity of freshwater diatoms to Na^+ as well as ionic concentration are as yet undetermined (Potapova and Charles, 2003).

Similar to the pioneer diatoms, the proportion of the adnate diatom *Rhopalodia gibba* of the motile guild also significantly increased with increasing alkalinity, Na^+ and total relative evapotranspiration (ETo). *R. gibba* is well adapted to waters with elevated ionic content and alkalinity such as the boreal alkaline ponds in Alberta, Canada, which are high in sulphates (Timoney et al., 1997). Irradiance may be reduced as a result of a decrease in water-level and an increase in sediment resuspension from wind mixing during the drying period, thus providing suitable conditions for epiphytic motile diatoms capable of migrating. This result is in agreement with those reported in a temporally changing Hungarian stream where motile guilds were resistant to disturbances such as lower irradiance during flooding through their ability to migrate to the surface layer of the biofilm and maintain access to light (Stenger-Kovács et al., 2013).

Studies have described intracellular spheroid bodies within *R. gibba*, and these bodies have been hypothesised as nitrogen-fixing symbionts which are able to provide nitrogen to their host diatom (Prechtl, 2004). Prechtl (2004) predicted that the genome of the spheroid bodies are closely related to that of, *Cyanothece* sp., a cyanobacterium species. During resuspension in shallow eutrophic lakes, the low N to P ratios can favour nitrogen-fixing cyanobacteria over other algal assemblages (Niemistö et al., 2008). It is thus conceivable that the low water-level and high sediment resuspension in our wetlands could promote the growth of *R. gibba* which harbour these nitrogen-fixing symbionts.

Abundance of tube-living diatoms, dominated by *Encyonema mesianum*, showed a significant positive correlation to Na^+. The *Encyonema* genus is generally regarded as sensitive to environmental stressors such as high organic loads and nutrient enrichment (e.g., Rumeau & Coste, 1988; Leira et al., 2009; Berthon et al., 2011). Nevertheless, Rimet & Bouchez (2011) observed an increase in tube-living diatoms under intense chemical disturbances in pesticide-contaminated mesocosms. Rimet & Bouchez (2011) hypothesised that the tubule, composed of exopolysaccharide matrices, may protect the cells living within from dissolved chemicals in the water, which could explain their resistance to increasing Na^+ concentrations here. The matrices include carbohydrates containing negatively charged groups which bind to positively charged cations, reducing the cation income into the cell (Masmoudi et al., 2013).

At site S1, tubule diatoms showed a rapid increase in abundance during autumn-winter 2011 followed by an abrupt decline in mid-summer 2012. S1 had the shortest hydroperiod of all three sites; thus, any disturbances such as an increase in Na^+ concentration could have an important influence on diatom composition. Variation in major ions is more acute in smaller wetlands than in larger wetlands because smaller wetlands are more affected by changes in the hydrologic budget which have the capacity to change wetland water-level and water chemistry (Caramujo & Boavida, 2010). The mean Na^+ concentrations were substantially higher at S1 compared to S2 and S3 (Chapter 2; Table 2.1, Figure 2.3), hence tubule diatoms could conceivably be subject to more competition from other species at S2 and S3 in a lower Na^+ environment. This may explain why the proportion of tube-living life-forms was comparatively lower at S2 and S3 although we have no direct evidence and further investigations are required to verify this.

Diatoms of the planktonic guild, mostly *Fragilaria tenera*, and the rosette diatom *Ulnaria acus* (planktonic guild) were significantly correlated to increasing alkalinity. The rosette life-form was also positively related to ETo. Planktonic diatoms can attach to the biofilm but understanding of their dynamics within the complex biofilm structure and how they respond to temporally changing conditions in stagnant waters is limited (Goldenberg Vilar et al., 2014). The abundance of planktonic diatoms has been correlated to high turbidity and low light conditions (B-Béres et al., 2014; Rimet and Bouchez, 2012b). Interestingly, there were clear successional phases observed at each site where the planktonic guild replaced the mobile life-forms although these successions did not always occur simultaneously at all three sites. This may be attributed to differences in physical characteristics among the sites (e.g., basin area and depth; see Section 2.2.1), that may affect their hydrology, and in turn, have a major influence on their water chemistry and ecological processes (e.g., rate of organic matter decomposition and carbon capture) (Brooks, 2009). *F. tenera*, an opportunistic r-strategist species (i.e. species whose ecology is defined by a high growth rate, *r*) can dominate phytoplankton communities (bloom) when conditions are favourable in the surface waters e.g., high light penetration through the water column and high nutrient availability (Black, 2008). We hypothesise that a proportion of seasonal blooms of *F. tenera* in the water column may have been trapped in the biofilm which could perhaps explain these bursts in planktonic abundance throughout the study.

In contrast to the rosette and adnate life-forms, and the low profile and planktonic guilds, the high profile guild, mostly comprised of the genus *Gomphonema* (pedunculate diatoms) were significantly reduced by higher alkalinity content. These findings corroborate the results of other studies in which the high profile diatoms were the most sensitive groups to disturbances (e.g., water-level changes: Leira et al., 2015, elevated ionic content and flooding: Stenger-Kovács et al., 2013; B-Béres et al., 2014, low light and nutrient availability: Berthon et al., 2011; Lange et al., 2011, and grazing pressure: Luttenton et al., 1986). As to why the high profile guild was negatively related to alkalinity is uncertain. It is possible that some variable highly correlated with ionic concentration is the driver of these functional group shifts in diatom communities, rather than ionic concentration itself. For example, Stokes (1986) found grazing pressure to decline with a decrease in pH. Stalked epiphytes are more prone to loss from grazing as they are positioned high within the biofilm which is more susceptible to grazing (Agrawal, 1999; Rimet et al., 2009), which could explain their preference for lower pH conditions here. On the contrary, low profile diatoms which have greater attachment strength, are strong competitors in these environments as they have good resistance to high grazing pressure (Goldenberg Vilar et al., 2014).

At the end of summer 2011, there was a gradual decline in high profile diatoms at sites S1 and S3, which were steadily replaced by low profiles (e.g., *A. minutissimum*) and motile guild (e.g., prevalent *Nitzschia acidoclinata*) until the end of sampling. In contrast, at S2, the motile diatoms, *Nitzschia acidoclinata* and *Nitzschia gracilis* generally dominated the biofilm throughout the study. S2 was substantially Ca^{2+} richer and Na^+ poorer compared to the other two sites (Chapter 2; Table 2.1, Figure 2.3). As discussed in Section 2.4.1, Ca^+ is important in providing the nutrient requirements for motility and growth of the *Nitzschia* genus (Cohn & Disparti, 1994). It is possible that the distribution of motile diatoms, *Nitzschia acidoclinata* and *Nitzschia gracilis*, were influenced by the higher concentrations of Ca^+ at Site S2. These taxa had relatively low optima for Na^+. Although we did not investigate their optima for Ca^{2+}, it is conceivable that the monovalent (Na^+) to divalent (Ca^+) cation ratio may also be an important factor influencing the distribution of these taxa (Potapova & Charles, 2003).

3.5 CONCLUSION

Our study demonstrates the sensitivity of life-forms and ecological guilds to seasonal changes along a hydrological and hydrochemical gradient in temporary depressional wetlands without compromising any loss of ecological information when using a broader taxonomic resolution.

Low profile guilds dominated by pioneer life-forms showed the strongest relationship with higher disturbance levels (i.e. increasing Na^+, alkalinity with a decrease in depth). Similarly, the planktonic guild and tube-living, rosette and adnate life-forms dominated at higher disturbance levels whereas the high profile diatoms displayed the reverse trend.

The strong ecological responses of life-forms and guilds to changing environmental conditions observed in this study suggests that with further research, diatom functional groups may be used in biological assessments and monitoring in the region, where wetland species taxonomy is poorly described. The use of functional groups may also be advantageous where budgets are limited but a reliable ecological status of the wetlands is still required, given its time and cost effective approach.

Nevertheless, we highlight the importance of complementing functional groups with traditional species-based diatom assessment tools, even if species taxonomy is uncertain. The relationship between the taxonomical and functional composition using a broad set of traits can provide insight into the mechanisms influencing diatom community structure and further our understanding of depressional wetland functioning. Numerous functional-related studies have focused solely on ecological guilds and their response to environmental conditions in wetlands (Goldenberg Vilar et al., 2014), lakes (Gottschalk & Kahlert, 2012; Leira et al., 2015), rivers (B-Béres et al., 2014) streams (Passy, 2007a; Stenger-Kovács et al., 2013) and mesocosm experiments (Lange et al., 2011; Passy & Larson, 2011). However, we advocate the inclusion of life-forms which provide additional information on the species' capacity to obtain resources and tolerate disturbance.

The functional groups which were good indicators of environmental changes and the important environmental variables identified can serve as a reference condition for further research on assessing impairment and restoration in similar wetlands subjected to environmental modifications. We recommend investigations of a variety of depressional wetlands in the region that encompass a wide range of disturbances, both natural and human-induced, in order to refine our findings and determine whether a simplified functional-based approach can discriminate between natural and anthropogenic disturbances.

CHAPTER 4

DEVELOPMENT OF A DIATOM-BASED MULTIMETRIC INDEX FOR ACID MINE DRAINAGE IMPACTED DEPRESSIONAL WETLANDS

ABSTRACT

Acid mine drainage (AMD) from coal mining in the Mpumalanga Highveld region of South Africa has caused severe chemical and biological degradation of aquatic habitats, specifically depressional wetlands, as mines utilise these wetlands for storage of AMD. Diatom-based multimetric indices (MMIs) to assess wetland condition have mostly been developed to assess agricultural and urban landuse impacts. No diatom MMI of wetland condition has been developed to assess AMD impacts related to mining activities. Previous approaches to diatom-based MMI development in wetlands have not accounted for natural variability. Natural variability among depressional wetlands may influence the accuracy of MMIs. We developed epiphytic diatom MMIs sensitive to AMD for a range of depressional wetland types to account for natural variation in biological metrics. For this, we classified wetland types based on diatom typologies. A range of 4-15 final metrics were selected from a pool of ~140 candidate metrics to develop the MMIs based on their: (1) broad range, (2) high separation power and (3) low correlation among metrics. Final metrics were selected from three categories: similarity to reference sites, functional groups, and taxonomic composition, which represent different aspects of diatom assemblage structure and function. We evaluated MMI performances according to their precision in distinguishing reference sites, responsiveness to discriminate reference and disturbed sites, sensitivity to human disturbances and relevancy to AMD-related stressors. Each MMI showed excellent discriminatory power, whether or not it accounted for natural variation. However, accounting for variation by grouping sites based on diatom typologies improved overall performance of MMIs. Our study highlights the utility of diatom-based metrics and provides a model for the biological assessment of depressional wetland condition in South Africa and elsewhere.

4.1 INTRODUCTION

Multimetric indices (MMIs) based on diatom assemblage responses to human disturbance have mostly been developed to assess the biological condition of lotic environments, specifically US streams and rivers (e.g., Wang et al., 2005; Cao et al., 2007; Potapova & Carlisle, 2011; Tang et al., 2016). Diatom-based MMIs developed for lentic systems have recently received increasing research attention, where efforts to develop MMIs for national-scale assessments of lakes (Stevenson et al., 2013) and ecoregional-scale assessments of freshwater wetlands (Miller et al., 2016) have benefited from US Environmental Protection Agency’s National Aquatic Resource Surveys initiated programmes. On a relatively smaller scale, MMI development for diatom assemblages has been successful for statewide-scale assessments of depressional wetlands (those surrounded by upland) in the state of Florida (Lane & Brown, 2007) and for riverine and depressional wetlands in the Casco Bay watershed in Maine (Wang et al., 2006).

However, no diatom MMI developed for wetlands has controlled for effects of natural variation on index values, where natural variability of environmental factors among sites (i.e. factors least affected by human disturbances) can reduce accuracy of the index (Cao et al., 2007; Stoddard et al., 2008; Hawkins et al., 2010a). This is particularly vital for wetland habitats which are inherently highly variable, even within wetland types (such as depressional wetlands), which can vary significantly over small spatial scales (Bird et al., 2013). Wetlands differ widely due to intra- and inter- regional differences in naturally varying environmental factors (e.g., climate, geology, soils, topography, hydrology, water chemistry and vegetation) that can attribute to natural differences in their biological assemblages (e.g., USEPA, 2016). Consequently, surveys with high heterogeneity among wetlands will likely result in poorly performing MMIs if indices are not controlled for the effects of natural gradients, as was found by Miller et al. (2016) when developing a diatom-based MMI for a range of northeastern US wetlands. Ecologically robust biological assessment indices should account for natural variability in biological assemblages so as to provide a more accurate assessment of deviation from reference condition (minimally disturbed conditions) as a result of human disturbances (Hughes et al., 1986; Hawkins et al., 2000).

Various approaches have been applied to MMIs to partition the effects of natural variability. One common approach is an a priori classification using geographic regionalisations, such as ecoregions, or abiotic factors such as altitude, geology, climate and water chemistry, to classify reference sites into environmentally homogenous types or classes (discrete categories) (Barbour et al., 1999). However, this approach has generally proven to be less effective in accounting for enough natural variation in community composition (e.g., Hawkins et al., 2000; Heino et al., 2002; Herlihy et al., 2006). A more successful approach to account for natural variation is to group reference sites by biological typology, in which sites are grouped based on similarity in composition of biological assemblages (Tison et al., 2005; Davies & Jackson, 2006; Grenier et al., 2006, 2010; Lavoie et al., 2014; Vander Laan & Hawkins, 2014). Classification by biological typology has accounted for more natural variability among reference sites and improved MMI accuracy than grouping sites by ecoregions (Hawkins, 2006; Tang et al., 2016).

MMIs offer a simple tool that can summarise complex biological systems, where a gradient of anthropogenic disturbance can be successfully evaluated using a collection of individual community metrics combined into a single index of human disturbance (Karr & Chu, 1997). A multimetric approach can be more effective in assessing biological condition than using individual metrics because they represent a variety of taxonomic and functional groups within the community which have varying responses to different stressors and thus, can indicate the overall biological condition in a more comprehensive manner (Gerritsen, 1995; Karr, 1999).

Diatom-based MMIs to assess wetland condition have mostly been developed to quantify nutrient loading, elevated salinity and changes in pH resulting from agricultural and urban landuse (e.g., Wang et al., 2006; Lane & Brown, 2007; Miller et al., 2016) but no diatom index of wetland condition has been developed to quantify acid mine drainage (AMD) impacts related to mining activities.

AMD is a primary stressor in depressional wetlands of the Mpumalanga Highveld region of South Africa, where coal mines utilise these wetlands for dirty water storage by pumping AMD directly into the wetland (Ochieng et al., 2010). AMD can also enter the wetland via seepage and surface runoff from contaminated mine tailings dumps and stockpiles. AMD pollution arises when pyrite, a sulphur bearing mineral found in coal deposits, is exposed to oxygenated water and consequently produces sulphuric acid (Johnson & Hallberg, 2005). The acidic water becomes neutralised by dilution and various chemical reactions with natural alkaline waters often found in permanent depressional wetlands used to store mine wastewater in the Mpumalanga Highveld. Nevertheless, some elements have high solubilities and in such cases, persist in the water; this is particularly true for sulphate (McCarthy, 2011). Consequently, AMD impacted depressional wetlands in the region are typically sulphate-rich and extremely saline. Such alterations to the physical and chemical environment may render the water toxic to varying degrees and inhospitable to aquatic biota (Sabater, 2000; Hirst et al., 2002).

Numerous studies, focused mostly on streams, have demonstrated that the distribution of diatom communities can be significantly explained by dominant indicators of AMD, such as pH, alkalinity, conductivity, sulphate, and metals (e.g., Ferreira da Silva et al., 2009; Zalack et al., 2010; Gray & Vis, 2013; Oberholster et al., 2013). An effective diatom MMI used to assess the impacts of AMD has already been successfully applied to mine-impacted Appalachian streams (Pool, 2010; Gray & Vis, 2013). Thus, the use of diatoms as indicators of wetland condition responsive to mining impacts is a promising alternative to the traditional approach employed in South Africa, using macroinvertebrates and macrophytes, which have shown weak associations with wetland impairment (Corry, in press; Bird & Day, 2010; Day & Malan, 2010; Ferreira et al., 2012).

