Division of Science and Engineering
Department of Environmental Biology

Diatoms and Invertebrates as Indicators of pH in Wetlands of the South-west of Western Australia

Erin J Thomas

This thesis is presented for the Degree of
Doctor of Philosophy
of
Curtin University of Technology

November 2007

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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Date: 22 November 2007

Acknowledgements

Abstract

Increased groundwater usage, rainfall decline and activities such as mining have resulted in the acidification of certain wetlands in the south-west of Western Australia. This study investigated the influence of pH, the factor most commonly associated with acidification, on the invertebrate and diatom community structure of 20 wetlands in the south-west region of Western Australia. Few studies in Western Australia have investigated both biotic groups, particularly in relation to pH. Consequently, this study examined the comparative sensitivity of the two biotic groups to pH in order to identify the most effective biotic tool for assessing the ecological impacts of pH decrease.

The wetlands included in this study displayed a wide range of pH from acidic (pH < 6.5) to alkaline (pH > 7.5). Other environmental parameters were also variable. Separation of the wetlands into three pH groups; Group 1 – acidic, Group 2 – circumneutral and Group 3 – alkaline, demonstrated that the acidic Group 1 wetlands generally had higher electrical conductivity than the remaining groups. This was probably due to the association of many Group 1 sites with mining and acid sulphate soils. Seasonal trends in environmental variables across the three pH groups were mostly unclear although some trends were evident within the individual pH groups.

The study showed that invertebrate community structure differed in response to pH. However, the results also demonstrated that invertebrate distribution patterns were influenced by other factors. Potential indicator species identified from the study included *Macrothrix indistincta* and *Tanytarsus fuscithorax/semibarbitarsus* which were abundant in acidic waters and *Alona quadrangularis* which was common in circumneutral sites. Taxa such as *Calamoecia tasmanica subattenuata* were common over a wider range of pH (acidic to circumneutral) but may still have potential to act as indicators of pH decline.

Diatom community structure was also shown to be influenced by pH, with the variable identified as a major determinant of diatom distribution patterns. *Nitzschia paleaeformis* and *Navicula* aff. *cari* were generally recorded from acidic wetlands and are potentially useful as indicators of low pH conditions. *Brachysira brebissonii*

and *Frustulia magaliesmontana* were also identified as species with the potential to indicate pH decline. In contrast, taxa including *Gomphonema parvulum*, *Staurosira construens* var. *venter* and *Nitzschia palea* were generally associated with moderate to high pH levels.

A comparative study of the two biotic groups using multivariate analyses revealed that diatoms were more sensitive to pH than invertebrates. Further investigation with a larger number of environmental variables would be necessary to ascertain the other factors primarily influencing invertebrate community structure. Nonetheless, the findings imply that diatoms and invertebrates differ in their responsiveness to various environmental factors and may provide complementary information on the integrity of a system.

Multivariate analyses on an expanded data-set of 40 sites found that pH accounted for the greatest amount of variation in the data and was conducive to the development of a diatom-based pH inference model. The strongest model was produced using weighted averaging (WA) with classical deshrinking. While the model displayed a high correlation coefficient, the prediction error was also relatively high, probably as a result of the comparatively small and heterogeneous data-set. Incorporation of the data into a larger training set would be likely to improve the predictive ability. Applications for the model include pH reconstructions or use in monitoring programs.

The current study has shown that pH is an important variable influencing both invertebrate and diatom community structure in wetlands in the south-west of Western Australia. However, the greater sensitivity of diatoms to pH suggests that they would be the most effective tool for the biological monitoring of pH in wetlands threatened or impacted by acidification. An integrated monitoring program including both diatoms and invertebrates may provide additional information on the impacts of pH decline and the overall integrity of the systems and should be investigated further.

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Chapter 1: Introduction

1.1   Scope of thesis

Acidification is a growing problem for wetlands in the south-west of Western Australia (EPA 2006), especially in the context of recent climate change (Smol and Douglas 2007). Among the potential impacts are major ecological changes such as decreased biodiversity (EPA 2006). The process is of particular concern as many wetlands in the south-west region have already been lost or degraded (Halse 1989; Davis and Froend 1999), thereby highlighting the conservation value of remaining wetlands.

The acidification of wetlands in the region has been primarily linked to the exposure and oxidation of acid sulphate soils resulting from lowered water tables (McHugh 2004; DoE 2005). The decline in water levels has been attributed to factors such as dewatering, groundwater extraction and patterns of reduced rainfall (Appleyard *et al.* 2004; EPA 2006). The temperature increases and continued rainfall decline predicted by climate change models (CSIRO 2001) are likely to result in more prolonged exposure of acidic materials in the future and lead to a greater number of acidified water bodies. Other potential sources of acidification in Western Australia are acidic groundwater discharge in agricultural areas (Davis 2004; Smith *et al.* 2004; Rogers and George 2005) and the use of ammonium based fertilisers (Helyar 1991). Mining may also contribute to the contamination of waters in the region (McCullough and Lund 2006).

The threat of acidification to wetlands in the south-west of Western Australia has created an urgent need to identify effective tools for assessing the ecological impacts of pH. This thesis aims to address the issue by focusing on the use of invertebrates and diatoms as biological monitors. The study investigates the distribution of the two groups of biota in relation to pH and evaluates their relative merits as potential indicators of acidification in the south-west of Western Australia.

1.2 Thesis structure

This thesis is presented in eight chapters. The current chapter provides a background to the study with a literature review on relevant topics including the relationship between pH and acidity, acidification of water bodies, the sensitivity of biota to pH and the use of diatoms and invertebrates as biological monitors. The justification for the study and the objectives of the research has also been included in this chapter. Chapter 2 describes the environmental conditions of the areas included in the study. The chapter also provides a description of the pH groupings used to classify the study sites and gives general outline of the sites contained in each pH group. Chapter 3 examines the environmental variables of the selected wetlands with particular reference to pH.

Chapter 4 explores the distribution of invertebrates in relation to environmental variables with an emphasis on pH and discusses the sensitivity of various invertebrates to pH. Chapter 5 analyses the distribution of diatoms in relation to environmental variables, specifically pH and outlines potential indicator species. Chapter 6 compares the sensitivity of the two biotic groups to pH and assesses their relative potential as biological monitors of pH change. Chapter 7 focuses on the development of a diatom-based pH inference model for wetlands of the south-west of Western Australia and examines the pH optima and tolerances of diatoms in the region. The final chapter outlines the major findings of the study, discusses limitations of the work and presents recommendations for future research.

1.3 Background

1.3.1 The relationship between pH, acidity and alkalinity

Acidity, alkalinity and pH are interrelated variables (Abel 1989) which are often used in reference to water quality. Acidity can be described as the total concentration of titratable acids expressed in milligrams per litre of equivalent calcium carbonate (Boyd 1996). It is considered a measurement of the base neutralisation capacity of a given volume of water. Three forms of acidity exist: an organic form associated with dissolved organic compounds, mineral acidity associated with dissolved metals and proton acidity associated with pH (Hedin *et al.* 1994). Alkalinity is a measure of the acid-neutralizing capacity of water (Patnaik 1997). The presence of bicarbonate

(HCO_3^-), carbonate (CO_3^{2-}) or hydroxide (OH^-) is generally responsible for the alkalinity of water (Tebbutt 1992), although dissolved ammonia and the anions of boric, phosphoric and silicic acids may also contribute (Baird 1999).