Depressional wetlands are one of the most valuable wetland types in the Mpumalanga Highveld region in terms of providing habitat for vulnerable species (e.g., the Blue Crane, the African Grass Owl and the Golden Mole) and socio-economic benefits to local communities. Remarkably, these wetlands (approximately 2600 of them in the region), have received relatively limited attention despite their importance and the ever-increasing threat to these systems from rapid mining development. In this study, our primary goal was to develop an MMI for depressional wetland condition in the Mpumalanga Highveld region based on epiphytic diatom assemblages that would be sensitive to mining impacts. To account for natural variability among reference sites, we constructed separate MMIs for classes of depressional wetland types. Our objectives were (1) to evaluate the performance of MMIs in their ability to discriminate between wetland diatom communities in AMD-impacted sites and non-AMD impacted reference sites; and (2) to determine whether accounting for natural variation in assemblages by diatom typology based classifications will improve MMI performance. We compared performance of MMIs in order to test the hypothesis that accounting for natural variation based on site classification by diatom typologies will improve MMI performance.

4.2 MATERIALS AND METHODS

4.2.1 Study area and site selection

This study undertakes an analysis of diatom communities of permanent depressional wetlands in the Mpumalanga Highveld region since wetlands used for AMD storage tend to be permanently inundated. Using information provided by wetland specialists around South Africa, we selected forty-six depressional wetlands that reflect an AMD pollution gradient based on historical water chemistry, vegetation and diatom data. Sites were also selected based on inundation conditions (permanence of surface waters) which we examined using Google Earth Pro and time-sequenced satellite imagery (Google, 2015) and verified with information from previous studies and local landowners. We delineated catchment boundaries of each site based on a 5m resolution Digital Elevation Model using ArcGIS 10.1 (ESRI, 2011).

Of the forty-six wetlands selected, thirty-four sites (range = 3 - 309ha) were accessible for sampling in May-August of 2015. We sampled once during the dry season to avoid possible seasonal effects on the structure and composition of diatom communities, such as differences in climate and hydrology that control wetland water chemistry, or the influence of surface runoff contaminants from other landuse activities (mostly agriculture) in the catchment (Fig. 4.1). We identified fifteen reference sites based on the following criteria - no mining in the catchment, surrounded by overgrazed pastures, with the exception of one site which was surrounded by both overgrazing and dryland cultivation, and no stressors in the surface water from mining-derived groundwater contamination or pollutants associated with human activities. The nineteen wetlands not meeting the above criteria were classified as disturbed sites.

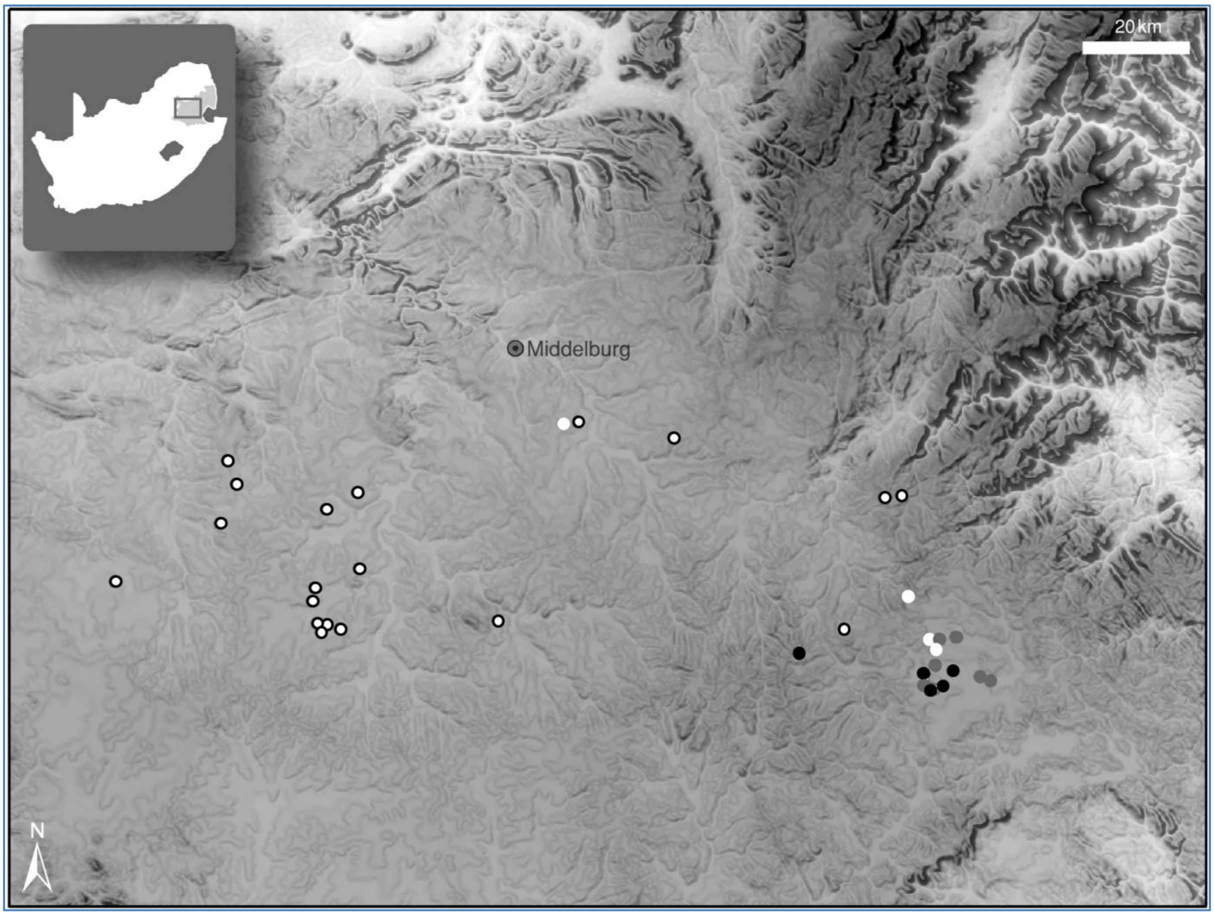

Figure 4.1 Map of the study area showing location of reference and disturbed sites; inset shows study area location in South Africa. Of the reference sites, the white circles with no borders represent reed depressions, grey circles are salt depressions, black circles are open-water and grass depressions combined, and the disturbed sites are represented by white circles with black borders.

4.2.2 Data collection

We collected one sample of diatom material and surface water per site in the inner part of the littoral zone. For disturbed sites, the sampling location within each wetland was selected based on proximity to visible disturbance from mining (e.g., mine-drainage discharge). We collected water chemistry samples and measured physical parameters at the same location and time as the diatom sampling. We measured conductivity and pH at each site using a Hach HQ40D Dual Input Multi-Parameter meter. Turbidity was measured in situ using a Hach 2100P portable turbidimeter; we also measured Secchi disk transparency. Wetland water (1 L) was collected in an acid-cleaned, high-density polyethylene bottle and stored in the dark on ice until return to the CSIR Environmental Laboratory in Stellenbosch, where samples were analysed for NH_3, TN, TP, PO_4^{2-}, Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^-, SO_4^{2-}, F^-, dissolved organic carbon (DOC), turbidity, alkalinity and metals (Al, Fe, Mn) following standard procedures (APHA, 1998).

Following the preferred method for sampling diatoms in shallow lakes (Blanco & Bécares, 2006; Stenger-Kovács et al., 2007), we sampled epiphytic diatoms from one emergent macrophyte species at each site (either *Phragmites australis*, *Typha capensis* or *Schoenoplectus* spp.). We collected young stalks (4-6 fragments of stems of 10cm length) in the inner part of the littoral zone, 2-5cm below the water surface, avoiding densely packed mats (shading effect). For sites devoid of vegetation (two hypersaline sites), we sampled stones according to guidance protocols described in King et al. (2006). Diatom samples were returned to the laboratory where they were acid cleaned and mounted on microscope slides (Battarbee et al., 2001). We counted and identified 500 frustules to the lowest feasible taxonomic level using standard European diatom floras (e.g., such as Krammer & Lange-Bertalot, 1986-1991; Lange-Bertalot, 2000-2002; Lange-Bertalot et al., 2001), several papers on the Southern African flora by Cholnoky, Schoeman and Archibald (e.g., Schoeman and Archibald, 1976-1980), and books and recent papers on *Gomphonema* taxonomy (e.g., Reichardt & Lange-Bertalot, 1999; Rose & Cox, 2014; Reichardt, 2015). We then checked the current nomenclature using AlgaeBase (www.algaebase.org).

4.2.3 Classification of reference sites

We made an a priori classification of reference sites into depression-type groups based on environmental factors (least affected by human disturbances), from published information (Allan, 1987; Allan et al., 1995; Cowan & van Riet, 1998). We assigned sites to four depression-type groups: (1) Reed depressions - typically saline, comprised of dense central stands of emergent *Phragmites*, encircled by a narrow band of open water, thickly underlain with submergents *Lagarosiphon* and *Potamogeton*. (2) Salt depressions - extremely saline, comprised of hypersaline substrata, which can appear bright white due to the high reflectiveness of the salt; sedge *Schoenoplectus triqueter* is typically found around the shorelines. (3) Open-water depressions - fresh to slightly saline, shallow soil or exposed rock, devoid of vegetation, excluding the shorelines where grass, *Cynodon dactylon*, sedges (*Cyperus* species and *Scirpus dioecus*), and several *Juncus* species can be found. (4) Grass depressions - comprised of dense growths of hygrophilous grasses and sedges and other low-growing terrestrial vegetation, with similar salinities to the open-water depressions.

Next, we defined depression-type groups based on diatom typology using cluster analysis (PC-ORD 5.10; McCune & Mefford, 2006) on square-root transformed diatom community data. We retained three depression-type groups for the development of the following MMIs: (1) MM1-1 using all 15 reference sites; (2) MMI-2 using 4 reed reference depressions; (3) MMI-3 using 6 salt reference depressions; and (4) MMI-4 using 5 reference depressions for both open-water and grass depression-types combined.

4.2.4 Development of MMIs

4.2.4.1 Candidate diatom metrics

We examined a total of 154 candidate metrics, assigned to four categories (diversity, similarity to reference sites, functional group and taxonomic composition) for possible inclusion in the MMIs, derived from Wang et al. (2005), Stevenson et al. (2013), Passy (2007a,b), and Rimet and Bouchez (2012b) (Supplementary Table S4.1). Diversity metrics included species richness, evenness, Shannon diversity and the relative abundance of the most dominant taxon in a sample (% dominance).

Similarity to reference metrics included the number of distinct reference taxa, and % reference and % tolerant taxa and individuals (species typical of reference and disturbed sites); all were identified by indicator species analysis (Dufrene & Legendre, 1997) using PC-ORD 5.10. We calculated the percentage of reference taxa and individuals found in reference sites that occurred in disturbed sites, and Bray-Curtis percent similarity to reference sites (Bray & Curtis, 1957) using PRIMER 6 (Clarke and Gorley, 2006).

Candidate functional group metrics were based on two types of functional groups a), life-forms and b), ecological guilds, in accordance with Passy (2007a,b) and Rimet and Bouchez (2012b) (Supplementary Table 4.1). We assigned diatom taxa at the species or genus level to the various life-form groups (e.g., pioneer, pedunculate or ribbon colony) and ecological guild groups (high profile, low profile, motile or planktonic guilds). We calculated functional group metrics as the relative abundance of taxa or individuals divided by the sum of taxa or individuals for each functional group within a sample. Similarly, taxonomic composition metrics were calculated as the relative abundance of taxa or individuals in relation to the number of taxa or individuals for each genus within a sample.

4.2.4.2 Metric selection

We selected metrics from the pool of candidate metrics using the following step-wise screening procedure (Stoddard et al., 2008). (1) Range test - we discarded metrics with an insufficient range; metrics with medians of 0 in both reference and disturbed sites. (2) Responsiveness test - we selected metrics with a Mann–Whitney Z value ≥2, considered to be a strong separation power indicator between reference and disturbed sites (Stevenson et al., 2013). (3) For similar metric pairs (e.g., % reference taxa and % reference individuals), we selected the metric with the highest separation power indicator (Z-value). If metric pairs shared the same Z-value, we retained the metric with the lowest coefficient of variation (CV); the lower the CV, the more precise the metric (Cao et al., 2007). (4) Redundancy test - in order to provide an independent set of metrics for the MMI, we identified redundant metrics within the reference sites ($r \geq 0.70$) using Spearman correlations (Hughes et al., 1998), and for redundant metric pairs, we selected the metric with the highest Z-value. Spearman correlation and the Mann–Whitney test were conducted using InfoStat (Di Rienzo et al., 2011)

4.2.4.3 Metric re-scaling and MMI scoring

In order to standardise the range of selected metrics, we rescaled each metric to a score between 0 and 1 following Blocksom (2003). We used the 5th and 95th percentile of all sites (reference and disturbed) within each metric. Each metric value was rescaled using the formula:

$$\text{Rescaled metric value} = \frac{\text{original metric value} - 5^{th}}{95^{th} - 5^{th}}$$

If rescaled metric values were less than zero, we adjusted these values to 0 and if greater than 1, we adjusted to 1. For metrics that increased at disturbed sites and decreased at reference sites, we subtracted the rescaled metric value from 1 in order to reverse the scale so that high metric values represented better biotic condition.

We calculated the MMI using a two-step approach by Stevenson et al. (2013) to provide a final MMI with a 0 to 100 scaling system. (1) We calculated composite metrics for each metric category (composed of ≥1 metrics) as the mean of the rescaled metric values within each metric category. (2) We then calculated the MMI as the mean of all composite metrics multiplied by 100. This approach produces an MMI in which each metric category is given equal weight independent of the number of metrics within each category. For an MMI with only one metric category, we calculated the MMI as the mean of the rescaled metric values multiplied by 100.

4.2.5 Evaluating performance of MMIs

We evaluated the performances of the MMIs according to four measures: precision, responsiveness, sensitivity and relevancy (Hawkins et al., 2010a; Stevenson et al., 2013; Tang et al., 2016). (1) We measured precision by calculating the coefficient of variance (CV) for MMI values within the reference sites; thus, an MMI with a lower CV is more precise. (2) We tested MMI responsiveness by calculating the difference in mean MMI values between the reference and disturbed sites and using Welch's *t* tests to compare those means in order to determine the extent to which each MMI discriminated between reference and disturbed sites; the greater the *t*-value, the more responsive the MMI. (3) We measured MMI sensitivity as the percentage of disturbed sites that were evaluated as non-reference; that is the MMI score at each disturbed site was less than the 25th percentile of reference site MMI values. (4) We evaluated MMI relevancy following Stevenson et al. (2013) by analysing the relationship between the MMI and the results of the first principal axis scores (PC1) from a Principal Components Analysis (PCA) of all sites using log-transformed chemical variables which discriminate reference from disturbed sites. We used two variables, Ca^{2+} and $\%SO_4^{2-}$:Cl, which we predicted as predominant coal mining-related stressors in depressional wetlands. AMD is treated with lime composed of calcium hydroxide, $Ca^{2+}(OH)_2$. We observed considerably higher concentrations of Ca^{2+} in our disturbed sites compared to reference sites (mean 177.6 and 17.3 mgL^{-1}, respectively, Supplementary Table 4.2) which potentially indicate the impact of AMD. SO_4^{2-} concentrations were high in the highly disturbed sites (mean 1740.6 mgL^{-1}) yet, similar concentrations also occurred naturally in salt depression reference sites (mean 1291 mgL^{-1}, Supplementary Table 4.2). For this reason, sulphate would not be a reliable indicator of AMD pollution. We did, however, observe that the ratio of SO_4^{2-}:Cl was consistently lower among reference sites than among mine-impacted sites (see also Abiye, 2014) and therefore we included SO_4^{2-}:Cl, calculated as a percentage, in the PC1 disturbance gradient. Metals Fe, Mn, Al were not included as stressors for two reasons, firstly, they were found in both reference and disturbed sites in low concentrations and therefore showed poor discrimination ability, and secondly, were correlated with the PC1 axis in the opposite direction to Ca^{2+} and % SO_4^{2-}:Cl. Including metals would, therefore, only weaken the strength of the relationship between MMIs and PC1 disturbance gradients. MMI relevancy was measured using simple linear regression where we regressed MMI values with PC1 disturbance gradients. We checked that all models met the assumptions of linear regression including normality of residuals and homoscedasticity using R (R Development Core Team, 2008) (all assumptions were met). A well-performing MMI should be linearly correlated across the entire disturbance gradient, and in doing so, demonstrates its ability to discriminate across varying levels of degradation (Tao et al 2016, Hawkins et al 2010a). We predicted MMIs to be negatively related to the measurement of disturbance (PC1), whereby the greater the amount of variability in an MMI explained by the disturbance variables, the more relevant the MMI.