The pH is a measure of the hydrogen ion concentration (Vesilind 1975) and is used to indicate the intensity of the acid or alkaline state of a solution (Yen 1999). It is defined as the negative of the logarithm to the base 10 of the hydrogen ion concentration and is expressed as pH = -log10[H^+] (Campbell *et al.* 1999). The scale used to represent pH ranges from 0 – 14 (Tebbutt 1992) with a solution of pH 7 classified as neutral. Values lower than 7 indicate acidic conditions while higher values characterise an alkaline or basic state (Kennedy 1992; Anderton *et al.* 1996). Most natural waters have pH values between 5 and 10, with the greatest frequency falling between 6.5 and 9 (Boyd 1996).

Classifications of water bodies based on pH generally separate them into acidic, near neutral or circumneutral (Patrick *et al.* 1981) and alkaline. From a chemical standpoint, acidic waters have zero or negative alkalinity or a pH $\leq$ 5.5. Studies on aquatic organisms however, have shown that the biological effects of acidification begin around pH 6.5 (Psenner 1994), with a number of biota responding to changes in proton concentration between pH 6.5 and 6 (Psenner and Catalan 1994). Subsequently, the pH levels used to define the different pH categories often vary between studies. In their work on the classification of wetlands and deepwater habitats of the United States, Cowardin *et al.* (1979) defined waters with a pH < 5.5 as acidic, pH 5.5 - 7.4 as circumneutral and > 7.4 as alkaline. Fjellheim and Raddum's summary on Norwegian invertebrates and acidification (1990) also classified wetlands with pH < 5.5 as acidic but did not define other pH categories. Conversely, authors including Foged (1978), Stokes *et al.* (1989) and Herrmann *et al.* (1993) have defined acidic waters using a higher pH cut-off. For example, Foged's study of diatoms in Eastern Australia (1978) categorised waters with pH readings of < 6.5 as acidic and those with pH 6.5-7.5 as circumneutral. The alkaline grouping was similar to the Cowardin *et al.* (1979) classification with waters displaying a pH of 7.5 considered alkaline. The present study adopts a pH classification system adapted from Foged 1978.

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1.3.2 Acidification

Acidification is defined as an increase in hydrogen ions (H^+) and is generally expressed as the pH value of environmental media (United Nations 1997). The process affects both soils and waters (Howells 1995) and while it can occur naturally, it is often induced or accelerated by human activities (Patrick *et al.* 1981; Stokes *et al.* 1989). Waters acidified through anthropogenic practices are characterised by a low pH (4.5-5.5), low levels of organic acids, high transparency and increased levels of dissolved aluminium (Al) (Meybeck *et al.* 1989). The sensitivity of surface waters to acidification is largely dependant on the soils and underlying geology of the surrounding area (Thomas *et al.* 1992; Hornung *et al.* 1995). The ability of soils to neutralize acidity is reliant on processes such as mineral weathering, anion uptake by vegetation and exchangeable base cation retention (Essington 2004). Increases in the concentration of sulphate or nitrate anions in soil solution must be accompanied by an equivalent amount of cations to prevent acidity transport. If the supply of cations is limited, H^+ and Al species may fill the deficit and facilitate the transport of acidity from soil to drainage water (Reuss 1991). Therefore waters located in catchments with base-poor soils are at greater risk of acidifying than those located in catchments with high buffering capacities (Meybeck *et al.* 1992).

Human induced acidification of waters may result from direct inputs of acidic solutions either from point sources such as sewers or through diffuse sources such as the leaching of mine tailings. Alternatively, acidification may result from indirect sources such as atmospheric deposition (Meybeck *et al.* 1992). Acidic drainage associated with mining activities has affected water bodies in countries including South Africa (Naicker *et al.* 2003), Japan (Sasaki *et al.* 2005), China (Liu *et al.* 2003) and Spain (Olías *et al.* 2006). Agricultural practices have also been implicated in acidification with extensive drainage of sulphidic soils impacting surface waters in countries such as Finland (Joukainen and Yli-Halla 2003) and Vietnam (Brinkman 1982; Nguyen and Wilander 1995). Afforestation, particularly using conifers is a further land-use that has been linked with low pH waters (Meybeck *et al.* 1989).

Acidification associated with acidic deposition or the transfer of acids and acid forming substances from the atmosphere to the earth's surface (Laws 2000; Driscoll

et al. 2001), is a major issue for freshwaters (Thomas *et al.* 1992). Countries including Norway, Scotland, USA and Canada (Howells 1995) have all experienced acidification of surface waters related to acidic deposition and there is concern that parts of Asia may be susceptible in the future (Monteith and Evans 2005).

Activities such as the burning of fossil fuels and practices associated with agriculture are mostly responsible for the emission of acidifying compounds (Kelly 1979; Meybeck *et al.* 1989; Kennedy 1992; Driscoll *et al.* 2003). These compounds include H_2SO_4 derived from sulphur dioxide emissions (SO_2), nitric acid (HNO_3) from nitrogen oxides (Harrison 1990; Pawlowski 1997; Yen 1999) and ammonium (NH_4^+) (Schindler *et al.* 1985b; Howells 1995). Deposition can result in changes to the chemical composition of soils (Reuss and Walthall 1989) and acidification of surface waters (Alewell *et al.* 2001). In conjunction with decreases in pH and acid neutralizing capacity (ANC), impacted water bodies may also experience increases in inorganic monomeric Al concentrations (Chen and Driscoll 2004).

1.3.3 Acidification in Australia

Although acidic deposition causes considerable environmental damage in many countries, it is not a problem faced in Australia. Instead, the greater threat to Australian wetlands lies with acidity created through exposure of acid sulphate soils, mining (Boulton and Brock 1999) and agricultural practices (Australian State of the Environment Committee 2001). Nonetheless, the impacts of acidification from these various sources are often similar and valuable information can be gained from studies in other regions (Boulton and Brock 1999).

1.3.3.1 Acid sulphate soils

Sediments containing iron sulphides are commonly known as acid sulphate soils (ASS) (Indraratna *et al.* 2001). Unoxidised sulphidic materials are commonly known as potential ASS (Ward *et al.* 2004) while soils containing acid sulphate materials are defined as actual acid sulphate soils (Brinkman and Pons 1973).

Acid sulphate soils are produced when metal sulphides, predominantly iron pyrite (FeS_2), are formed in organically rich, water logged sediments (Sammut and Lines-Kelly 2000). These soils commonly occur in coastal regions in association with low

lying vegetated areas such as floodplains (Sammut *et al.* 1996) and brackish marshes (Brinkman and Pons 1973; Dent 1986). They are also known to occur inland and have been identified from peaty sediments associated with freshwater wetlands, mining deposits such as coal (DoE 2006) and non-tidal seepage and marsh areas in regions impacted by dryland salinity (Fitzpatrick *et al.* 1996).

The FeS_2 that accumulates in potential ASS is only stable under anaerobic conditions (Dent 1986). Extended periods of drought (White *et al.* 1997; Indraratna *et al.* 2001) and activities such as drainage, water and sand extraction, ploughing, excavation and construction may expose these materials to oxygen (MacDonald *et al.* 2002; Powell and Martens 2005). Upon exposure, the metal sulphides undergo an oxidation reaction which subsequently generates sulphuric acid (H_2SO_4) (Lin *et al.* 2004). The chemical reactions involved in the process are as follows (Singer and Stumm 1970):

$$FeS_2 + 7/2\ O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+$$

$$Fe^{2+} + 1/4O_2 + H^+ \rightarrow Fe^{3+} + 1/2H_2O$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH_3) + 3H^+$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15\ Fe^{2+} + 2SO_4^{2-} + 16H^+$$
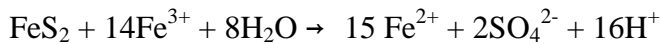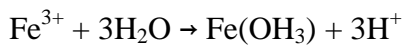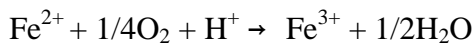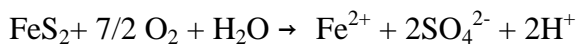
The release of dissolved ferrous iron (Fe^{2+}) and acidity follows the oxidation of the sulphide of the pyrite to sulphate (Stumm and Morgan 1981). The ferrous iron released by the oxidation process is further oxidised to ferric iron (Fe^{3+}). If the pH is suitably low, the oxidation is catalysed by iron-oxidising microorganisms (Moreno and Neretnieks 2006) such as *Thiobacillus ferrooxidans* and *Ferrobacillus ferrooxidans*. Fe^{3+} hydrolyses to form ferric hydroxide ($Fe(OH_3)$) and release further acidity (Stumm and Morgan 1981). Fe^{3+} can also be reduced by FeS_2, subsequently generating additional Fe^{2+} and acidity (Singer and Stumm 1970). The H_2SO_4 produced may lower the soil pH (van Breemen 1993), potentially to readings as low as 2 (Dent and Pons 1995). Moreover, as the acid produced moves through the soil it can break down the soil structure stripping metals such as Al and Mn (Sammut and Lines-Kelly 2000).

Environmental impacts associated with the production of acid include acidic scalds (Beavis *et al.* 2005), decreased crop yield (Lin and Melville 1994) and corrosion of infrastructure (Indraratna *et al.* 2001). Additionally, the translocation of oxidation products following the exposure of ASS has been identified as a major cause of acidification in receiving waters (Glamore and Indraratna 2004). Wetlands receiving acidic drainage have experienced decreased biodiversity (Powell and Martens 2005) and studies have also demonstrated a probable link between the presence of acid sulphate soils on floodplains, major rainfall events and seasonal fish mortalities (Callinan *et al.* 1993). Moreover, acidic drainage has been implicated in the increased incidence of the fish disease epizootic ulcerative syndrome (EUS) (Willett *et al.* 1993).

It has been estimated that there are over two million hectares of ASS in Australia. These soils have been identified from areas such as the coastal regions of New South Wales and Queensland (Sammut and Lines-Kelly 2000) while Western Australia accounts for more than 30% of the country's acid sulphate soils. Particular areas of concern in Western Australia include floodplains, damplands and estuarine regions along the south-west coast as well as tidal and intertidal flats in northern areas such as the Pilbara and Kimberley coasts (DoE 2006). The peat deposits associated with groundwater-dependent wetlands in the Perth Metropolitan Region are also of concern. Relatively high levels of oxidiseable sulphur are contained within these materials and could potentially cause major groundwater acidification (Appleyard *et al.* 2004). In the suburb of Stirling, disturbance of peaty wetland sediments during housing development has already been implicated in the acidification of local groundwater and excavated wetlands. Increased groundwater abstraction for irrigation and dewatering for development have lead to reductions in the water table. Moreover, the stock piling of large amounts of peat has resulted in the oxidation of sulphidic materials and the subsequent production of H_2SO_4 (WRC and DEP 2002). Groundwater pH values in the area have declined to readings as low as 1.9 and a pH of < 3 was recorded from a lake in the vicinity (Appleyard *et al.* 2004).

Patterns of decreasing rainfall (Smith *et al.* 2000; IOCI 2002) have also been linked to the increased incidence of drought induced acidification for wetlands in areas such as the Gnangara Mound, an area included in the current study (DoE 2005). Wetlands

such as Lake Jandabup and Lake Mariginiup have already undergone episodic acidification resulting from the drying and oxidation of acid sulphate sediments (McHugh 2004). Other sites in the region impacted by acidification include Lexia 86, 186a and b and Melaleuca Park (Clark and Horwitz 2004) with more wetlands considered to be at risk (DoE 2005).

The main cereal-growing region of Western Australia, commonly known as the Wheatbelt, is approximately defined as the area between the 300 mm and 600 mm average annual rainfall isohyets (Halse *et al.* 2003; Cale *et al.* 2004). ASS has been identified from saline inland areas and is a major concern for parts of the Wheatbelt impacted by secondary salinisation (DoE 2006). For example, saline sulphidic seeps have been documented from areas including the Blackwood Catchment near Darkin (Fitzpatrick *et al.* 2003). The formation of sulphuric materials in open drains constructed to manage salinity is a further concern, requiring increased research and planning (Fitzpatrick *et al.* 2005). The presence of acidic drainage in the region is of particular relevance because most of the receiving areas are alkaline in nature (Rogers and George 2005).

1.3.3.2 Agricultural Acidity

Acidification in agricultural systems is caused by processes that interfere with the natural cycling of carbon and nitrogen (Helyar and Porter 1989; NLWRA 2001). These processes decrease the soil pH through creation of residual H^+ ions (Robinson and Helyar 1996). Nitrate leaching is one factor that commonly contributes to the acidification of agricultural land (Helyar 1991; Fitzpatrick 2002). Nitrate is very soluble in water and can easily be lost through run-off or leaching below the root zone of plants (Helyar and Porter 1989; NLWRA 2001). The process may be accelerated through either the application of ammonium fertilisers or through the clearing of land (Helyar and Porter 1989; Dolling *et al.* 2001). If newly established plants cannot utilise available nitrate following clearing, severe acidification may ensue as a result of nitrate leaching (Helyar and Porter 1989). Moreover, crops planted in acidic soils are not able to use fertilisers efficiently and may subsequently require higher concentrations (Archer 2001). The addition of fertiliser can be problematic though, as the use of certain products may compound the acidity (Reuss and Walthall 1989; AACM International 1995; Moody and Aitken 1997; Dolling *et*

al. 2001). In particular, the nitrification of ammonium (NH_4) based fertilisers is known to produce soil acidity (Helyar 1991; Kennedy 1992; Pawlowski 1997).

Aside from the terrestrial impacts of agricultural acidity, a further issue is the potential degradation of receiving environments. The Australian State of the Environment report (2001) noted that surface water acidification can occur as a result of the acidification of soils in agricultural areas. The report found that increases in land acidification had been reflected in associated water bodies, with a positive relationship between soil and water acidity evident in some catchments (Australian State of the Environment Committee 2001). Given that between 12 and 24 million hectares of Australian soils are considered highly acidic (pH of 4.8 or less) with the possibility of that figure rising to as much as 60 million hectares if left untreated (NLWRA 2001), surface waters in many catchments may be at risk.

Acidic groundwater is another potential threat to surface waters in the agricultural region of Western Australia. Groundwaters of this nature are widespread in the Wheatbelt and have been proposed as a major source of off-site risk to receiving environments (Rogers and George 2005). Research suggests that the acidity is generally caused by ferrolysis or hydrolysis of Fe (Mann 1983; McArthur *et al.* 1991). During this process reduced Fe in the groundwater undergoes oxidation following exposure to oxygen in the atmosphere or infiltrating waters, producing H^+ and iron oxyhydroxide precipitates (Douglas and Degens 2006). Installation of drains to treat waterlogging in salinised areas may facilitate the transport of acidic groundwater (Smith *et al.* 2004) and could potentially lead to the acidification of receiving waters (Davis 2004). Moreover, the resulting acidification of previously alkaline or neutral water bodies in the region is likely to substantially alter the community structure of the associated biota (Halse *et al.* 2003).