4.3 RESULTS

4.3.1 Physical-chemical and diatom data

A wide range of physico-chemical variables were observed among the reference sites (Supplementary Table 4.2). Open-water/grass depressions were characterised by relatively low conductivity and alkalinity (mean conductivity: $57 mSm^{-1}$, mean alkalinity: $204 mgL^{-1}$) in contrast to the hypersaline, highly alkaline (carbonate-rich), salt depressions (mean conductivity: $1378 mSm^{-1}$, mean alkalinity: $2067 mgL^{-1}$). Nutrient concentrations were low at most reference sites, except for two salt depression sites, where the presence of flamingo colonies at these sites was a likely explanation for the high nutrient loading (Max TP: $11 mgL^{-1}$ and Max TN: $32 mgL^{-1}$).

With regards to the diatom data, a total of thirty-four samples were analysed and 52 genera and 186 species were identified. From this total, 47 genera and 152 species were identified in disturbed sites. Of all the reference depression-types, reed depressions were the most diverse (25 genera and 70 species), followed by open-water/grass depressions (21 genera and 46 species), and salt depressions (15 genera and 32 species). Supplementary Table 4.3 shows the results of the indicator species analysis showing taxa to be significantly ($P < 0.05$) present in reference (Ref) and disturbed (Dist) sites. These taxa were used to calculate the following metrics for each MMI: number of distinct reference taxa, and % reference and % tolerant taxa and individuals.

4.3.2 Metric screening and selection of final metrics

Our metric screening procedure reduced candidate metrics from around 140 to a range of 4-15 final metrics to construct the MMIs (Table 4.1, Table 4.2). The range test for zero median values in both reference and disturbed sites eliminated the largest number of candidate metrics for possible inclusion in the four MMIs, all of which belonged to the taxonomic composition category. The diversity category failed the screening process for all MMIs. Most diversity metrics had $|Z| < 2$ and thus, were eliminated by the responsiveness test (Supplementary Table 4.4 a,b), except for MMI-2 (reed depression-types) in which diversity metrics failed the evaluation for redundancy.

Table 4.1 Results of the screening procedure for metrics used in the development of the four MMIs. N = number of sites. Range = medians > 0 for both reference and disturbed sites. Responsiveness = Z value ≥ 2. Removal of similar metrics = metric with lowest coefficient of variation (CV) retained when metric pairs have same Z-value. Redundancy = Spearman correlations for redundancy.

MMI	N	No. of metrics retained after test:				
		Start	Range	Responsiveness	Removal of similar metrics	Redundancy
MMI-1	34	148	56	19	12	8
MMI-2	23	144	74	21	17	4
MMI-3	25	140	62	39	23	15
MMI-4	24	142	66	34	17	6

Table 4.2 Final metrics used to develop composite metrics comprised of ≥ 1 metrics within each metric category. (x) Metrics selected for each MMI best discriminated between references and disturbed sites. Direction of metric response to disturbance is shown by + or – which represents an increase or decrease to disturbance, and (v) a variable response.

Category/ Final metric	MMI-1	MMI-2	MMI-3	MMI-4	Response
Similarity to reference sites					
% reference taxa	x	x	x	x	-
% tolerant taxa	x	x	x	x	+
% similarity to reference sites	x	x	x		-
% reference taxa found in reference sites that occurred in impaired sites			x	x	-
% reference individuals found in reference sites that occurred in impaired sites	x				-
No. of distinct reference taxa		x	x	x	-
Functional group					
Mobile % taxa	x				-
Adnate % taxa			x		-
Pad (attached to substrate) % taxa	x		x		+
Non-colonial % taxa			x		-
Ribbon % taxa			x		+
Ribbon % individuals	x				+
High profile guild % taxa			x		+
Taxonomic composition					
% Encyonopsis taxa			x		+
% Cocconeis taxa			x		+
% Craticula taxa			x		-
% Ctenophora taxa	x		x		+
% Gomphonema taxa			x	x	v
% Nitzschia individuals				x	-

4.3.3 MMI performance

To establish whether accounting for natural variation by diatom typology based site classifications improved MMI performance, we compared evaluations in performance between an MMI without site classification (MMI-1) and MMIs with site classification (MMI-2, MMI-3 and MMI-4). We used a visual comparison of MMI scores, examining the degree of overlap between interquartile ranges and medians of MMI distributions to evaluate MMI performances (Fig. 4.2). All four MMIs displayed excellent discriminatory power between reference and disturbed sites by having no overlap between the interquartile ranges within reference and disturbed sites. Indeed, the entire MMI distributions within reference and disturbed sites, developed for MMI-2, MMI-3 and MMI-4, did not overlap, suggesting an improvement in performance using diatom typology based classification to account for natural variation among sites.

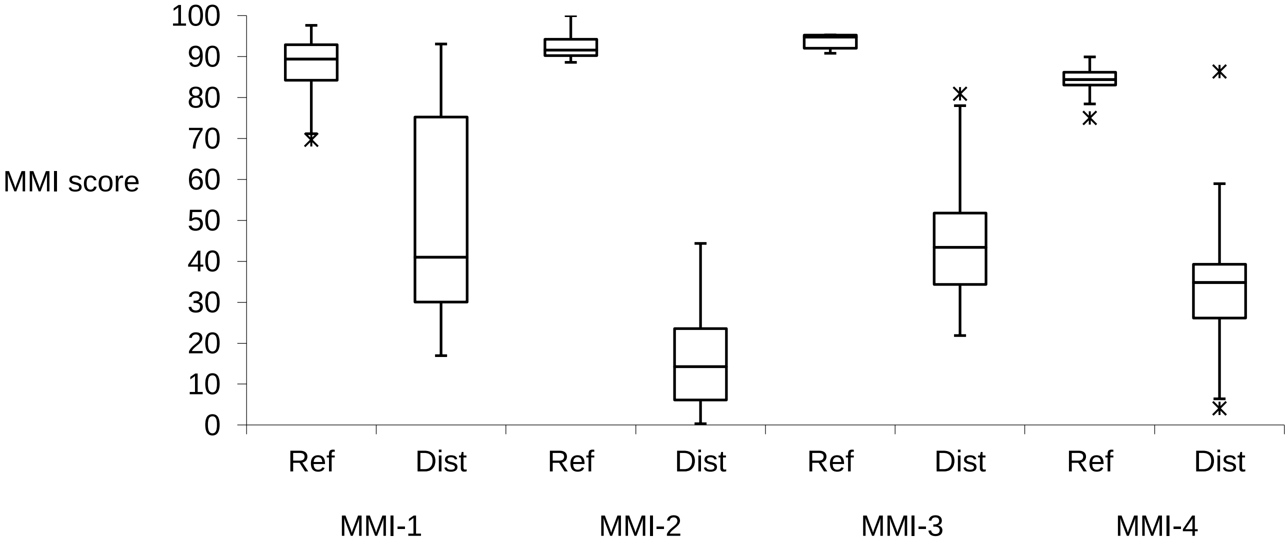

Figure 4.2 Box plots showing the range of scores for four MMIs at reference (Ref) and disturbed sites (Dist) under 0–100 scaling system. Lines represent the medians, boxes represent the interquartile ranges (25th and 75th percentiles), whiskers represent 1.5 interquartile ranges and * represent outliers.

Results of the visual comparison of MMI performance corroborated further evaluations of performance (Table 4.3). Site classification by diatom typology increased precision of MMIs indicated by lower CVs (2.2-6.6%) than an MMI without site classification (8.4%). CVs for all MMIs were low compared to other diatom MMIs (e.g., Tang et al., 2016: CV 14.8-30.7%; Cao et al., 2007: 13-14%). This suggests that there was low natural variability within and among reference depression types. MMIs with site classification had considerably greater responsiveness than an MMI without site grouping, as indicated by the higher t-values to discriminate reference and disturbed sites (from 5.76 to 10.33-20.93). All four MMIs showed high sensitivity, although MMIs with site classification had better sensitivity (range, 94.7-100%) than without classification (89.5%). However, using site classification did not improve MMI relevancy. The results of the linear regressions indicated a stronger negative intercorrelation between an MMI without site classification and the human disturbance gradient (PC1; $R^2_{adj} = 0.71$) than MMIs with site classification (PC1; $R^2_{adj} = 0.63 – 0.23$).

Differences in performances were observed among the MMIs with site classification; MMI-2, for reed depression-types, had the highest overall performance indicated by better

MMI responsiveness, sensitivity and relevancy, in contrast to MMI-4, for open-water/grass depression-types, which had the lowest performance.

Table 4.3 Results of MMI performance based on: precision (coefficient of variation (%CV) of reference site index values), responsiveness (mean difference and Welch's t test value for comparison between mean reference and disturbed site index values), sensitivity (% of disturbed sites evaluated as in non-reference condition), and relevancy (R^2 (adj) as explained variance of MMI regression with principal component axis 1 (PC1) disturbance gradient. Significance of each relationship is indicated by * $P \leq 0.01$ and **$P < 0.0001$.

MMI	Precision	Responsiveness		Sensitivity	Relevancy		
	(%CV)	Mean difference	*t*-value	(%)	PC1		
					R^2 (adj)		Slope
MMI-1	8.4	35.96	5.76	89.5	0.71	**	-8.26
MMI-2	5.3	76.99	20.93	100	0.63	**	-8.19
MMI-3	2.2	49.70	13.30	100	0.58	**	-7.76
MMI-4	6.6	50.26	10.33	94.7	0.23	*	-5.36

Three wetland condition classes were established based on the 25th and the 5th percentiles of the reference-site distribution of MMI scores, benchmarks recommended by Paulsen et al. (2008): good (scores >25th% of the reference distribution), fair (scores between 5th% and 25th% of the distribution), poor (scores <5th% of the distribution) (Table 4.4). Based on these category scores, all four indices classified 74-100% of disturbed sites as Poor, 16% as Fair, and 6-11% as Good.

Table 4.4 25th and 5th percentiles of the reference-site distribution of MMI scores which can be used as benchmarks to partition sites into depressional wetland condition categories Good (>25th% of the reference distribution), Fair (between 25th% and 5th%) and Poor (< 5th% of the distribution). Number of sites that were categorised as Good, Fair, and Poor are shown for each MMI for reference (Ref) and disturbed (Dist) sites.

MMI	25th%	5th%	Good	Fair	Poor	Total
MMI-1	84.2	76.1	2	3	14	19
MMI-2	90.2	88.9	0	0	19	19
MMI-3	92.1	90.9	0	0	19	19
MMI-4	83.1	76.6	1	0	18	19

4.4 DISCUSSION

4.4.1 Metric selection

We developed MMIs based on epiphytic diatoms that provided a significant negative linear response to a gradient of human disturbance in depressional wetlands. For this we used metrics from three categories: similarity to reference sites, functional groups and taxonomic composition, reflecting different aspects of assemblage structure and function. Metrics most responsive to discriminate between reference and disturbed sites (i.e. those with the highest Z-value) were generally higher for species-level metrics (e.g., % reference and % tolerant taxa, % similarity to reference sites) than for genus-level metrics within the functional-group and taxonomic composition category (Supplementary Table 4.4a,b). Similar observations were reported by Lavoie et al. (2009a) who demonstrated that using genus-level taxa resulted in substantial loss of information, but that reference sites and disturbed sites were still correctly identified. Moreover, Stevenson et al. (2013) when developing an MMI of lake diatom condition, expected species-level metrics to be more responsive because species-level is generally associated with lower genetic and physiological variability than with higher taxonomic levels. Regardless, many genus-level metrics showed significant ability to discriminate reference from disturbed sites, as was also found by Stevenson et al. (2013) and investigators of depressional wetland condition (Wang et al., 2006; Lane, 2007). Genus-level taxonomy does not require high-level species identification which can be time consuming and require specialised taxonomic expertise, which in South Africa is largely lacking (Dalu & Froneman, 2016). The use of genus-level metrics may therefore allow for a simplified, time and cost effective assessment of wetland condition while maintaining important ecological information.

Of the final metrics useful in identifying heavily AMD impacted depressional wetlands in this study, five of those metrics were important in assessing AMD polluted Appalachian streams (Zalack et al., 2010). These five metrics were all from the similarity to reference site category, including % reference and % tolerant taxa, % similarity to reference sites and number of distinct references taxa; and have been recognised as being sensitive to a range of anthropogenic disturbances (Stevenson & Smol, 2003; Wang et al., 2005, 2006; Stevenson et al., 2008, 2013). As was expected from the literature, these metrics showed negative responses to increasing human disturbance, except for % tolerant taxa, which showed the opposite trend.

The response of diatom functional groups and taxonomic composition to anthropogenic disturbances (typically nutrient enrichment) has been mostly investigated in running waters, while no studies have examined their response to AMD pollution in lentic waters, as carried out in this study for the Mpumalanga Highveld depressional wetlands. We found that in MMI-3 (salt depressions), increasing AMD impacts was associated with an increase in attached diatoms, either by mucilage pad, dominated by *Ctenophora* and *Encyonopsis*, or by valve face, adnate diatoms, dominated by *Cocconeis*, as well as an increase in pad-attaching, ribbon-shaped colonies (comprised of *Synedra fasciculata*). Our results are in line with other studies which have reported the same genera in highly disturbed environments (e.g., Wang et al., 2006; Passy, 2007a; Stenger-Kovács et al., 2013; B-Béres et al., 2014), including AMD impacted waters (e.g., Potapova & Charles, 2003; Archibald & Taylor, 2007; Luís et al., 2009). We also observed a positive correlation between AMD pollution and high profile diatoms which include *Gomphonema*, *Ctenophora* taxa and *Synedra fasciculata*. Yet, in MMI-4 (open-water/grass depressions), *Gomphonema* responded inversely (negatively) to increasing pollution. *Gomphonema* taxa are widely acknowledged as ubiquitous with a wide morphological range and a broad ecological tolerance, found in polluted waters but also a wide variety of water qualities (e.g., Abarca et al., 2014; Jüttner et al., 2013; Rose and Cox, 2014). Different species of the same genera can vary greatly in environmental sensitivities and tolerances (Hill et al., 2001). This may explain the lack of sensitivity of *Gomphonema* to discriminate disturbed wetlands in MMI-1, which was developed from a range of depression reference types including salt and open-water/grass depressions. Thus, we highlight the importance of accounting for natural differences among wetland types when developing an MMI of wetland condition. The response of functional and taxonomic groups to human disturbance for a given wetland type may improve our understanding of how species diversity influences ecosystem processes (Nock et al., 2016), which may otherwise be masked in an MMI that does not control for natural variation among wetlands. Exactly what physiological mechanisms govern interactions between diatom functional groups and levels of AMD stressors in depressional wetlands are, however, still not known and deserve further investigation.