1.3.3.3 Mining

Mining disturbs the land (Brown 1997) and creates an increased risk of pollutants being released into the air, land and water (Australian Bureau of Statistics 2003). The effects of mining on surface water quality typically result from acid mine drainage, release of effluent from milling and waste heaps and the leaching of substances from ore and waste rock (Johnson 1997).

Acid Mine Drainage (AMD) or Acid Rock Drainage (ARD) is produced when mining activities expose sulphidic materials to oxygen and water (Parker and Robertson 1999). Along with the production of H_2SO_4 (Ripley *et al.* 1996), the input of an oxidant into a sulphidic system causes mobilising reactions such as a decrease in pH and increased mobility of heavy metals (Wisotzky 2000; Klapper and Geller 2001). Sulphide oxidation can occur naturally, taking place slowly as the earth erodes. During mining however, this process is greatly accelerated as waste rock, overburden and tailings are exposed to air (Harries 1997). Sulphides are present in materials such as coal and lignite deposits, metallic ores, black shales and overburden sediments (Wisotzky 1998, 2000). Pyrite (FeS_2) is a common example of a sulphide present in overburden materials (Brown 1997). During a two to three month average exposure up to 10% of the pyritic sulphur content in the material will be oxidised, with equivalent amounts of SO_4, Fe^{2+} and H^+ ions being produced (Wisotzky 1994 cited in van Berk and Wisotzky 1995).

Although any sulphide containing material may potentially be a source of acid mine drainage, some types of mining are more prone to the problem (Kelly 1988). Coal mining for example, is a common source of acid mine drainage (Hustwit *et al.* 1992). The mining process promotes the oxidation of pyrites and results in drainage containing high concentrations of Al, Fe and Mn (Hedin *et al.* 1994). In addition, the drainage produced by the pyrite oxidation is highly acidic with values from pH 2 (Evangelou 1998). Potential sources of transport for the drainage include runoff from open pits, leachate from waste rock dumps, ore stock piles and mill tailings. It may also be exported as effluent and spent ore from heap leach operations (Johnson 1997).

While any pollution can be harmful to the environment, mine drainage differs from most industrial pollution in that it can continue indefinitely, even after the cessation of mining (Parker and Robertson 1999). It has been identified as a major issue in Australia with some waterways severely impacted by previous mining operations (Sheehy and Dickie 2002). The release of acidic leachate from the abandoned Captain's Flat mining area near Canberra into the Molonglo River is one well known example (Hore-Lacy 1992). In Tasmania, the Queen and King Rivers have been adversely affected by acidic drainage generated from the Mt Lyell copper mine

(Klessa *et al.* 1997; Koehnken 1997). The abandoned Rum Jungle mine in the Northern Territory and Mt Morgan in Queensland are also among the mining operations that have caused substantial environment damage (Hamblin 2001).

In Western Australia, many of the mine sites are located in the arid to semi-arid regions and the low annual rainfall and extended droughts may lessen the risk of acid mine drainage. Nonetheless, the potential to affect native vegetation, local groundwater and streams remains (WRC 2000). In contrast, the generation of acidic waters is an important issue for the coal-mining industry in the higher rainfall region of the south-west and for a few discrete metalliferous mines (Johnson and Wright 2003).

There are approximately 1800 mine voids in Western Australia and more than 150 mines operating below the water table (Johnson and Wright 2003). In the Collie Basin in the state's south-west, voids have been created since the onset of open-cut coal-mining in 1943 (Stronach 1988). Following the cessation of open-cut mining and associated dewatering, abandoned voids are often left to fill with both groundwater and surface water (Doyle and Runnells 1997; Shevenell 2000). In the Collie Basin, one of the area included in the present study, this process has led to the formation of acidic void or pit lakes (Commander *et al.* 1994; CWAG 1996). The mining techniques used in the region have been implicated in the generation of acidity, with contact between waste rock, spoil and water potentially leading to solute leaching (Johnson and Wright 2003).

In regions such as Collie, which display relatively high rainfall and low evaporation, the flow of groundwater into void water bodies exceeds evaporation. Consequently, uncontaminated groundwater may be degraded as it passes through the lakes (McCullough and Lund 2006). This suggests that the risk to nearby waters is not confined to run-off from waste heaps and instead may also include contamination from the acidic void lakes.

1.3.4   The effect of pH on aquatic organisms

The pH of an aquatic ecosystem is an important water quality variable and drastic changes in this parameter have the potential to adversely affect aquatic organisms.

For example, the pH decline or acidification of a water body is generally accompanied by reductions in species diversity and overall productivity (Abel 1989; ANZECC and ARMCANZ 2000). Increased proportions of dominant taxa and reduced complexity of food webs can also occur (Hall *et al.* 1980). Changes such as these can result from direct or indirect effects of pH on organisms (Patrick 1948). The high H^+ concentrations present in low pH waters can directly impact organisms at a cellular level (Gross 2000). Organisms that possess gills or external structures with large surface areas for gas exchange are particularly vulnerable to H^+ toxicity (Havens 1993).

In contrast, heavy metal toxicity is an indirect effect of pH decline (Hall *et al.* 1980). Acidic waters often contain comparatively high concentrations of metals, attributable to the increased mobilization of cations such as Al in acidified watersheds and aquatic sediments (Hall *et al.* 1985; Schindler 1988; Harvey 1989; Cronan and Schofield 1990). This may result from the increased solubility of the cations under low pH conditions (Meybeck *et al.* 1992) or through their replacement by H^+ ions during cation exchange (Hall *et al.* 1980). The toxic effects of elevated metal concentrations, specifically Al, on biota in low pH conditions have been documented by studies including Baker and Schofield (1982), Johnson *et al.*, (1987), Havens and Heath (1989; 1990), Havens (1991), Vuori (1996) and Russell and Helmke (2002).

Organisms may also respond indirectly to pH change through shifts in the food web (Schindler *et al.* 1985a; Webster *et al.* 1992). For example Bendell and McNicol (1987) suggested that variation in macroinvertebrate community composition may have been partly correlated with changes in fish populations rather than the toxic effects of low pH. In addition, very acidic waters commonly contain fewer organisms than circumneutral systems, a factor which may lead to a limited supply of oxygen and carbon dioxide and further restrict the type of organisms inhabiting the low pH waters (Patrick 1948).

Although general trends have been described in the literature, different groups of organisms display varied responses to pH and related water chemistry changes. For example, species of bacteria, ciliates and rotifers are known to inhabit waters with low pH levels (pH <3) (Wollman *et al.* 2000). Conversely, shell bearing organisms

such as snails and clams are highly sensitive to acidification. It is possible that the depression of waters to pH < 5.2 may result in a lethal disturbance of the organism's Ca^{2+} metabolism (Fellenberg 2000). Other sedentary organisms such as plants are likely to experience stunted growth or possibly death under acidic conditions (Sammut and Lines-Kelly 2000). Amphibians are another group considered intolerant of low pH, particularly during their immature life stages (Harvey 1989).