4.4.2 MMI performance

All four MMIs were excellent indicators of disturbance in depressional wetlands, as shown by the non-overlapping interquartile ranges within reference and disturbed categories which implies high discrimination efficiency (Barbour et al., 1996). Further evaluations of MMI performance demonstrated that our MMIs, whether controlled for natural variation or not, performed considerably well. However, overall MMI performance improved, particularly the precision, responsiveness and sensitivity to human disturbances, when we classified sites by diatom assemblages to account for natural variation.

Accurate biological assessments should account for the natural variability in biological metrics (Cao et al., 2007) and one of the most effective approaches to control for natural variation is using biological typologies. Indeed, Tang et al. (2016), studying US streams, showed site classification by diatom assemblages gave a better MMI performance than geographic stratifications (e.g., ecoregions). Similarly, other authors (e.g., Hawkins & Vinson, 2000; Van Sickle & Hughes, 2000; Snelder et al., 2012) have demonstrated for invertebrate and fish assemblages, that classification by biological typology revealed stronger site classifications than by ecoregions and other geographic regionalisations (e.g. hydrological catchments). The fact that grouping by biological typology accounted for more of the natural variation in assemblages than ecoregions is not surprising, since grouping by ecoregion is based on the entire ecosystem, which includes biotic and abiotic components, rather than grouping by typology which is based on one ecosystem component (Tang et al., 2016).

Alternatively, using a predicted site-specific modelling approach, which is suitable for larger reference datasets than ours, has proven extremely effective for partitioning out the effects of natural variability among assemblages (e.g., Cao et al., 2007; Hawkins et al., 2010a; Vander Laan et al., 2013), including those related to seasonal effects (Chen et al., 2014; Macedo et al., 2016). Classification and regression trees (CART) have been employed to remove effects of natural variation from individual metrics (Cao et al., 2007). MMIs based on predictive models may be preferable over typological approaches because of their ability to establish site-specific thresholds, which may lead to more appropriate management decisions (Hawkins et al., 2010b; Mazor et al., 2016). This approach may also be more advantageous for further MMI development in the Mpumalanga Highveld, where an MMI with site-specific models for depressional wetlands in this region may be easier to implement than having to use an MMI for a specific wetland type, as presented here. However, this would demand many more reference sites than were accessible for this study.

4.4.3 Priorities for future research

Our study has two important limitations, firstly, the relatively small sample size, preventing the use of a separate dataset to test the MMIs, as suggested by other authors (e.g., Wang et al., 2005; Stoddard et al., 2008; Zalack et al., 2010). While our MMI performances showed considerably high precision and accuracy, we need to sample additional sites in the region in order to test and evaluate their applicability to coal mining impacted depressional wetlands. Secondly, sampling in one season did not allow for identifying natural temporal variation in diatom assemblages. Although our study wetlands were permanent, and therefore relatively stable compared to seasonally-fluctuating temporary wetlands (Calhoun et al., 2016), the effects of seasonal changes on environmental factors, such as temperature, organic matter availability and catchment runoff, may directly or indirectly influence diatom assemblage structure in permanent depressional wetlands. A robust MMI should account for both natural spatial and temporal variation in assemblages to allow for accurate assessment of site condition and repeatable sampling, independent of time (Blocksom, 2003). Our standardised sampling protocol and index methodology enables future research to build on this database by sampling more reference and disturbed sites, and more seasons that will include greater natural variation and help create an assessment tool that performs well across a range of environmental settings.

Future research should also address temporary depressional wetlands in the region to determine whether our diatom metrics can accurately reflect temporary wetland condition, where the uses of other biological assemblages have performed poorly. For example, several authors (Day & Malan, 2010; Ferreira et al., 2012; Bird et al., 2013) found macroinvertebrate-based metrics unsuitable in assessments of temporary wetland condition in South Africa because the influence of natural variability on macroinvertebrate assemblages was far greater than the influence of human-induced variability. Another option is to take all samples during the same season/period. This approach is interesting because it allows for an inter-annual comparison of water quality for a given period.

Finding suitable reference sites will, however, be challenging in a region characterised by highly transformed landscapes from mining and agricultural development, in which a significant proportion of wetlands have already been heavily modified. Eutrophication related to agricultural practices is another major stressor affecting the condition of depressional wetlands in the Mpumalanga Highveld. It would be useful to develop an MMI based on metrics that each responds to a different type of stressor indicative of AMD pollution and agricultural impacts, such as nutrient loading and pesticide contamination. This may help elucidate how AMD pollution affects depressional wetland condition with high and low agricultural impacts, thus providing an important tool for resource managers and relevant stakeholders.

4.5 CONCLUSIONS

In this study, we developed diatom-based MMIs sensitive to coal mining impairment for permanent depressional wetlands in the Mpumalanga Highveld region. All MMIs performed considerably well, although accounting for natural variation by diatom typology site classification increased overall MMI performance. This is a preliminary approach and for further improvement in MMIs for depressional wetlands in the region and elsewhere, we recommend an approach that accounts for natural gradients. The strong ecological responses of epiphytic diatom assemblages to AMD pollution, measured by changes in diatom structure and function, suggests that with further research, diatom assemblages present a viable alternative in the biological assessment of depressional wetlands in South Africa, where the use of other types of biological indicators have proven to be ineffective. The metrics presented here may be most suitable for regional use in optimising wetland conservation in the context of management, protection and rehabilitation, but may also be transferable among regions, although this would require further testing.

CHAPTER 5

DIATOMS AND DEPRESSIONAL WETLANDS: SYNTHESIS AND CONCLUSIONS

5.1 INTRODUCTION

In the previous chapters we presented new information about diatom structure and function in Mpumalanga Highveld temporary and permanent depressional wetlands. Specifically, we examined the utility of epiphytic diatoms as indicators of different stressors and disturbances related to: 1) natural seasonally changing environmental conditions in temporary depressions, and 2) human-induced environmental changes in permanent depressions. In this final chapter we summarise this new information in a broader African and global context, with particular reference to the use of diatoms as a touchstone for remediation and conservation of South African wetlands. We conclude this chapter with directions for further advances in diatom assessments of depressional wetlands, and recommend diatoms as a criterion for biological assessments of aquatic resources in African and other developing countries.

5.2 OVERVIEW

Biological assessments of freshwater aquatic resources in South Africa and sub-Saharan Africa routinely use the species composition of macroinvertebrates (e.g., South African Scoring System: Chutter, 1998; Mbaka et al., 2014; Bere, 2016a), fish (e.g., Fish Assemblage Integrity Index: Kleynhans, 1999; Kadye, 2008), and macrophytes (e.g., Riparian Vegetation Index: Kemper, 2001). Only in the last two decades has the use of diatoms become more popular for water quality monitoring and assessments in South Africa (de la Rey et al., 2004, 2008; Taylor et al., 2007) and sub-Saharan countries (e.g., Zimbabwe: Bere et al., 2014, Malawi: Kaonga & Monjerezi, 2012, Kenya: Ndiritu et al., 2006); though it seems surprising, considering the use of diatoms as water quality indicators in African waters goes as far back as the mid-twentieth century (e.g., Cholnoky, 1958).

In particular, scientific communities and national regulatory authorities in Africa have focused considerable attention on developing diatom tools for assessing the biological condition of rivers and streams (see review in Dalu & Froneman, 2016), while little effort has been assigned toward developing diatom tools to assess wetland condition. Such is the case of South Africa, in which the diatom index (SPI) developed for running waters in Europe has been included for use in wetland biological assessments, as part of the requirements for Wetland Reserve Determinations (Rountree et al., 2013), despite having not been tested and assessed for its applicability in lentic waters across the country.

In developed countries, diatoms are widely used for routine assessments of wetland condition in compliance with water legislation statutory requirements, such as the European Water Framework Directive 2000/60/EC (WFD) and US Environmental Monitoring and Assessment Program - Surface Waters (EMAP-SW). It seems almost inconceivable that amongst such a wealth of information from published European and American diatom studies resulting from these initiatives, and the increasing use of diatoms in some African countries, that diatoms and their utility in biological assessments of wetlands in Africa is still largely unknown. This was the main impetus for the current study. In this thesis, I aimed to address one such knowledge gap; the applicability of diatoms for biological assessments in depressional wetlands in the Mpumalanga Highveld region of South Africa.

This research is opportune since it extends on the recent interest in developing a reliable biological assessment tool for depressional wetlands in the Mpumalanga Highveld region and other parts of South Africa (e.g., Ferreira, 2012; Bird et al., 2013; Bird & Day, 2014) and addresses a clear gap in our knowledge of diatom ecology and taxonomy in depressional wetlands on the African continent.

5.3 GENERAL DISCUSSION

5.3.1 Diatoms and temporary depressional wetlands

5.3.1.1 Temporal dynamics in taxonomic composition of epiphytic diatom communities

The first task in investigating the utility of epiphytic diatoms for the biological assessment of temporary depressional wetlands in a new region is to establish baseline information on the temporal dynamics of epiphytic diatom assemblages and their natural controlling factors. The results from Chapters 2 show that epiphytic diatoms are good indicators of changing environmental condition inherent in temporary depressional wetlands. Overall, temporal variability in alkalinity, Na^+ and Cl^-, regulated by hydrological factors, water depth and total relative evapotranspiration (ETo), were key drivers influencing the distribution of diatom communities in three least-disturbed temporary depressional wetlands during various stages of inundation. This corroborates earlier investigations on temporary wetlands in other parts of Africa and Australia, where ionic concentration and composition have explained a substantial part of the variance of diatom community structure as a result of seasonal and climate-driven changes (e.g., Gasse et al., 1995; Gell et al., 2002; Tibby et al., 2007).

We also observed that the community dynamics of the three wetlands were heterogeneous, however it is still unknown as to what extent the ionic concentration and composition influenced the diatom assemblage structure at each site. One of the limitations in the dataset is insufficient within-site variation that would produce long enough environmental gradients and allow for the use of ordination methods to examine the physicochemical-species relationships within each depressional wetland. Consequently, we were unable to quantify, via ordination techniques such as CCA, how seasonal variability in major ions influences the dynamics of diatom assemblages within each site.

It is well established that concentrations of certain cations and anions have a strong relationship with the distribution of individual diatom species (Patrick & Reimer, 1966b, 1975; Cholnoky, 1968). A less explored but perhaps just as important factor influencing diatom communities is the ratio of monovalent to divalent cations (M : D) in the water. Potapova & Charles (2003) found that numerous diatoms with comparatively high optima for divalent cations, Ca^{2+} and Mg^{2+}, had low optima for monovalent cations, Na^{+} and K^{+}, while those with high optima for monovalent cations had low optima for divalent cations. The results of Chapter 2 showed *Nitzschia acidoclinata* and *Nitzschia gracilis* to be dominant at a study site that was significantly Ca^{2+} richer and Na^{+} poorer compared to the other study sites. These taxa had relatively low optima for Na^{+}; although we did not investigate their optima for Ca^{2+}, it is conceivable that the M : D ratio may also be an important factor influencing the distribution of these individual taxa, and possibly other diatom taxa in the study. That the distributions of certain diatoms are not only affected by proportions of individual cations and anions, but also the ratio of monovalent to divalent cations (Potapova & Charles, 2003), is an interesting direction for future enquiry of diatom structure in relation to ionic composition.

In the same chapter, we presented species indicator values (optima and tolerances) for alkalinity, Na^{+} and water depth because a simple WA with inverse deshrinking produced reasonably robust models for these variables. These species optima may be useful as indicators to assess pollution severity and habitat alterations (i.e. stressors) in temporary depressional wetlands with similar water chemistry characteristics in the region.

One obvious limitation in the dataset is the small selection of temporary depressional wetlands sampled. Similar to other studies (e.g., Mackay et al., 2003; Tibby et al., 2007), we were able to derive quantitative diatom autecological data from reasonably robust weighted-averaging models using data from a small number of intensively monitored temporary wetlands. Nevertheless, the autecological characteristics presented here are reliant on the limited range and distribution of the physicochemical variables in the dataset, and may not be suitable for sites with differing physical and chemical attributes. For this reason, we recommend future work to build on this existing dataset by sampling additional temporary depressional wetlands in the region that encompass a broader range of environmental conditions. This would provide more reliable diatom autecological data for use in regional biological assessments of temporary depressional wetlands.

The results presented in Chapter 2 contribute to our understanding of how epiphytic diatoms are distributed along a hydrochemical and hydrological gradient in temporary depressional wetlands in the Mpumalanga Highveld region. We demonstrated the responsiveness of epiphytic diatoms to natural temporally changing disturbances in temporary depressional wetlands in the region. However, one avenue of future enquiry that will be most valuable is to investigate whether the epiphytic diatom species composition can discriminate between the confounding effects of natural environmental gradients and human disturbance gradients. For example, earlier studies on temporary depressional wetland macroinvertebrates in the Mpumalanga Highveld region and other regions of South Africa, found that the influence of natural gradients on macroinvertebrate assemblages influenced macroinvertebrate responses to a larger degree than human disturbances (Day & Malan, 2010; Ferreira et al., 2012; Bird et al., 2013). Determining whether diatom assemblages have the ability to discriminate the effects of natural variability from human-induced variability in temporary depressional wetlands is perhaps the most important next step to evaluate the utility of diatoms as an effective indicator of ecological condition in these wetlands.

5.3.1.2 Temporal dynamics in functional group composition of epiphytic diatom communities

In this second part of the study, we investigated the utility of functional-based approaches, beyond traditional species-level metrics alone, for understanding community responses to natural disturbances in temporary depressional wetlands. The use of functional-based approaches in water quality assessments are increasing since (a) it does not involve finer species-level taxonomy which can be challenging; b) species can be easily identified based on obvious morphological traits; and c) functional group metrics have proven to perform as well as metrics based on species-level identification (Stevenson & Bahls, 1999; Berthon et al., 2011).

Diatom taxa use various adaptations to tolerate environmental disturbances, such as grazing pressure, or changes in the water-level which may affect light and nutrient availability and major ion content (Lange et al., 2011; Stenger-Kovács et al., 2013; Leira et al., 2015); and this was observed in the results of Chapter 3 through distinct changes in the abundance of ecological guilds (low profile, high profile, motile or planktonic) and life-forms (such as tube-living diatoms; see Table 3.1). The guilds relate to the strategies of sharing of a similar resource by species in a competitive context while the life-form groups illustrates the ecosystem processes these species eventually perform through resource exploitation (Rimet & Bouchez, 2011).

Previous studies have demonstrated the usefulness of ecological guilds and life-forms as biological indicators of nutrient concentrations and organic pollution, with most of the research attention on rivers and streams (e.g., Passy, 2007; Berthon et al., 2011; Rimet & Bouchez, 2011). Other functional traits such as cell sizes have been tested in diatom water quality assessments for inferring trophic status, although their use have proven to be less successful (Pringle, 1990; Lavoie et al., 2010).

Function-related responses to changing environmental conditions are poorly understood for depressional wetlands. In Chapter 3 we attempted to address this knowledge gap. We compared the performances of both the taxonomic composition and functional group composition (ecological guilds and life-forms) to assess changes in temporary depressional wetland condition and found that they performed equally well. We demonstrated that changes in alkalinity, Na^+ and Cl^- as a result of seasonal fluctuations in water depth and ETo in three least-disturbed temporary depressional wetlands not only explained most of the variation in the diatom taxonomic composition, but also the largest part of the variance in the functional group composition. These results corroborate the findings in previous studies where ecological guilds and life-forms were able to assess gradients of ionic composition, organic and nutrient concentrations as good as diatom indices based on species (Passy, 2007; Berthon et al., 2011; Rimet & Bouchez, 2011).

In this study, low profile guilds dominated by pioneer life-forms, *Achnanthidium minutissimum*, showed the strongest relationship with higher disturbance levels; specifically, an increase in alkalinity and Na^+ associated with a decrease in water depth during the drying out phase. It is well known that low profile pioneer diatoms, *A. minutissimum*, is an early coloniser resistant to various disturbances including extreme fluctuations in lake water-level (Leira et al., 2015), low light exposure and low nutrient conditions in lakes (Gottschalk & Kahlert, 2012) and shear stress (Peterson & Stevenson, 1992). Similarly, the planktonic guild and tube-living, rosette and adnate life-forms dominated at higher disturbance levels. These guilds and life-forms have features to resist disturbances, for example, the thick exopolysaccharide matrices surrounding diatoms living in mucous tubules may protect the diatom from incoming dissolved chemicals in the water, such as Na^+. Rimet and Bouchez (2011) reported results that could support this hypothesis where increased pesticide contamination increased tube-living diatoms.

Moreover, the nitrogen-fixing symbionts found in adnate diatoms, *Rhopalodia gibba*, may access nutrients under low nutrient conditions caused by sediment resuspension while water levels are dropping (Niemistö et al., 2008). In contrast, high profile diatoms, mostly comprised of the genus *Gomphonema* (pedunculate diatoms), were most sensitive to disturbance, specifically increasing alkalinity. Such finding are in line with many other studies in which the high profile guild has been the most sensitive group to disturbance including water-level changes: Leira et al. (2015), elevated ionic content and flooding: Stenger-Kovács et al. (2013); B-Béres et al. (2014), low light and nutrient availability: Berthon et al. (2011); Lange et al. (2011), and grazing pressure: Luttenton et al. (1986).

The results of Chapter 3 demonstrate the possibility of several coarser-level diatom functional groups for use in temporary depressional wetland condition assessments, and suggest that differences between functional group composition and taxonomic composition are not so large that we need to resort to finer species-level taxonomy. This will have particular relevance in many African countries where information on local diatom flora may be limited or high-level taxonomic expertise is lacking (Dalu & Froneman, 2016). A functional-based approach may allow for a simplified assessment of aquatic resource condition while retaining ecologically meaningful information (Goldenberg Vilar et al., 2014), although Rimet & Bouchez (2012b) advocate the use of a fine (species) taxonomic resolution where precise biological assessments are required.

Determining whether a functional-based approach is suitable for reliable pollution assessments of temporary depressional wetlands is an important area for future research. Further studies should address, as for the taxonomic composition, whether functional groups of epiphytic diatom communities can discriminate between gradients of human disturbance and natural gradients.

Most functional-related studies include only a few traits, mostly those belonging to ecological guilds (e.g., Passy, 2007; Lange et al., 2011; Passy & Larson, 2011; Gottschalk & Kahlert, 2012; Stenger-Kovács et al., 2013; B-Béres et al., 2014; Goldenberg Vilar et al., 2014; Leira et al., 2015). We highlight the inclusion of life-forms as they can provide additional information on the underlying ecological processes related to resource exploitation that is not available from ecological guild data. Investigating the response of diatom communities using a comprehensive set of metrics can provide more insight into the mechanisms that drive diatom community composition, particularly where there are multiple stressors at play (Lange et al., 2016).

5.3.2 Diatoms and AMD polluted permanent depressional wetlands

The impact of AMD on aquatic resources in the coal-rich region of the Mpumalanga Highveld is of increasing concern not only to national and local governments but also coal mine owners (Munnik et al., 2010). The use of water for mining activities is regulated by the National Water Act (NWA) which is aimed at the protection of water resources including water quality and quantity, and ecosystem condition (condition of the aquatic biota and habitat). The Act requires mines to produce an Environmental Management Programme Report (EMPr), with the main objective to ensure that sufficient resources are allocated for rehabilitation, monitoring and auditing of aquatic resources.

Biological assessments are necessary to evaluate the benefit of these actions, but thus far, biological assessment methods for aquatic resources in South Africa have only been developed for rivers and streams, not for wetlands. Considering the well established relationship of (river and stream) diatoms with AMD (e.g., Zalack et al., 2010; Gray & Vis, 2013; Oberholster et al., 2013) and the fact that other biological indicators (macroinvertebrates, macrophytes) have proven ineffective for assessing wetland pollution in South Africa, diatoms may be suitable candidates to quantify AMD impacts in wetlands.

In this final part, we investigated the utility of epiphytic diatoms for assessing AMD impacts in permanent depressional wetlands in the Mpumalanga Highveld. AMD has severely affected biological diversity and ecosystem functioning of permanent depressional wetlands in the region since mines utilise these wetlands for storage of AMD. This thesis aimed to address the urgent need to develop a strategy for impact assessment and management of depressional wetlands in the region. In Chapter 4, we developed a diatom-based multimetric index (MMI) for AMD impacted permanent depressional wetlands in the region, which is also the first diatom index to quantify AMD impacts in wetland habitats.

Depressional wetlands can vary considerably in natural environmental factors such as hydrology, water chemistry and vegetation, over small spatial scales, which in turn, can attribute to natural differences in their biological assemblages (Bird et al., 2013). High heterogeneity among wetlands will likely result in poorly performing MMIs if indices are not controlled for the effects of naturally varying environmental factors. For this reason, we created separate MMIs for classes of depressional wetland types in order to account for natural variation among reference sites, and compared their performance with an MMI that did not account for natural variation. In this study, we used reference site classification defined by diatom typologies to account for natural variation and hypothesised that by using this approach, we would improve MMI performance.

Data collected from 34 sites that represented a range of conditions along an AMD gradient within the Mpumalanga Highveld was used to select responsive diatom metrics which we combined into a multimetric index. A range of 4-15 final metrics were selected from a pool of ~140 candidate metrics to develop the MMIs, based on their broad ranges, an ability to discriminate reference from disturbed depressions, and low correlation among metrics. The final metrics selected for the MMIs belonged to three metric categories: similarity to reference sites, functional groups, and taxonomic composition, which reveal different features of biological condition (Stevenson et al., 2013).

To evaluate MMI performance we used measures of precision, responsiveness, sensitivity and relevancy to coal mining impairment. Overall, all MMIs performed considerably well, although grouping sites by diatom typology to account for natural variation increased MMI precision, responsiveness and sensitivity to disturbance. The results presented here demonstrate the potential use of diatom-based metrics as biological indicators of impact and recovery in depressional wetlands affected by AMD.

This, however, is a preliminary approach that has two important limitations in the development of the MMIs: the limited number of sites sampled prevented us from testing and assessing the applicability of the indices to AMD impacted depressional wetlands in the region. Furthermore, a single sampling visit did not allow us to assess the temporal stability of the metrics. Future work in MMI development for depressional wetlands would benefit from testing additional sites in the region and sampling multiple seasons to account for natural temporal variation in biological metrics, as recommended by others (e.g., Hering et al., 2006b; Zhang et al., 2012; Chen et al., 2014). Sampling more reference and disturbed sites and more seasons would cover greater natural variation and produce for a more robust MMI (Cao et al., 2007; Mazor et al., 2016). A large reference dataset is also advantageous since it will allow for the development of one MMI that is adjusted for natural gradients by modeling the effects of natural environmental gradients on assemblage composition. The modeling approach is a highly effective means of increasing MMI accuracy and precision (Cao et al., 2007; Stoddard et al., 2008; Stevenson et al., 2013). The application of one MMI for all depression types is much more favourable than having to choose from a selection of MMIs which are developed for a particular depression type. Finally, we recommend identifying metrics that respond to different types of pollution in the Mpumalanga Highveld, where more than one source of human disturbance dominates (e.g., mining and agricultural activities). This should improve mine land remediation and biomonitoring programmes for depressional wetlands by providing a more precise diagnosis of the type and severity of pollution.

5.4 CONSERVATION IMPLICATIONS

Freshwater ecosystems are recognised as possibly the most threatened ecosystems in the world (Dudgeon et al., 2006). The protection of aquatic resources is of vital importance for preserving ecosystem functions and biodiversity (Strayer & Dudgeon, 2010). Historically, wetland conservation initiatives have concentrated on the protection of rare or unique sites, such as Ramsar sites, that are managed as discrete units (Amezaga et al., 2002). However, wetland conservation efforts have gone beyond the protection of isolated sites to develop conservation strategies at multiple scales of wetland networks (Baguette et al., 2013), recognising that often it is the cumulative loss of many wetlands, including small, depressional ones, that can significantly affect biodiversity (Leibowitz, 2003). Since depressional wetlands are generally small in size, it might be assumed that their loss may have minor importance. However, earlier studies have highlighted that the degree of functional loss is not relative to size (e.g., Gibbs, 1993; Robinson, 1995; Semlitsch & Bodie, 1998). Much of the significance associated with depressional wetlands is attributed to biodiversity. A rich biota is often associated with these wetlands which studies have shown to be partly attributable to the effects of hydroperiod duration (Gaiser & Johansen, 2000; Dimitriou et al., 2009). They may also host endemic species because of their isolation. Consequently, loss of depressional wetlands may have a disproportionate effect on regional biodiversity in comparison with other wetland types (Leibowitz, 2003).

An example of how depressional wetland loss could significantly impact regional biodiversity is in the near pristine area of the Mpumalanga Highveld region, known as the Mpumalanga Lakes District (MLD). Here, we sampled depressional wetlands representing non-AMD impacted reference sites (Chapter 4). This area is host to around 320 depressions which provide habitat for more than one hundred and fifty bird species and where, in the wet season, bird populations may exceed 20,000. However, proposed coal mining within the catchment of the MLD presents a serious threat to this pristine and biodiverse wetland system (McCarthy et al., 2007). It is possible that local extinction of species as a result of wetland loss and degradation from mining activities in the MLD may also result in regional loss of those species. Wetland loss or degradation from mining activities (e.g., lowering the water table and AMD related stressors) could reduce landscape connectivity and increase isolation; consequently, lower connectivity would reduce recolonisation since there would be fewer individuals available for dispersal and the average distance between remaining wetlands would increase (Gibbs, 1993; Semlitsch & Bodie, 1998).

Studies on the aquatic flora and fauna of depressional wetlands in the MLD is surprisingly limited (de Klerk et al., 2012; Ferreira, 2012), with poorly described diatom flora (Hutchinson et al., 1932). The epiphytic diatom flora and the physical and chemical data we collected from a range of reference depression types in this area (reed, salt, open-water, grass depressions) can provide information about natural baseline conditions against which potential changes in ecological condition as a result of human disturbances can be assessed.

5.5 CONCLUSIONS

The main objective of this thesis was to evaluate the feasibility of using diatoms for the biological assessment of depressional wetlands in South Africa for the first time. This work has demonstrated that epiphytic diatom communities are strong indicators of changing environmental conditions in temporary and permanent depressional wetlands, and highlights the potential of diatoms for accurate ecological assessments of depressional wetlands. Throughout this chapter, we have underlined areas for future research that will be most useful and insightful for further advances in biological assessments of depressional wetlands and management of these aquatic resources in the Mpumalanga Highveld region. In a broader context, this research highlights the potential use of diatom-based indices in wetland condition assessments in South Africa, and in other countries which currently have no well-developed method for assessing wetland condition; and/or where diatoms are not included in the suite of biological indicators (macroinvertebrates, fish and macrophytes) used routinely for assessment of aquatic resources (e.g. US: Davis et al., 1996; Barbour et al., 1999; Johnson et al., 2006, Europe: WFD; European Union, 2000). The methodology to develop the multimetric index presented in this thesis could be used to develop diatom-based multimetric indices in other ecoregions.

Diatom assessments of ecological condition commonly have focused on inferring physical and chemical conditions using indices derived from species composition of diatoms (Kelly et al., 2007; Stenger-Kovács et al., 2007). However, the use of species-based indices present major challenges for use in African countries and many other developing countries: 1) they require high-level taxonomic expertise and well described diatom flora which is largely lacking; and 2) they include a large diversity of species found in very different geographic and climate zones (mostly in the Northern hemisphere). This may not be transferable to Southern Hemisphere countries such as for example, the Democratic Republic of Congo, which have unique diatom flora and ecological conditions (Cocquyt et al., 2013; Cocquyt & Taylor, 2015).

These challenges have contributed to the difficulty of implementing diatoms in biological assessment programmes in many sub-Saharan countries (Dalu & Froneman, 2016). Consequently, diatoms are viewed in this region as being the most technically difficult of all the biological indicators (Bere, 2016b).

In this thesis, we present a biological assessment approach using simple diatom metrics of biological condition. Most of these metrics are developed using the identification of just a few diatom species (e.g., % reference or % tolerant taxa metrics), or applying intermediate taxonomy using genus-level identifications or assignation of easily measurable morphological traits (functional groups) to species. Following a similar approach as for macroinvertebrate biological assessments, African countries and other developing countries could adopt coarse diatom taxonomy which would be easier, time and cost effective and demand less skilled personnel. For a quick and robust diatom assessment of ecological condition, a coarser level of identification has proven satisfactory (e.g., Hill et al., 2001; Raunio & Soininen, 2007; Rimet & Bouchez, 2012a). This approach may allow the inclusion of diatom-based metrics along with metrics for macroinvertebrates, fish and macrophytes commonly used in ecological condition assessment of aquatic resources, or in the case of South African wetlands, diatom-based metrics could become the significant component in assessments of wetland ecological condition and the protection and restoration of these habitats.

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SUPPLEMENTARY TABLES

CHAPTER 2

Table S2.1 SIMPER results indicating the cumulative contribution of the taxa contributing most to the Bray-Curtis similarity in diatom composition within each site. These taxa contributed up to 50% of the total average similarity between surveys within each site.

S1 Average similarity 54.3%	Cum.%
Nitzschia acidoclinata Lange-Bertalot	23.3
Encyonema mesianum (Cholnoky) D.G. Mann	42.4
Eunotia bilunaris (Ehrenberg) Schaarschmidt	56.1
S2 Average similarity 58.4%	Cum.%
Nitzschia acidoclinata Lange-Bertalot	33.7
Nitzschia gracilis Hantzsch	50.4
S3 Average similarity 51%	Cum.%
Nitzschia acidoclinata Lange-Bertalot	14.5
Gomphonema spec. aff. *angustatum* (Kützing) Rabenhorst	27
Encyonema mesianum (Cholnoky) D.G. Mann	37.1
Achnanthidium minutissimum (Kützing) Czarnecki	46.5
Eunotia bilunaris (Ehrenberg) Schaarschmidt	54.1

Table S2.2 Optima and tolerances of the most common taxa for weighted averaging (WA) inference models for Na^+, alkalinity and depth. The total number of occurrences (N), the effective number of occurrences (Hill's N2) and maximum percent abundance (Max) are shown. Log-transformed tolerances were added and subtracted from the log-transformed optima and all 3 numbers back transformed to mgL^{-1} for Na^+ and alkalinity. Depth is measured in cm. Tolerance is presented as lower limit to upper limit.