While generally more tolerant than shelled animals, fish are still considered relatively sensitive to pH (Fellenberg 2000) and will move away from low pH waters, if possible (Sammut and Lines-Kelly 2000). The H^+ and Al ion species present at low pH interfere with Na-K ATPase in the gills, thereby disturbing regulation of plasma sodium and chloride, possibly resulting in the death of both larval and adult fish (Harvey 1989). Death may also result from asphyxiation following the deposition of aluminium oxide on the gill filaments as it precipitates out of solution in acidified waters (Thomas *et al.* 1992). There are some species of fish however including *Tribolodon hakonensis* (Satake *et al.* 1995) and *Perca flavescens* or yellow perch that are considered tolerant of acidic conditions (Bendell and McNicol 1987).

The taxa richness of zooplankton generally decreases in response to acidification (Stokes *et al.* 1989). Phytoplankton tend to follow a similar pattern, with acidified lakes mostly displaying lower species numbers than higher pH waters (Lydén and Grahn 1985; Mason 1991). Along with shifts in phytoplankton community structure, many acidified systems display an obvious increase in the biomass of filamentous green algae, either attached to substrates or floating in the littoral zone (Stokes *et al.* 1989). The green alga *Mougeotia* species in particular*,* is frequently recorded from low pH waters (Müller 1980; Havens and Heath 1990; Pillsbury and Lowe 1999; Greenwood and Lowe 2006).

As evidenced by various studies (Hall *et al.* 1980; Johnson *et al.* 1987; Webster *et al.* 1992), a decline in pH can adversely impact the organisms inhabiting aquatic systems. The wetlands in the south-west of Western Australia currently under threat of acidification such as those on the Gnangara Mound, would be likely to experience loss of taxa, increased dominance of fewer species and reductions in acid sensitive

taxa in response to pH change. The use of effective biological monitoring tools would assist in the early detection of ecological changes related to acidification.

1.3.5   Biological monitoring of aquatic environments

Biological monitoring can be defined as the observation of biotic responses to evaluate the suitability of a system for living organisms (Cairns Jnr. and Pratt 1993). Aquatic biota integrate the environmental effects of their habitats and are therefore able to reflect the total state of the ecosystem (Gower 1980; Lowe and Pan 1996). Consequently, while physical and chemical monitoring are still accepted as necessary components of water quality monitoring, in recent years there has been an emerging emphasis on the use of aquatic biota to assess conditions more directly (Norris and Norris 1995).

The ability of changes in biota to provide a continuous record of the conditions in a water body may reveal the occurrence of significant environmental changes that might otherwise not have been detected (Abel 1989; Cox 1991). In contrast, physical and chemical measurements can only provide information on the conditions that exist at the time of sampling (Rosenberg *et al.* 1986). Chemicals in water are known to fluctuate widely over short periods, making it likely that biologically important peak concentrations will be missed, regardless of frequent sampling (Abel 1989). A further concern when using physical and chemical monitoring alone is that detection may occur after the ecosystem has been substantially impacted (Cairns Jnr. *et al.* 1993). Additionally, there are many physical and chemical parameters that could be important determinants of ecosystem health, and measuring each one would be impractical. Conversely, biological indicators may respond to changes in physical and chemical conditions that have not been measured (Stevenson and Pan 1999). Ideally, a combination of physico-chemical monitoring and biomonitoring should be used (van Dam *et al.* 1998) as the analysis of both chemical and biological data may provide complementary information (Abel 1989).

The modern concept of biological monitoring was devised in the early twentieth century (Rosenberg and Resh 1993) and biological monitoring programs are now used in regions such as Australia (Humphrey *et al.* 1995; Smith *et al.* 1999; John 2000), the United States (McCormick and Stevenson 1998; Lane and Brown 2007),

South America (Marques and Barbosa 2001) and Europe (Raddum and Fjellheim 1995; Wright 1995; Sabater 2000; Poulícková *et al.* 2004).

A wide range of organisms including fish, macroinvertebrates, macrophytes, algae and microorganisms can be used as biological monitors (Schofield and Davies 1996) although macroinvertebrates and algae are the two most commonly recommended groups (Hellawell 1986; Rosenberg and Resh 1993). Environmental stresses are reflected differently by each group of biota (Schofield and Davies 1996) and the selection of indicators should be largely dependent on the aims of the study (Norris and Norris 1995). Indicators need to be appropriate to the particular situation (Stewart *et al.* 1999) as certain organisms are potentially more useful in regard to biomonitoring different environments (Hellawell 1986).

Organisms selected for biomonitoring programs should be present in abundance (Round 1991) and display a cosmopolitan distribution (Hellawell 1986). This allows the group to be utilised for monitoring over a wide area. However, indicator species should also have narrow and specific tolerances to environmental conditions (Johnson *et al.* 1993) and display sensitivity to local impacts (Pratt and Bowers 1999). It is therefore preferable that the organisms are sedentary or have only limited mobility (Abel 1989).

Organisms used for biomonitoring should ideally be unaffected by life-cycle stages that may leave periods of time where they are not present. Ease of identification is a further issue to consider when assessing the suitability of a group for biomonitoring. Ideal indicators should be easily identified and quantifiable (Cox 1991; Round 1991).

1.3.6   Macroinvertebrates as biomonitors

Fish, algae and macroinvertebrates are three of the groups frequently recommended for use in water quality assessment (Lenat and Barbour 1994; Chessman *et al.* 1999). However, in practice macroinvertebrates remain the most widely used (Hawkes 1979; Abel 1989; Norris and Norris 1995; Resh *et al.* 1995), possessing many of the characteristics necessary for indicator organisms (Abel 1989; Rosenberg and Resh 1993). They inhabit both lentic (still) and lotic (running) waters (Gooderham and Tsyrlin 2002) and occupy a variety of niches within those water bodies. Benthic

invertebrates are associated with bottom substrates including sediments, logs and debris. Other taxa favour open waters (Rosenberg and Resh 1993) and invertebrate grazers generally prefer vegetative cover (Boulton and Brock 1999).

The diversity of the group is one of the factors that contribute to their popularity as biomonitors (Cairns Jnr. and Pratt 1993). The wide range of organisms represented increases the probability of response to environmental change (Hellawell 1986; Abel 1989). Macroinvertebrates are ubiquitous and can therefore reflect environmental disturbances in different types of water bodies (Rosenberg and Resh 1993). The restricted mobility of many taxa justifies the use of the group in spatial analyses (Abel 1989) while their relatively long life-cycles (eg. months to years) allow for the assessment of temporal changes in community structure (Hellawell 1986; Cairns Jnr. *et al.* 1993).

Macroinvertebrates are able to integrate the effects of many environmental parameters (James 1979) and are considered sensitive to physico-chemical stresses (Dills and Rogers 1974). Moreover, the specific ecological tolerances or preferences of many invertebrates are known (Sládeček *et al.* 1982). For example, studies have investigated the association between macroinvertebrates and factors including salinity (Bunn and Davies 1992; Kefford *et al.* 2003), nutrient levels (Chambers *et al.* 2006; Smith *et al.* 2007) and mine pollution (Faith *et al.* 1995).

The sampling techniques used are relatively simple with well developed methodology, which allows for good qualitative samples. The availability of taxonomic keys for most groups is another advantage of using macroinvertebrates as biomonitors (Hellawell 1986; Abel 1989). In terms of biomass and number, macroinvertebrates make up a large proportion of the biota with habitats ranging from benthic to pelagic. Additionally, macroinvertebrates form an integral part of the aquatic food chain, providing a major food source to fish, frogs and birds (Napier and Fairweather 1998).