				Na^+		Alkalinity		Depth	
Taxon	N	Max	N2	Optimum	Tolerance	Optimum	Tolerance	Optimum	Tolerance
Nitzschia acidoclinata Lange-Bertalot	63	18.1	54.8	4.9	1.8 - 13.6	68.1	45.1 - 102.7	62.4	33.8 - 91
Encyonema mesianum (Cholnoky) D.G. Mann	59	14.8	47.0	7.6	3.7 - 15.7	69.1	46.5 - 102.5	54.7	32.5 - 76.9
Gomphonema parvulum Kützing	55	11.1	41.1	6.4	3 - 13.6	78.7	48.7 - 127	52.2	29.0 - 75.4
Eunotia bilunaris (Ehrenberg) Schaarschmidt	60	17.6	41.0	5.4	2 - 14.7	60.8	38.7 - 95.4	60.2	33.1 - 87.3
Nitzschia gracilis Hantzsch	56	14.0	40.5	4.6	1.8 - 12	76.8	48.5 - 121.7	56.3	29.5 - 83.1
Fragilaria tenera (W.Smith) Lange-Bertalot	44	7.8	37.0	6.2	2.7 - 14.4	73.3	50.0 - 107.4	58.2	35.3 - 81.1
Achnanthidium minutissimum (Kützing) Czarnecki	46	14.9	31.9	9.5	5.1 - 17.7	83.6	55.3 - 126.4	47.0	27.0 – 67.0
Gomphonema parvulius (Lange-Bertalot & Reichardt) Lange-Bertalot& Reichardt	46	8.8	31.7	4.6	1.9 - 11.5	59.1	40.1 - 87	72.3	44.9 - 99.7
Gomphonema exilissimum (Grunow) Lange-Bertalot & Reichardt	36	3.9	31.5	3.5	1.3 - 9.4	60.3	40.2 - 90.5	72.3	44.8 - 99.8
Gomphonema spec. aff. *angustatum* (Kützing) Rabenhorst	43	11.7	31.1	3.3	1.3 - 8.5	58.6	43 - 79.9	77.5	53.6 - 101.4
Nitzschia fruticosa Hustedt	37	13.9	24.4	6.2	2.3 -16.2	82.7	53.9 - 127.0	47.4	25.3 - 69.6
Navicula tridentula Krasske	35	4.4	27.4	5.0	1.6 - 16.1	64.8	43.9 - 95.7	58.6	27- 90.2
Nitzschia palea var. debilis (Kützing) Grunow	32	3.2	29.0	3.7	1.4 - 9.8	57.6	45.8 - 72.4	71.8	49.8- 93.8
Gomphonema parvulum (Kützing) Kützing sensu lato	32	4.4	27.1	8.8	4.6 - 16.6	78.7	48.7 - 127	52.5	29.8 - 75.2
Gomphonema spiculoides H.P.Gandhi	31	6.6	24.3	7.8	3.9 - 15.8	56.2	39.2 - 80.6	63.2	36.3 - 90.1
Gomphonema auritum A. Braun ex Kützing	30	11.6	18.7	4.2	1.8 - 9.4	49.9	36 - 69.2	76.0	53.5 - 98.4
Gomphonema parvulum (Kützing) Kützing sensu lato Nr.2	28	4.0	24.1	7.7	3.6 - 16.7	66.9	46.1 - 97.0	58.4	33.8 - 83.1
Nitzschia acicularis (Kützing) W.M.Smith	27	9.8	18.8	5.2	2.2 - 12.4	75.9	52.5 - 109.8	61.8	39.9 - 83.6
Gomphonema gracile Ehrenberg	27	5.4	20.8	6.5	3.0 - 14.2	54.8	37.1 - 81.0	68.4	43.6 - 93.2
Brachysira neoexilis Lange-Bertalot	23	5.0	19.0	12.0	7.3 - 19.8	68.7	47.0 - 100.5	43.8	23.2 - 64.4
Nitzschia palea (Kützing) W.Smith	22	4.4	17.1	3.9	1.3 - 11.3	67.7	44.0 - 104.1	60.2	38.3 - 82.1
Gomphonema exilissimum (Grunow) Lange-Bertalot & Reichardt sensu lato	20	5.8	16.9	2.7	1.1 - 7.0	54.9	42.7 - 70.6	83.1	61.8 - 104.5

Pinnularia subgibba Krammer var. *subgibba*	19	3.6	15.3	7.2	2.9 - 17.8	66.0	39.6 - 110.0	56.5	27.5 - 85.4
Gomphonema spec. aff. *stonei* Reichardt ssp.	17	4.6	13.7	3.0	1.1 - 8.4	48.9	36.1 - 66.3	71.6	47.7 - 95.6
Pinnularia spec. aff. *graciloides* Hustedt	15	2.2	13.8	8.6	3.1 - 24.1	69.0	44.3 - 107.6	44.2	15.6 - 72.8
Gomphonema Ehrenberg spec. Nr. 3	15	4.0	12.4	4.0	1.8 - 8.8	52.2	40.2 - 67.9	79.3	58.8 - 99.8
Ulnaria acus (Kützing) Aboal	12	4.4	9.7	3.6	2.3 - 5.7	86.7	58.4 - 128.8	56.5	39.5 - 73.4
Gomphonema pseudobohemicum Lange-Bertalot& Reichardt	12	7.1	9.4	7.0	3.8 - 12.8	61.0	41.2 - 90.2	66.4	40.9 - 91.8
Gomphonema cf. *parvulum* (Kützing) Kützing	12	3.2	11.0	4.5	1.8 - 11.2	97.3	55.8 - 169.5	48.6	27.6 - 69.7
Achnanthidium saprophilum (Kobayasi et Mayama) Round & Bukh.	11	6.4	7.4	11.4	5.8 - 22.3	91.7	67.0 - 125.6	45.8	26.7 - 64.9
Nitzschia subacicularis Hustedt	11	5.8	10.4	10.7	7.8 - 14.9	83.3	65.5 - 105.9	52.7	38.1 - 67.3
Gomphonema spec. aff. *bohemicum* Reichelt & Fricke	11	7.2	8.6	4.8	2.7 - 8.3	47.3	41.1 - 54.3	84.0	68.8 - 99.1
Hantzschia amphioxys (Ehrenberg) Grunow	10	2.0	9.3	7.9	3.3 - 19.0	115.5	63.9 - 208.7	38.1	18.6 - 57.7
Eunotia minor (Kützing) Grunow	9	2.2	8.4	10.3	6.3 - 17.0	113.9	64.0 - 202.9	38.4	20.1 - 56.7
Gomphonema spec. aff. *angustatum* (Kützing) Rabenhorst spp. 2	8	4.8	5.8	3.1	1.8 - 5.5	51.3	37.8 - 69.6	76.7	58.9 - 94.6
Pinnularia viridiformis Krammer var. *minor* Krammer	8	1.4	7.9	5.6	1.9 - 16.5	85.7	41.0 - 179.4	43.6	21.7 - 65.6
Pinnularia pseudogibba Krammer	8	2.0	7.6	9.4	4.4 - 20.1	65.2	52.3 - 81.3	42.5	23.2 - 61.8
Gomphonema Ehrenberg spec. Nr. 4	8	3.5	7.3	11.0	8.3 - 14.7	85.5	74.5 - 98.0	50.4	41.0 - 59.8
Rhopalodia gibba (Ehrenberg) O. Muller	7	4.8	5.6	7.9	6.5 - 9.7	70.1	65.4 - 75.2	63.7	56.9 - 70.6
Pinnularia spec. aff. *tirolensis* (Metzeltin & Krammer) Krammer	7	2.2	6.2	3.0	0.8 - 11.1	55.7	34.3 - 90.3	65.5	29.0 - 102.0
Navicula cryptocephala Kützing	7	2.2	6.3	4.0	1.8 - 8.8	109.4	61.8 - 193.7	41.6	26.1 - 57.2
Sellaphora joubaudii (Germain) Aboal	6	2.6	5.3	11.3	3.5 - 36.7	85.9	48.7 - 151.6	29.4	5.5 - 53.3
Gomphonema spec. aff. *angustatum* (Kützing) Rabenhorst spp. 5	6	3.7	4.4	6.2	3.0 - 12.6	50.3	42.5 - 59.4	77.6	52.2- 103.0
Pinnularia subcapitata Gregory var. *elongata* Krammer	5	2.0	4.6	6.6	2.6 - 17.3	83.3	41.8 - 166.2	51.6	21.4 - 81.7
Pinnularia acrospheria W. Smith	5	1.0	5.0	9.4	7.5 - 11.8	52.4	45.3 - 60.5	57.7	38.5 - 76.9
Pinnularia Ehrenberg spec. Nr. 1	5	1.0	5.0	5.9	4.6 - 7.5	64.0	49.1 - 83.4	68.3	54.8 - 81.8
Gomphonema spec. aff. *angustatum* (Kützing) Rabenhorst spp. 4	5	3.3	4.1	4.0	2.4 - 6.6	86.9	67.3 - 112.3	53.8	39.3 - 68.4
Gomphonema spec. aff. *angustatum* (Kützing) Rabenhorst spp. 3	5	2.8	4.5	9.4	6.6 - 13.4	71.7	58.6 - 87.8	48.6	38.7 - 58.4
Gomphonema Ehrenberg spec. Nr. 2	5	1.7	4.7	9.2	6.3 - 13.4	70.7	51.0 - 98.0	54.4	39.9 - 68.9
Gomphonema Ehrenberg spec. Nr. 8	5	1.4	4.9	3.3	1.4 - 7.8	71.3	52.7 - 96.5	56.4	33.2 - 79.6

Table S2.3 Pearson correlation coefficients between the most common taxa and the best performance WA model variables. Significance of each comparison is indicated by *P ≤ 0.05, ** P ≤ 0.01 and *** P ≤ 0.001.

	Na^+		Depth		Alkalinity	
Nitzschia acidoclinata Lange-Bertalot	-0.19		0.10		0.09	
Encyonema mesianum (Cholnoky) D.G.Mann	**0.67**	***	**-0.43**	***	0.12	
Gomphonema parvulum Kützing	**0.29**	*	**-0.47**	***	**0.52**	***
Eunotia bilunaris (Ehrenberg) Schaarschmidt	0.04		-0.06		**-0.32**	*
Nitzschia gracilis Hantzsch	-0.18		**-0.26**	*	**0.43**	***
Fragilaria tenera (W.Smith) Lange-Bertalot	0.21		-0.14		**0.25**	*
Achnanthidium minutissimum (Kützing) Czarnecki	**0.63**	***	**-0.55**	***	**0.52**	***
Gomphonema parvulius (Lange-Bertalot & Reichardt) Lange-Bertalot & Reichardt	-0.13		**0.41**	***	**-0.30**	*
Gomphonema exilissimum (Grunow) Lange-Bertalot& Reichardt	**-0.43**	***	**0.41**	***	**-0.25**	*
Gomphonema spec. aff. *angustatum* (Kützing) Rabenhorst	**-0.48**	***	**0.60**	***	**-0.31**	*
Nitzschia fruticosa Hustedt	0.13		**-0.42**	***	**0.39**	***
Navicula tridentula Krasske	-0.05		-0.09		-0.07	
Nitzschia palea var. debilis (Kützing) Grunow	**-0.33**	**	**0.36**	***	**-0.33**	**
Gomphonema parvulum (Kützing) Kützing sensu lato	**0.47**	***	**-0.29**	*	0.12	
Gomphonema spiculoides H.P.Gandhi	**0.33**	**	0.05		**-0.33**	**
Gomphonema auritum A.Braun ex Kützing	-0.16		**0.36**	**	**-0.45**	***
Gomphonema parvulum (Kützing) Kützing sensu lato Nr.2	**0.32**	**	-0.09		-0.01	
Nitzschia acicularis (Kützing) W.M.Smith	-0.004		0.01		0.19	
Gomphonema gracile Ehrenberg	0.16		0.19		**-0.33**	**

CHAPTER 4

Table S4.1 List of metric categories and candidate metrics used in the development of four MMIs for reference and disturbed depressional wetlands in the Mpumalanga Highveld region.

Category/Candidate metric	Description and source
Diversity	Stevenson et al. (2013)
Hurlbert's evenness (EHurlbert)	Evenness of taxa
	Wang et al. (2005)
Species richness (S)	No. of taxa in samples (richness)
Shannon diversity (H)	Evenness and richness of taxa
% dominance	Relative abundance of the most commonly observed taxon in a sample
Similarity to reference sites	Stevenson et al. (2013)
% reference taxa	Taxa characteristic of reference sites based on relative abundance of reference taxa to total no. of taxa in the sample
% tolerant taxa	Taxa characteristic of impaired sites by mining based on relative abundance of tolerant taxa to total no. of taxa in the sample
% reference individuals	Relative abundance of reference individuals characteristic of reference sites based on relative abundance of all individuals (valves) typical of reference sites to total no. of individuals in the sample
% tolerant individuals	Relative abundance of tolerant individuals characteristic of impaired sites by mining based on relative abundance of all individuals (valves) typical of disturbed sites to total no. of individuals in the sample
	Wang et al. (2005)
% similarity to reference sites	Bray-Curtis similarity in taxa composition to all reference sites

% reference taxa found in reference sites that occurred in impaired sites	Relative abundance of taxa found in reference sites that occurred in impaired sites
% reference individuals found in reference sites that occurred in impaired sites	Relative abundance of individuals (valves) found in reference sites that occurred in impaired sites
No. of distinct reference taxa	No. of taxa found predominantly in reference sites not in impaired sites
Functional group	Passy (2007a, b); Rimet and Bouchez (2012b)
Mobile % taxa and individuals	Relative abundance of taxa and individuals which are free moving e.g. some species vertically migrate into the sediments to acquire nutrients
Teratology % taxa and individuals	Relative abundance of taxa and individuals having deformed frustules, often caused by metals and trace elements
Pioneer % taxa and individuals	Relative abundance of taxa and individuals which colonise bare substrates faster than other species
Adnate % taxa and individuals	Relative abundance of taxa and individuals which grow parallel to the substrate, attached by their valve face
Pad (attached to substrate) % taxa and individuals	Relative abundance of taxa and individuals which grow upright to substrate, attached by a mucilage pad
Stalk (attached to substrate) % taxa and individuals	Relative abundance of taxa and individuals which grow upright to substrate, attached by a stalk
Colonial % taxa and individuals	Relative abundance of taxa and individuals that can form colonies of multiple cells, possessing tall stature characteristics or if comprised of just a few cells, having short stature characteristics; can form various types of colonies e.g., filamentous-, ribbon- and rosette colonies.
Non-colonial % taxa and individuals	Relative abundance of taxa and individuals that are unicellular
Tube-living % taxa and individuals	Relative abundance of taxa and individuals which live in mucous substance within which they can move freely
Filamentous % taxa and individuals	Relative abundance of taxa and individuals that form long visible chains of attached cells; forms protrude above the biofilm extending into the water column

Rosette % taxa and individuals	Relative abundance of taxa and individuals which produce a short stalk, forming colonies which are similar to a fan/rosette shape
Ribbon % taxa and individuals	Relative abundance of taxa and individuals attached to one another either by interlocking spines or by a layer of mucous on their valve face, forming long, ribbon-like colonies
High profile guild % taxa and individuals	Relative abundance of taxa and individuals of tall stature, including erect, filamentous, branched, chain-forming, tube-forming, pedunculate and colonial centrics
Low profile guild % taxa and individuals	Relative abundance of taxa and individuals of short stature, including prostrate, adnate, small erect, solitary centrics, slow-moving species
Motile guild % taxa and individuals	Relative abundance of taxa and individuals of fast-moving species
Planktonic guild % taxa and individuals	Relative abundance of taxa and individuals which are solitary or colonial centrics, pennates
Taxonomic composition	Wang et al. (2005)
% taxa in genera	Relative abundance of taxa within each genus based on relative abundance of no. of taxa in the genus to total no. of taxa in the sample
% individuals in genera	Relative abundance of individuals within the genus based on relative abundance of all individuals in the genus to total no. of individuals in the sample

Table S4.2 Mean, standard deviation (SD), minimum (min) and maximum (max) values of physical-chemical variables of reference (Ref) and disturbed (Dist) sites used to develop the four MMIs: MM1-1 using all 15 reference sites; MMI-2 using 4 reed depressional reference sites; MMI-3 using 6 salt depressional reference sites; and MMI- 4 using 5 open-water and grass depressional reference sites. All 19 disturbed sites were used to develop each MMI.

Variables	MMI-1				MMI-2				MMI-3				MMI-4							
	Ref (*n* = 15)				Ref (*n* = 4)				Ref (*n* = 6)				Ref (*n* = 5)				Dist (*n* = 19)			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
Cond (mSm^{-1})	612	933	16	3700	158	91	84	280	1378	1194	425	3700	57	75	16	190	337	336	28	1340
pH	8.77	1.16	6.32	10.47	7.65	1.39	6.32	9.35	9.61	0.44	9.21	10.47	8.65	1.01	6.97	9.66	8.37	0.98	6.69	9.84
Turbidity (NTU)	65.1	50	4.2	192	20.2	19.7	4.2	48.6	115.3	37.6	100	192	40.9	27.7	7.8	78.1	206.8	367.1	1.8	999
NH$_3^{a}$	0.32	0.27	0.06	1.1	0.46	0.43	0.19	1.1	0.36	0.23	0.12	0.69	0.16	0.09	0.06	0.29	1.1	3.17	0.03	14
TN^{a}	6.63	7.05	1.9	32	3.5	1.17	1.9	4.6	10.55	10.8	3	32	4.44	0.91	3.2	5.6	5.12	7.73	0.25	32
TP^{a}	2.68	3.49	0.03	11	0.29	0.31	0.03	0.64	6.4	2.97	3.5	11	0.13	0.11	0.03	0.28	1.15	1.97	0.03	7.5
PO$_4^{2-a}$	2.31	3.11	0.03	9	0.06	0.07	0.03	0.17	5.68	2.5	3.3	9	0.06	0.05	0.03	0.14	0.61	1.33	0.03	5.7
Ca^{2+a}	17.3	9.8	7.3	46	17.3	4.3	11	20	19.7	13.1	12	46	14.4	10.6	7.3	33	177.6	246.9	7.2	929
Mg^{2+a}	15.2	14.7	5	63	18	6.7	9.8	26	10.8	8.8	5	27	18.2	25.1	5.6	63	268.8	634.5	9.4	2700
Na^{+a}	1548.7	2670.2	14	10700	293	191.1	133	555	3596.7	3597.8	1040	10700	95.8	154.1	14	370	444.4	583.7	13	2510
K^{+a}	76.5	106.8	3.4	372	14.1	5.2	7.2	19	175.3	121	41	372	7.7	3	3.4	11	31.2	35.9	1.7	151
Cl^{-a}	1455.5	2548.7	15	10200	358.5	252.4	149	698	3358.8	3487.1	823	10200	49.2	51.5	15	139	218.9	409.7	11	1830
SO$_4^{2-a}$	528.2	983.4	4.3	3720	10.6	10.3	4.3	26	1291	1317.8	284	3720	27	26.9	9.8	73	1740.6	2988.2	13	12300
F^{-a}	1.5	1.1	0.3	4	0.7	0.2	0.5	0.9	2.7	0.8	2	4	0.6	0.3	0.3	1	3.7	6.1	0.2	20
DOC^{a}	91.8	104.3	24	457	36.5	15.2	24	55	156.7	152.7	45	457	58.2	26.1	27	98	43.2	70.1	0.25	300
Alkalinity^{a}	959	1563	26	6430	242	67	182	335	2067	2188	777	6430	204	321	26	776	381	588	44	2023
Al^{a}	0.03	0.06	0.01	0.2	0.01	0.00	0.01	0.01	0.05	0.08	0.01	0.2	0.04	0.08	0.01	0.19	0.91	2.77	0.01	12
Fe^{a}	0.15	0.27	0.01	0.92	0.22	0.33	0.02	0.71	0.02	0.03	0.01	0.09	0.24	0.39	0.01	0.92	1.26	4.56	0.01	20
Mn^{a}	0.05	0.07	0.01	0.23	0.08	0.07	0.01	0.14	0.02	0.03	0.01	0.08	0.06	0.1	0.01	0.23	0.13	0.34	0.01	1.5

^{a} mgL^{-1}. Alkalinity as CaCO$_3$

Table S4.3 Indicator species analysis results showing taxa to be significantly ($P < 0.05$) present in reference (Ref) and disturbed (Dist) sites. Taxa typical of reference and disturbed sites were used to calculate the following metrics for each MMI: number of distinct reference taxa, and % reference and % tolerant taxa and individuals.

	MMI-1		MMI-2		MMI-3		MMI-4	
Taxon	Ref	Dist	Ref	Dist	Ref	Dist	Ref	Dist
Achnanthidium minutissimum (Kützing) Czarnecki							X	
Encyonema mesianum (Cholnoky) D.G.Mann							X	
Eunotia bilunaris (Ehrenberg) Schaarschmidt	X						X	
Craticula buderi (Hustedt) Lange-Bertalot			X			X		
Ctenophora pulchella (Ralfs ex Kützing) D.M.Williams & Round		X		X		X		X
Encyonopsis minuta Krammer & E.Reichardt		X						
Eolimna minima (Grunow) Lange-Bertalot			X					
Fragilaria fasciculata (C.Agardh) Lange-Bertalot		X		X		X		X
Fragilaria nanana Lange-Bertalot		X						
Gomphonema parvulum Kützing			X					
Halamphora coffeiformis (C.Agardh) Levkov				X		X		X
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski		X						
Navicula adamantiformis Archibald				X		X		X
Nitzschia acidoclinata Lange-Bertalot							X	
Nitzschia archibaldii Lange-Bertalot			X					
Nitzschia etoshensis Cholnoky					X			
Nitzschia frustulum (Kützing) Grunow		X				X		X
Nitzschia gracilis Hantzsch						X		X
Nitzschia lacuum Lange-Bertalot					X			
Nitzschia liebethruthii Rabenhorst					X			
Nitzschia nana Grunow		X						
Stauroneis phoenicenteron (Nitzsch) Ehrenberg			X					
Gomphonema spec. aff. *angustatum* (Kützing) Rabenhorst	X						X	
Gomphonema auritum A.Braun ex Kützing	X						X	
Gomphonema spec. aff. *stonei* Reichardt ssp.			X					
Pinnularia spec. aff. *tirolensis* (Metzeltin & Krammer) Krammer			X					
Rhopalodia gibberula (Ehrenberg) Otto Müller	X				X			

Table S4.4a Candidate metrics of MMI-1 – MMI-3 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included. ( ) Metric not used in the development of the MMI.

	MMI-1				|Z|	P	CV	MMI-2				|Z|	P	CV	MMI-3				|Z|	P	CV
	Reference		Disturbed					Reference		Disturbed					Reference		Disturbed				
Category/Candidate metric	Min	Median	Min	Median				Min	Median	Min	Median				Min	Median	Min	Median			
Diversity																					
Hurlbert's evenness (EHurlbert)	0.1	0.5	0.2	0.5	0.61	0.544	0.38	0.5	0.7	0.2	0.5	2.11	0.035	0.32	0.2	0.5	0.2	0.5	0.26	0.799	0.34
Species richness (S)	8.0	16.0	12.0	19.0	1.67	0.096	0.44	20.0	31.0	12.0	19.0	1.71	0.088	0.36	8.0	11.5	12.0	19.0	2.93	0.003	0.43
Shannon diversity (H)	0.3	1.7	0.7	1.6	0.24	0.808	0.43	1.7	2.5	0.7	1.6	2.07	0.039	0.36	0.7	1.3	0.7	1.6	1.08	0.279	0.38
% dominance	21.4	45.4	26.0	60.4	0.21	0.835	0.42	23.2	26.1	26.0	60.4	2.43	0.015	0.41	33.0	56.1	26.0	60.4	0.48	0.633	0.37
Similarity to reference sites																					
% reference taxa	0.0	7.7	0.0	0.0	3.79	< 0.001	1.61	16.7	23.6	0.0	10.3	3.63	< 0.001	2.12	16.7	19.4	0.0	0.0	3.85	< 0.001	1.55
% tolerant taxa	0.0	0.0	0.0	11.8	3.96	< 0.001	1.11	0.0	0.0	0.0	0.6	2.61	0.009	0.84	0.0	0.0	0.0	10.3	2.91	0.004	1.04
% reference individuals	0.0	1.2	0.0	0.0	3.77	< 0.001	2.15	34.6	40.9	0.0	1.0	3.63	< 0.001	2.27	4.0	38.1	0.0	0.0	3.72	< 0.001	2.05
% tolerant individuals	0.0	0.0	0.0	3.2	3.57	< 0.001	2.02	0.0	0.0	0.0	5.9	2.61	0.009	1.60	0.0	0.0	0.0	2.4	2.91	0.004	1.89
% similarity to reference sites	10.4	22.6	2.8	11.7	3.83	< 0.001	0.48	41.8	55.2	4.1	9.3	3.08	0.002	0.92	30.0	42.0	0.2	4.7	3.37	< 0.001	1.02
% reference taxa found in reference sites that occurred in impaired sites	55.6	81.8	46.7	64.4	2.65	0.008	0.19	69.4	85.4	34.3	59.3	2.52	0.012	0.19	75.0	82.1	12.5	29.6	3.60	< 0.001	0.54
% reference individuals found in reference sites that occurred in impaired sites	53.0	95.8	12.4	84.0	2.72	0.006	0.26	89.4	93.8	12.4	76.6	1.95	0.051	0.30	87.0	95.0	6.6	27.8	3.05	0.002	0.66
No. of distinct reference taxa	0.0	1.0	0.0	0.0	3.73	< 0.001	1.63	5.0	6.5	0.0	2.0	3.63	< 0.001	2.17	2.0	3.0	0.0	0.0	3.79	< 0.001	1.40

Table S4.4a (contd.) Candidate metrics of MMI-1 – MMI-3 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included. ( ) Metric not used in the development of the MMI.

	MMI-1				|Z|	P	CV	MMI-2				|Z|	P	CV	MMI-3				|Z|	P	CV
	Reference		Disturbed					Reference		Disturbed					Reference		Disturbed				
Category/Candidate metric	Min	Median	Min	Median				Min	Median	Min	Median				Min	Median	Min	Median			
Functional group																					
Mobile % taxa	70.0	94.4	17.1	85.2	2.71	0.007	0.17	91.7	94.7	17.1	85.2	2.39	0.017	0.20	88.2	96.9	17.1	85.2	2.64	0.008	0.19
Mobile % individuals	55.0	98.6	27.0	91.6	2.03	0.042	0.17	92.8	98.3	27.0	91.6	1.22	0.224	0.19	96.6	99.5	27.0	91.6	2.39	0.017	0.18
Pioneer % taxa	0.0	2.8	0.0	3.7	0.39	0.699	1.01	0.0	2.8	0.0	3.7	0.86	0.389	0.99	0.0	0.0	0.0	3.7	1.64	0.101	1.16
Pioneer % individuals	0.0	0.8	0.0	0.6	0.18	0.86	1.68	0.0	5.2	0.0	0.6	0.21	0.838	1.82	0.0	0.0	0.0	0.6	2.10	0.036	2.07
Adnate % taxa	0.0	10.0	0.0	6.9	0.68	0.499	0.59	2.8	6.7	0.0	6.9	0.81	0.417	0.59	10.0	14.6	0.0	6.9	2.32	0.020	0.53
Adnate % individuals	0.0	9.6	0.0	3.8	0.43	0.664	1.47	2.0	4.5	0.0	3.8	0.28	0.776	1.57	9.6	23.7	0.0	3.8	2.10	0.036	1.31
Pad (attached to substrate) % taxa	0.0	0.0	0.0	11.8	3.38	< 0.001	1.04	0.0	4.7	0.0	11.8	2.27	0.023	0.80	0.0	0.0	0.0	11.8	3.27	0.001	1.00
Pad (attached to substrate) % indiv.	0.0	0.0	0.0	8.2	2.96	0.003	1.71	0.0	1.0	0.0	8.2	1.79	0.074	1.46	0.0	0.0	0.0	8.2	3.27	0.001	1.58
Stalk (attached to substrate) % taxa	0.0	20.0	0.0	11.1	1.25	0.211	0.99	19.4	21.1	0.0	11.1	2.27	0.023	0.80	0.0	0.0	0.0	11.1	2.27	0.023	1.06
Stalk (attached to substrate) %indiv.	0.0	9.4	0.0	4.2	0.04	0.972	1.38	8.4	13.9	0.0	4.2	0.89	0.372	1.40	0.0	0.0	0.0	4.2	2.94	0.003	1.75
Colonial % taxa	0.0	5.9	0.0	10.7	0.99	0.321	0.93	0.0	1.9	0.0	10.7	2.03	0.042	0.71	0.0	0.0	0.0	10.7	2.43	0.015	0.78
Colonial % individuals	0.0	0.6	0.0	5.8	2.14	0.032	1.87	0.0	0.7	0.0	5.8	1.79	0.073	1.49	0.0	0.0	0.0	5.8	2.37	0.018	1.56
Non-colonial % taxa	60.0	94.1	81.0	89.3	0.99	0.321	0.10	91.7	98.1	81.0	89.3	2.03	0.042	0.07	90.9	100.0	81.0	89.3	2.43	0.015	0.07
Non-colonial % individuals	34.0	99.4	49.8	94.2	2.14	0.032	0.16	98.6	99.3	49.8	94.2	1.79	0.073	0.13	96.6	100.0	49.8	94.2	2.37	0.018	0.12
Ribbon % taxa	0.0	0.0	0.0	5.9	1.40	0.163	1.08	0.0	1.9	0.0	5.9	1.51	0.131	0.90	0.0	0.0	0.0	5.9	2.73	0.006	1.10
Ribbon % individuals	0.0	0.0	0.0	2.0	2.16	0.031	1.79	0.0	0.5	0.0	2.0	1.39	0.165	1.53	0.0	0.0	0.0	2.0	2.73	0.006	1.67
High profile guild % taxa	0.0	13.9	0.0	13.8	0.04	0.972	0.94	10.0	19.5	0.0	13.8	0.77	0.441	0.61	0.0	0.0	0.0	13.8	3.46	< 0.001	0.94
High profile guild % individuals	0.0	6.0	0.0	4.2	1.01	0.313	1.52	6.6	8.3	0.0	4.2	1.01	0.310	1.05	0.0	0.0	0.0	4.2	3.46	< 0.001	1.45

Table S4.4a (contd.) Candidate metrics of MMI-1 – MMI-3 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included. ( ) Metric not used in the development of the MMI.

	MMI-1				|Z|	P	CV	MMI-2				|Z|	P	CV	MMI-3				|Z|	P	CV
	Reference		Disturbed					Reference		Disturbed					Reference		Disturbed				
Category/Candidate metric	Min	Median	Min	Median				Min	Median	Min	Median				Min	Median	Min	Median			
Functional group																					
Low profile guild % taxa	0.0	13.6	5.3	17.6	1.53	0.127	0.47	5.6	15.9	5.3	17.6	0.93	0.351	0.41	0.0	10.9	5.3	17.6	1.43	0.152	0.47
Low profile guild % indiv.	0.0	26.0	0.6	34.6	0.10	0.917	0.87	4.4	13.2	0.6	34.6	0.85	0.394	0.91	0.0	23.0	0.6	34.6	0.38	0.703	0.87
Motile guild % taxa	0.0	63.9	35.3	58.8	0.64	0.521	0.36	50.0	62.5	35.3	58.8	0.57	0.570	0.22	75.0	79.2	35.3	58.8	3.37	< 0.001	0.27
Motile guild % individuals	0.0	65.2	4.0	26.8	0.17	0.862	0.73	66.2	70.0	4.0	26.8	1.66	0.096	0.65	13.2	74.6	4.0	26.8	1.40	0.162	0.67
Planktonic guild % taxa	0.0	5.6	0.0	6.7	1.20	0.23	0.80	0.0	5.3	0.0	6.7	1.34	0.180	0.71	0.0	3.2	0.0	6.7	1.21	0.225	0.77
Planktonic guild % individuals	0.0	1.0	0.0	1.2	0.80	0.423	1.79	0.0	1.3	0.0	1.2	0.28	0.776	1.69	0.0	0.5	0.0	1.2	1.31	0.190	1.84
Taxonomic composition																					
% Achnanthidium taxa	0.0	5.6	0.0	6.3	0.40	0.689	0.75	5.0	5.6	0.0	6.3	0.08	0.935	0.70	0.0	0.0	0.0	6.3	1.64	0.102	0.90
% Achnanthidium individuals	0.0	1.4	0.0	1.2	0.14	0.889	1.65	1.4	5.9	0.0	1.2	0.77	0.440	1.77	0.0	0.0	0.0	1.2	2.51	0.012	2.02
% Cocconeis taxa	0.0	0.0	0.0	2.9	1.74	0.081	1.41	0.0	0.0	0.0	2.9	1.31	0.191	1.15	0.0	0.0	0.0	2.9	2.03	0.043	1.32
% Cocconeis individuals	0.0	0.0	0.0	0.2	1.62	0.106	3.53	0.0	0.0	0.0	0.2	1.10	0.271	3.37	0.0	0.0	0.0	0.2	2.03	0.042	3.57
% Craticula taxa	0.0	5.6	0.0	3.7	0.96	0.336	0.99	2.8	4.7	0.0	3.7	0.69	0.487	1.08	8.3	10.9	0.0	3.7	2.63	0.009	0.91
% Craticula individuals	0.0	0.8	0.0	0.4	0.74	0.462	1.51	2.0	5.1	0.0	0.4	2.13	0.034	1.44	0.2	0.7	0.0	0.4	0.74	0.462	1.71
% Ctenophora taxa	0.0	0.0	0.0	2.9	2.90	0.004	1.64	0.0	0.0	0.0	2.9	1.71	0.088	1.22	0.0	0.0	0.0	2.9	2.03	0.042	1.31
% Ctenophora individuals	0.0	0.0	0.0	0.2	2.90	0.004	2.44	0.0	0.0	0.0	0.2	1.71	0.088	1.94	0.0	0.0	0.0	0.2	1.05	0.294	1.59
% Cyclotella taxa	0.0	0.0	0.0	2.9	1.39	0.164	1.18	0.0	1.4	0.0	2.9	0.66	0.507	1.02	0.0	0.0	0.0	2.9	0.72	0.471	1.08
% Cyclotella individuals	0.0	0.0	0.0	0.2	1.03	0.303	2.02	0.0	0.4	0.0	0.2	0.21	0.836	1.85	0.0	0.0	0.0	0.2	1.05	0.294	1.59
% Encyonopsis taxa	0.0	0.0	0.0	3.3	2.90	0.004	1.79	0.0	0.0	0.0	3.3	1.31	0.191	1.28	0.0	0.0	0.0	3.3	2.03	0.043	1.45

Table S4.4a (contd.) Candidate metrics of MMI-1 – MMI-3 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included. ( ) Metric not used in the development of the MMI.