1.3.7 The relationship between invertebrates and pH

Hydrogen ion content or the pH of a water body is an important water quality variable for freshwater biota (Berezina 2001). Consequently, the effects of pH

change or acidification on invertebrate community structure have been investigated by numerous studies (Hall 1994; Dangles and Guérold 2000; Madarish and Kimmel 2000). One of the well documented responses to pH decline is decreased species richness (Kimmel *et al.* 1985; Simpson *et al.* 1985; Feldman and Connor 1992; Kullberg 1992). These reductions in species richness are partly attributable to the elimination of sensitive taxa. Schindler (1987) noted that lake acidification in the Sudbury area of Canada had resulted in the loss of invertebrates such as the crustaceans *Mysis relicta* and *Orconectes virilis*. Sommer and Horwitz (2001) documented the loss of the amphipod *Austrochiltonia subtenuis,* gastropods and mayflies of the family Caenidae following pH decline in Lake Jandabup in the south-west of Western Australia. Caenid mayflies were also identified as highly sensitive to acidification by Havens (1993) during laboratory based toxicity trials.

In lotic waters, the loss of intolerant taxa may be a function of increased drift (Hall *et al.* 1985). Other responses to pH decline include an increase in the frequency of acid-tolerant taxa (Simpson *et al.* 1985; Hall and Ide 1987; Dangles and Guérold 2000). A shift in functional feeding groups may also occur with increased numbers of shredders, predators and deposit feeders, replacing scrapers and collectors (Stokes *et al.* 1989). For example Kullberg (1992) noted that the shredding Trichoptera recorded during a study on the macroinvertebrate community structure of streams in Sweden appeared to benefit from lower pH. A reduced emergence of insects has also been documented under low pH conditions (Bell 1971).

As a result of their responses to pH change, invertebrates are considered to be useful biological indicators of acidification (Battarbee *et al.* 1992; Howells 1995). In Scandinavia, their frequent inclusion in programs monitoring acidification has led to the development of several biotic indices (Johnson *et al.* 1993). For example Norwegian studies have resulted in the formulation of an acidification index based on the tolerance limits of various species (Raddum and Fjellheim 1984; Raddum *et al.* 1988; Fjellheim and Raddum 1990). The system divides the invertebrates collected into four categories and assigns each category an acidification index. Category **a** has an acidification index of 1.00 and includes taxa which become extinct at pH values of < 5.50. Category **b** has an index of 0.5 and includes taxa which become extinct at pH < 5.00. Taxa which become extinct at pH levels of < 4.70 are

assigned to category **c**, with an index of 0.25 and taxa which tolerate pH of <4.70 have an index of 0 and are included in category **d**. Each sample is given a value based on the acidification index of the invertebrates present. Sites containing category **a** taxa are assigned a score of 1 and are classified as unacidified. Sites containing category **d** taxa receive a score of 0 and are classified as highly acidified (Raddum and Fjellheim 1984; Fjellheim and Raddum 1990).

While invertebrates are the most commonly used biological monitors in Australia (Chessman 1995; Growns *et al.* 1997; Linke *et al.* 1999; Smith *et al.* 1999; Chessman *et al.* 2002; Marchant and Hehir 2002), their application as indicators of pH decline has been limited in comparison with other regions. Nonetheless, growing concerns about the acidification of Australian waters through processes such as soil oxidation are likely to heighten interest in the group's potential to act in this capacity. Further investigation into the pH tolerances of the group is necessary before their effectiveness as biological monitors of acidification in the wetlands of Australia and specifically Western Australia can be assessed.

1.3.8 Diatoms as biomonitors

The unicellular microalgae diatoms (Bacillariophyceae) are ubiquitous (John 2007), occurring in various waters and damp habitats where there is adequate light for photosynthesis (Patrick and Reimer 1966). In these suitable environments, diatoms make a substantial contribution to productivity and often form the base of food chains (Cox 1996). Many diatom species exhibit specific ecological tolerances and preferences, allowing the group to be used as indicators of environmental conditions (Cox 1996; Schofield and Davies 1996). This is further supported by their rapid immigration and reproduction rates which enable the diatoms to respond quickly to environmental change (Cooper *et al.* 1999).

The diversity of the group and ease of sampling contribute to their suitability as biological monitors (Stevenson and Pan 1999). A further advantage of diatoms is their possession of cell walls embedded with silica, collectively referred to as the frustule. The siliceous cell walls are resistant to decay and generally remain in the sediment, making diatoms suitable for palaeoecological studies (Round *et al.* 1990). Moreover, features of the cell walls such as the numbers of rows or striae and the

presence of a raphe or fissure can often be used to identify specimens to species level (Battarbee *et al.* 2001). The mostly well-established taxonomy of the group is another advantage of using diatoms as biological monitors (Schofield and Davies 1996). The ability to identify many diatoms to species level increases the accuracy of indicators that may be revealed through variation in ecological preferences within a given genera (Stevenson and Pan 1999).In addition, diatoms are amenable to permanent slide preparations, providing long-term access for counting and comparative purposes (Cox 1991; Round 1991; Lowe and Pan 1996).

The use of diatoms as indicators of past and present water quality is prevalent in many regions including the USA, Canada and Europe (Austin and Deniseger 1985; Prygiel and Coste 1993; Kelly 1998; Sabater 2000; Ponader *et al.* 2007). While not as commonly used in Australia, biological monitoring with diatoms is gradually gaining momentum (John 1983; 1993; 1998; Chessman *et al.* 1999; John 2000; Blinn and Bailey 2001; Sonneman *et al.* 2001; Gell *et al.* 2002; Blinn *et al.* 2004).

1.3.9 The use of artificial substrates for diatom collection

Periphytic communities (attached biota) (Jarlman *et al.* 1996), in particular diatoms, are commonly used in water quality studies (Jüttner *et al.* 2003; Bak *et al.* 2004; Lai and Wang 2004). While these organisms can be collected from natural substrates such as rocks, many researchers choose to employ artificial substrata (Patrick 1968; Chessman 1985; Ács and Kiss 1993; Oliveira *et al.* 2001; Maznah and Mansor 2002). Glass slides are one of the most common artificial substrata (Aloi 1990; Barbiero 2000), and are often used in conjunction with suspended devices such as the Catherwood Diatometer (Patrick *et al.* 1954) or the JJ Periphytometer (John 1998). Tiles are another frequently used medium (Lamberti and Resh 1985; Lane *et al.* 2003).

Periphyton samples on natural substrata may be extremely variable (Stevenson and Pan 1999) and obtaining a quantitative sample can be difficult (Sládeček *et al.* 1982). An advantage of artificial substrata is that they provide a standardised time and surface for colonisation (Cattaneo and Amireault 1992). Moreover, the uniform surface allows for estimations of statistical variability (Barbiero 2000). Further

advantages include increased sampling efficiency (Reid *et al.* 1995) and reduced disruption of habitat (Lamberti and Resh 1985). Among the disadvantages are risk of vandalism, damage (Chessman and McCallum 1981) and the possibility that the artificial substrata do not accurately represent natural conditions (Reid *et al.* 1995).

A study by Lamberti and Resh (1985) using tiles as artificial substrates found that the community which developed after at least 28 days was representative of the natural community. Lowe and Gale (1980) suggested that artificial substrates were able to provide a reasonable estimate of the dominant periphytic taxa, while Cattaneo and Amireault (1992) reported that in particular, the diatom assemblages of artificial substrates were generally similar to natural communities. Lane *et al.* (2003) similarly found that the diatom composition of natural substrates was well represented by the assemblages on artificial substrata. Patrick, Hohn and Wallace (1954) demonstrated that around 75 to 85% of the diatom species inhabiting the natural environment during their study were also recorded on the artificial substrates. From their work they suggested that artificial substrates gave a suitable representation of the diatom species inhabiting water bodies.

Conversely, a review by Aloi (1990) suggested that the periphytic assemblages developing on artificial substrata did not necessarily simulate natural communities. However, the review acknowledged that artificial substrates were a valuable tool when comparing environments and the effect of environmental variables (Aloi 1990). Artificial substrates allow for precise assessments in highly heterogeneous conditions (Stevenson and Pan 1999) and the assemblages that develop are generally thought to reflect the ambient environmental conditions at the time of sampling (John 1998).

1.3.10 The relationship between diatoms and pH

The variable of pH is considered to be one of the primary factors influencing the composition of diatom communities (Patrick 1948; Planas 1996; Battarbee *et al.* 2001; Young 2001). For example, a recent study on the Cape Cod Peninsula in the United States identified pH as the primary chemical parameter controlling the distribution of diatoms in freshwater ponds (Siver *et al.* 2004).

Recognition of the strong relationship between pH and diatom community structure led to the development of the highly influential diatom classification system by Hustedt (1938-1939) (Battarbee *et al.* 1999). The system categorised diatoms based on their pH preference and has been widely referred to since its development (Foged 1978, 1979; Jones *et al.* 1989; Watanabe and Asai 2001). The categories are as follows:

Alkalibiontic: Occurring at pH values > 7

Alkaliphilous: Occurring at pH about 7 with widest distribution at pH > 7

Indifferent: Occurring equally on each side of pH = 7

Acidophilous: Occurring at pH about 7 with widest distribution at pH < 7

Acidobiontic: Occurring at pH values < 7 with the optimum distribution at pH $\leq$ 5.5 (Hustedt 1938-1939). In addition, some authors using the Hustedt system include a circumneutral category to classify diatoms which occurred at around pH 7 (Battarbee *et al.* 1999).

Aside from the well documented association with community composition, various studies have also shown a correlation between pH and diatom species richness. Kwandrans (1993) found that the number of diatom taxa declined with decreasing pH in acidic streams in Poland (pH 3.5 – 6) while a study on Finnish lakes (pH 4.4 - 7.6) revealed a similar relationship (Eloranta 1990). However over a larger pH range, the relationship is not necessarily linear. Patrick (1948) suggested that few species could inhabit waters with pH < 3.5 or pH >8 and during her studies on the Pocono Plateau (Patrick 1945), the greatest species diversity was recorded from circumneutral wetlands.

In the last few decades, the issue of human-induced acidification has led to an increase in the research and use of diatoms as indicators of pH. For example, van Dam *et al.* (1981) investigated the impact of acidification on diatoms in Dutch moorland pools and Battarbee *et al.* (1997) examined the relationship between diatoms and pH in an area of Norway considered geologically sensitive to acidification. More recent work has included a study of diatom community structure in acidified streams in the Adirondacks in the USA (Passy 2006). A further application of the group in reference to acidification has been in the field of

palaeolimnology. Studies including the Palaeolimnological Investigation of Recent Lake Acidification project (PIRLA), resulting in the PIRLA iconograph (Camburn *et al.* 1986); the Surface Water Acidification Project (SWAP) (Birks *et al.* 1990), Hinderer et al (1998) and Ek and Renberg (2001) have all used diatoms to reconstruct past pH conditions.

The association between diatoms and pH has been less comprehensively studied in Australia than in other regions of the world. This is likely to be a function of the fact that intensive research on this association is generally undertaken in countries impacted by acidic deposition. While Australia is not generally affected by acid deposition, other forms of acidification represent a growing threat. As a result, further research into the relationship between pH and potential indicators such as diatoms is required. This research would enable the implementation of effective monitoring programs.

1.3.11 Diatom-based inference models

Diatoms are considered to be useful tools for the quantitative reconstruction of environmental variables (Gasse *et al.* 1995). The first stage of the reconstruction is the regression, where the responses of the modern diatom taxa to the environmental variables are modelled (Birks 1994). These are generally derived from a modern data-set or training set consisting of environmental and diatom data from a number of sites in a geographical region, covering a range of the environmental variable of interest (Battarbee *et al.* 1999; Racca *et al.* 2004). The final stage is the calibration step where the modelled responses, also known as transfer functions (Gasse *et al.* 1995) or inference models (Michelutti *et al.* 2001) are applied to an assemblage to infer past environmental conditions (Birks 1994).

The relationship between the diatom patterns and environmental variables within a training set is commonly investigated prior to the development of an inference model using multivariate analyses such as canonical correspondence analysis (CCA) (ter Braak 1986). Development of a diatom-based transfer function for a given variable can be justified if the analyses determine that the particular factor accounts for a significant portion of the variation in the diatom data (Gasse *et al.* 1995).

Species data may display a linear relationship over a limited range of an environmental variable (ter Braak and Prentice 1988). However, species responses over large gradients, for example a pH gradient of 2.5, are often unimodal (Battarbee *et al.* 1997). Subsequently, the unimodal based technique of weighted averaging (ter Braak and van Dam 1989) is one of the most commonly used methods of deriving transfer functions for variables such as pH (Birks *et al.* 1990; Gasse *et al.* 1995; Dixit *et al.* 1999; Tibby *et al.* 2003); salinity (Fritz *et al.* 1993; Gell 1997; Sherrod 1999), electrical conductivity (Reed 1998; Davies *et al.* 2002) and total phosphorus (TP) ; (Hall and Smol 1992; Pan and Stevenson 1996). The theory of weighted averaging suggests that a species will be present in the highest numbers when the environmental variable in question is near the optimum for that species (ter Braak and Prentice 1988). Therefore, weighted averaging regression estimates the indicator value or species optimum of a variable by averaging the values of the particular environmental variable from those sites which contain the species weighted by the abundance of the species (ter Braak and Looman 1987). Weighted averaging calibration estimates the value of the environmental variable at a site by taking a weighted average of the indicator values (optima) of the species (ter Braak 1987).

The effective management of aquatic systems requires an understanding of natural variability and the response of systems to disturbance (Enache and Prairie 2002). The use of transfer functions to infer past environmental conditions can provide an improved understanding of such factors and is particularly relevant in light of threatening processes such as acidification.

1.4   Objectives of the study

Although work has been carried out in Australia and other countries using both invertebrates and diatoms as biological monitors, most studies in Australia and Western Australia in particular have focused on either diatoms or invertebrates individually. Published research involving both groups of organisms has generally been limited to restricted geographical regions or has been focused on environmental variables such as salinity. Furthermore, the relative effectiveness of invertebrates and diatoms as biological indicators of pH in the south-west of Western Australia has not been thoroughly investigated. To address these issues, the present study examined

the distribution patterns of both invertebrates and diatoms in response to pH over a relatively wide region of the south-west of Western Australia. Knowledge gained from this study can be used in the development of strategies for the monitoring and management of water bodies currently or potentially impacted by acidification.