	MMI-1				|Z|	P	CV	MMI-2				|Z|	P	CV	MMI-3				|Z|	P	CV
	Reference		Disturbed					Reference		Disturbed					Reference		Disturbed				
Category/Candidate metric	Min	Median	Min	Median				Min	Median	Min	Median				Min	Median	Min	Median			
% Encyonopsis individuals	0.0	0.0	0.0	0.2	2.90	0.004	3.48	0.0	0.0	0.0	0.2	1.01	0.312	2.81	0.0	0.0	0.0	0.2	2.03	0.042	2.96
% Eolimna taxa								2.8	3.3	0.0	0.0	1.90	0.057	1.52							
% Eolimna individuals								1.4	24.4	0.0	0.0	3.20	0.001	2.61							
% Epithemia taxa								0.0	1.4	0.0	0.0	0.53	0.598	1.78							
% Epithemia individuals								0.0	0.1	0.0	0.0	0.66	0.509	3.22							
% Fistulifera taxa															0.0	3.2	0.0	0.0	1.65	0.098	1.91
% Fistulifera individuals															0.0	1.8	0.0	0.0	1.76	0.078	2.34
% Fragilaria taxa								0.0	1.4	0.0	0.0	0.43	0.670	1.46							
% Fragilaria individuals								0.0	0.1	0.0	0.0	0.13	0.898	2.24							
% Gomphonema taxa	0.0	13.9	0.0	3.4	1.36	0.174	1.41	10.0	15.3	0.0	3.4	2.38	0.017	1.21	0.0	0.0	0.0	3.4	2.20	0.028	1.81
% Gomphonema individuals	0.0	2.8	0.0	0.4	1.41	0.158	1.80	5.2	7.8	0.0	0.4	2.95	0.003	1.60	0.0	0.0	0.0	0.4	2.20	0.028	2.14
% Halamphora taxa	0.0	5.0	0.0	4.8	0.66	0.508	0.74	2.8	3.3	0.0	4.8	0.65	0.515	0.75	0.0	7.3	0.0	4.8	1.60	0.110	0.73
% Halamphora individuals	0.0	6.2	0.0	1.6	0.64	0.52	1.61	1.4	4.5	0.0	1.6	0.85	0.393	1.72	0.0	22.8	0.0	1.6	1.50	0.134	1.45
% Mayamaea taxa								0.0	3.3	0.0	0.0	1.94	0.053	2.16	0.0	3.2	0.0	0.0	1.51	0.132	2.14
% Mayamaea individuals								0.0	0.2	0.0	0.0	1.80	0.071	4.07	0.0	0.3	0.0	0.0	1.40	0.163	3.43
% Navicula taxa	0.0	7.7	0.0	10.5	0.80	0.425	0.58	5.0	6.7	0.0	10.5	1.18	0.239	0.55	6.3	12.2	0.0	10.5	1.27	0.203	0.50
% Navicula individuals	0.0	3.0	0.0	1.8	0.28	0.781	1.87	0.8	1.2	0.0	1.8	0.24	0.807	1.90	3.0	5.0	0.0	1.8	1.50	0.135	1.66
% Nitzschia taxa	0.0	30.6	13.3	29.4	0.00	1.000	0.41	19.2	30.6	13.3	29.4	0.41	0.685	0.29	27.3	37.7	13.3	29.4	1.94	0.052	0.33

Table S4.4a (contd.) Candidate metrics of MMI-1 – MMI-3 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included. ( ) Metric not used in the development of the MMI.

	MMI-1				|Z|	P	CV	MMI-2				|Z|	P	CV	MMI-3				|Z|	P	CV
	Reference		Disturbed					Reference		Disturbed					Reference		Disturbed				
Category/Candidate metric	Min	Median	Min	Median				Min	Median	Min	Median				Min	Median	Min	Median			
% Nitzschia individuals	0.0	26.4	1.8	12.8	0.23	0.822	0.99	24.4	32.5	1.8	12.8	1.14	0.256	0.93	6.4	40.6	1.8	12.8	1.31	0.192	0.92
% Pinnularia taxa								0.0	1.4	0.0	0.0	0.90	0.369	1.87							
% Pinnularia individuals								0.0	0.1	0.0	0.0	0.90	0.368	2.38							
% Planothidium taxa								0.0	0.2	0.0	0.0	0.62	0.538	1.68							
% Planothidium individuals								0.0	1.4	0.0	0.0	0.35	0.725	1.57							
% Pseudostaurosira taxa								0.0	1.4	0.0	0.0	0.96	0.340	2.05							
% Pseudostaurosira individuals								0.0	0.2	0.0	0.0	0.96	0.340	2.92							
% Rhopalodia taxa															0.0	6.1	0.0	0.0	2.78	0.005	2.17
% Rhopalodia individuals															0.0	0.9	0.0	0.0	2.82	0.005	3.27
% Sellaphora taxa								0.0	1.0	0.0	0.0	1.77	0.076	2.66							
% Sellaphora individuals								0.0	3.9	0.0	0.0	1.34	0.180	1.36							
% Stauroneis taxa								0.0	1.4	0.0	0.0	2.20	0.028	3.35							
% Stauroneis individuals								0.0	0.1	0.0	0.0	2.20	0.028	4.27							

Table S4.4b Candidate metrics of MMI-4 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included.

	MMI-4				|Z|	P	CV
	Reference		Disturbed				
Category/Candidate metric	Min	Median	Min	Median			
Diversity							
Hurlbert's evenness (EHurlbert)	0.1	0.4	0.2	0.5	0.89	0.374	0.41
Species richness (S)	9.0	11.0	12.0	19.0	1.64	0.102	0.41
Shannon diversity (H)	0.3	0.9	0.7	1.6	1.10	0.271	0.45
% dominance	21.4	79.8	26.0	60.4	1.17	0.241	0.39
Similarity to reference sites							
% reference taxa	13.6	23.1	0.0	3.4	3.34	< 0.001	1.36
% tolerant taxa	0.0	0.0	0.0	11.8	2.68	0.007	0.93
% reference individuals	24.6	82.4	0.0	0.6	2.83	0.005	1.32
% tolerant individuals	0.0	0.0	0.0	2.6	2.61	0.009	1.88
% similarity to reference sites	24.3	66.4	0.8	3.7	3.02	0.003	1.14
% reference taxa found in reference sites that occurred in impaired sites	55.6	61.5	18.2	31.6	3.31	< 0.001	0.45
% reference individuals found in reference sites that occurred in impaired sites	53.0	95.8	6.0	47.2	2.67	0.008	0.62
No. of distinct reference taxa	2.0	4.0	0.0	1.0	3.31	< 0.001	1.15
Functional group							
Mobile % taxa	70.0	88.5	17.1	85.2	0.50	0.619	0.20
Mobile % individuals	55.0	96.6	27.0	91.6	0.43	0.67	0.20
Pioneer % taxa	3.8	9.1	0.0	3.7	1.75	0.08	0.85
Pioneer % individuals	0.8	79.8	0.0	0.6	2.47	0.014	1.37
Adnate % taxa	0.0	9.1	0.0	6.9	0.50	0.619	0.66
Adnate % individuals	0.0	0.4	0.0	3.8	1.71	0.088	1.71
Pad (attached to substrate) % taxa	0.0	7.7	0.0	11.8	1.25	0.213	0.78
Pad (attached to substrate) % individuals	0.0	3.0	0.0	8.2	0.82	0.413	1.36
Stalk (attached to substrate) % taxa	27.3	30.0	0.0	11.1	3.09	0.002	0.95
Stalk (attached to substrate) % individuals	25.4	94.0	0.0	4.2	2.56	0.01	1.16
Colonial % taxa	9.1	22.2	0.0	10.7	2.45	0.014	0.71

Table S4.4b (contd.) Candidate metrics of MMI-4 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included.

<table>
<tr><th></th><th colspan="4">MMI-4</th><th></th><th></th><th></th></tr>
<tr><th></th><th colspan="2">Reference</th><th colspan="2">Disturbed</th><th>|Z|</th><th>P</th><th>CV</th></tr>
<tr><th>Category/Candidate metric</th><th>Min</th><th>Median</th><th>Min</th><th>Median</th><th></th><th></th><th></th></tr>
<tr><td>Functional group</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Colonial % individuals</td><td>0.6</td><td>3.6</td><td>0.0</td><td>5.8</td><td>0.18</td><td>0.859</td><td>1.54</td></tr>
<tr><td>Non-colonial % taxa</td><td>60.0</td><td>77.8</td><td>81.0</td><td>89.3</td><td>2.45</td><td>0.014</td><td>0.10</td></tr>
<tr><td>Non-colonial % individuals</td><td>34.0</td><td>96.4</td><td>49.8</td><td>94.2</td><td>0.18</td><td>0.859</td><td>0.18</td></tr>
<tr><td>Ribbon % taxa</td><td>0.0</td><td>9.1</td><td>0.0</td><td>5.9</td><td>1.50</td><td>0.134</td><td>0.82</td></tr>
<tr><td>Ribbon % individuals</td><td>0.0</td><td>0.6</td><td>0.0</td><td>2.0</td><td>0.14</td><td>0.886</td><td>1.44</td></tr>
<tr><td>High profile guild % taxa</td><td>36.4</td><td>50.0</td><td>0.0</td><td>13.8</td><td>3.09</td><td>0.002</td><td>0.77</td></tr>
<tr><td>High profile guild % individuals</td><td>1.4</td><td>7.8</td><td>0.0</td><td>4.2</td><td>0.89</td><td>0.374</td><td>1.40</td></tr>
<tr><td>Low profile guild % taxa</td><td>3.8</td><td>13.6</td><td>5.3</td><td>17.6</td><td>0.71</td><td>0.477</td><td>0.43</td></tr>
<tr><td>Low profile guild % individuals</td><td>0.8</td><td>79.8</td><td>0.6</td><td>34.6</td><td>1.39</td><td>0.166</td><td>0.78</td></tr>
<tr><td>Motile guild % taxa</td><td>0.0</td><td>30.8</td><td>35.3</td><td>58.8</td><td>2.95</td><td>0.003</td><td>0.37</td></tr>
<tr><td>Motile guild % individuals</td><td>0.0</td><td>3.0</td><td>4.0</td><td>26.8</td><td>2.67</td><td>0.008</td><td>0.90</td></tr>
<tr><td>Planktonic guild % taxa</td><td>0.0</td><td>7.7</td><td>0.0</td><td>6.7</td><td>0.07</td><td>0.943</td><td>0.72</td></tr>
<tr><td>Planktonic guild % individuals</td><td>0.0</td><td>1.6</td><td>0.0</td><td>1.2</td><td>0.07</td><td>0.943</td><td>1.60</td></tr>
<tr><td>Taxonomic composition</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>% Achnanthidium taxa</td><td>3.8</td><td>9.1</td><td>0.0</td><td>6.3</td><td>0.93</td><td>0.355</td><td>0.68</td></tr>
<tr><td>% Achnanthidium individuals</td><td>0.8</td><td>79.8</td><td>0.0</td><td>1.2</td><td>2.38</td><td>0.017</td><td>1.35</td></tr>
<tr><td>% Cocconeis taxa</td><td>0.0</td><td>0.0</td><td>0.0</td><td>2.9</td><td>0.11</td><td>0.912</td><td>1.13</td></tr>
<tr><td>% Cocconeis individuals</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.2</td><td>0.04</td><td>0.971</td><td>2.97</td></tr>
<tr><td>% Craticula taxa</td><td>0.0</td><td>0.0</td><td>0.0</td><td>3.7</td><td>1.60</td><td>0.109</td><td>1.36</td></tr>
<tr><td>% Craticula individuals</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.4</td><td>1.20</td><td>0.229</td><td>1.84</td></tr>
<tr><td>% Ctenophora taxa</td><td>0.0</td><td>0.0</td><td>0.0</td><td>2.9</td><td>1.88</td><td>0.06</td><td>1.27</td></tr>
<tr><td>% Ctenophora individuals</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.2</td><td>1.88</td><td>0.06</td><td>1.99</td></tr>
<tr><td>% Cyclotella taxa</td><td>0.0</td><td>0.0</td><td>0.0</td><td>2.9</td><td>1.43</td><td>0.152</td><td>1.13</td></tr>
<tr><td>% Cyclotella individuals</td><td>0.0</td><td>0.0</td><td>0.0</td><td>0.2</td><td>1.10</td><td>0.27</td><td>1.57</td></tr>
</table>

Table S4.4b (contd.) Candidate metrics of MMI-4 showing the minimum and median values at reference and disturbed sites and evaluations of separation between reference and disturbed sites. |Z| = Mann-Whitney absolute value of Z statistic. P = significance of Z test. CV = coefficient of variation. Metrics with median values of zero for both reference and disturbed sites are not included.

	MMI-4						
	Reference		Disturbed		|Z|	P	CV
Category/Candidate metric	Min	Median	Min	Median			
Taxonomic composition							
% Encyonema taxa	0.0	3.8	0.0	0.0	2.15	0.031	2.28
% Encyonema individuals	0.0	0.2	0.0	0.0	2.15	0.031	4.59
% Encyonopsis taxa	0.0	0.0	0.0	3.3	1.88	0.06	1.40
% Encyonopsis individuals	0.0	0.0	0.0	0.2	1.88	0.06	2.89
% Eunotia taxa	0.0	4.5	0.0	0.0	2.15	0.031	2.10
% Eunotia individuals	0.0	0.2	0.0	0.0	1.90	0.058	3.20
% Gomphonema taxa	18.2	22.7	0.0	3.4	3.05	0.002	1.36
% Gomphonema individuals	0.8	4.8	0.0	0.4	2.66	0.008	1.99
% Halamphora taxa	0.0	4.5	0.0	4.8	0.14	0.886	0.82
% Halamphora individuals	0.0	0.4	0.0	1.6	1.11	0.267	1.89
% Navicula taxa	0.0	4.5	0.0	10.5	2.03	0.043	0.65
% Navicula individuals	0.0	0.2	0.0	1.8	2.03	0.042	2.06
% Nitzschia taxa	0.0	18.2	13.3	29.4	2.52	0.012	0.41
% Nitzschia individuals	0.0	2.0	1.8	12.8	2.92	0.004	1.24