This thesis aimed to provide greater insight into the pH tolerances of invertebrates and diatoms in wetlands of the south-west of Western Australia and to assess the sensitivity of the two groups as biological monitors of pH. The objectives of the study were:

- To investigate the environmental variables of the selected wetlands in the south-west of Western Australia over the three seasons and discern any relationships between pH and other environmental factors.
- To examine the distribution patterns of invertebrates in the wetlands and assess their sensitivity to pH.
- To investigate the influence of pH on diatom community structure in the wetlands and analyse the sensitivity of the group to pH.
- To compare the sensitivity of invertebrates and diatoms to pH and identify the group likely to be the most effective indicators of ecological impacts related to pH change.
- To develop a diatom-based inference model for pH for the south-west of Western Australia which can be used as a tool for pH reconstructions on palaeolimnological investigations and has potential applications in modern monitoring programs.

The following chapter provides an outline of the study sites for both the seasonal and inference model data-sets and provides some information on the physical setting of the areas sampled in the south-west of Western Australia.

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Chapter 2: Study sites

2.1 Introduction

The study was designed to include four components: seasonal sampling of invertebrates, seasonal sampling of periphytic diatoms; the additional once only sampling of periphytic diatoms for use in a diatom-based inference model and monitoring of selected water quality parameters. Wetland sites were selected to reflect the range of pH found in wetlands of the south-west of Western Australia. A total of 20 wetlands were sampled in the seasonal program with a further 20 sites included for the diatom inference model.

The seasonal sites were located within 250 km of Perth and were selected from three localities: the Perth Metropolitan Region, Wagerup and the Collie Basin (Figure 2.1; Appendix 2.1). The Wagerup area is located approximately 120 km south of Perth and 7 km south of Waroona. The Collie Basin is a sedimentary depression which has been preserved in the Precambrian surface of the Yilgarn Block (Lord 1979). The Basin adjoins the townsite of Collie, approximately 200 km south-east of Perth. The selection of wetlands in these areas provided a comprehensive range of pH in conjunction with providing the readily accessible sites required for the seasonal sampling.

The seasonal sampling regime was undertaken during each of the four seasons of 2001. However the autumn data-set was excluded as a high proportion of wetlands were dry and could not be sampled. Each site was visited twice per season to allow for the installation and retrieval of the periphyton samplers.
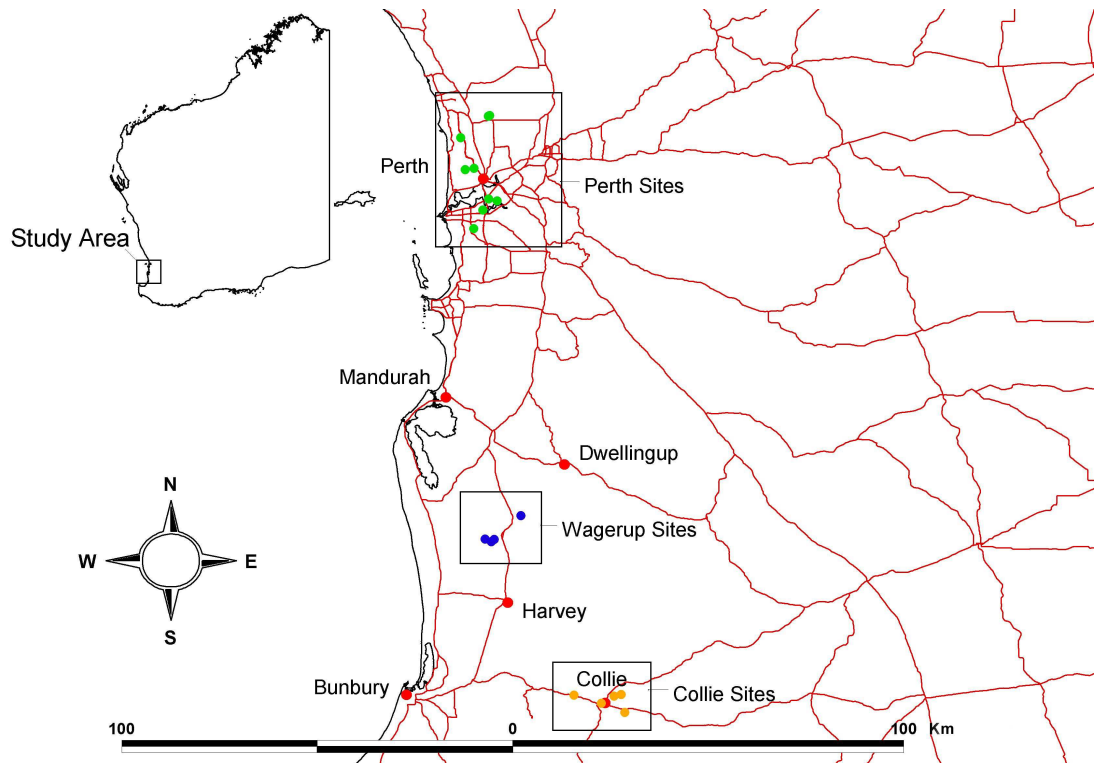
Figure 2.1: Location of the 20 sampling sites in the south-west of Western Australia selected for the seasonal study. Green points indicate the sites located in the Perth Metropolitan Region. Blue points indicate study sites located in the Wagerup area. Yellow points represent sites located in the Collie Basin. Red points represent the nearest neighbouring towns.

2.1.1 Climate and rainfall

The south-western region of Western Australia experiences a Mediterranean climate of cool, wet winters and warm to hot, dry summers (Beard 1990; Jaensch and Lane 1993). The highest rainfall in the region is concentrated in the extreme south (Tinley 1986) and there is a general decrease in rainfall as the distance from the ocean and the south-west corner increases (Jarvis 1986). For example, the town of Geraldton, situated near the northern limits of the south-west region receives approximately 467 mm of rainfall per year (Gentilli 1972). In contrast, the area between Augusta and Denmark in the state's extreme south-west has an annual rainfall of 1400 mm (Beard 1986).

The areas of Perth, Wagerup and Collie are all located in the winter-wet region of the south-west (Gentilli 1972). Perth receives an average rainfall in excess of 800 mm per year (BOM 2000) while Wagerup has an average annual rainfall of approximately 950 mm (ENVIRON Australia 2005). Similarly, the town of Collie receives an average of approximately 960 mm per year (BOM 1988).

2.1.2 Geology, soil type and topography

Coastal lowlands, plateau and plateau margins are the three distinguishable topographical regions of the south-west of Western Australia (Jarvis 1986). One of the main topographical features within these regions is the Darling Scarp. The scarp extends from Muchea to Donnybrook and geologically marks the division between the ancient granitic rocks of the Yilgarn Block to the east and the more recent sedimentary deposits to the west. The Darling Plateau encompasses the area to the east of the scarp (Pilgram 1979) and displays an average elevation of approximately 300 m (Beard 1990). The area between the coast and the plateau is a narrow lowland comprised of sand dunes and plains, limestone ridges, lakes, estuaries and rivers (Jarvis 1986) and is commonly described as the Swan Coastal Plain.

The town of Collie is located on the Darling Plateau whereas both Perth (Semenuik 1996) and Wagerup (ENVIRON Australia 2005) are situated on the Swan Coastal Plain. The plain reaches from the Moore River to Busselton (Erickson *et al.* 1973) and is described in geological terms, as the surface expression of a limited area of the Perth Basin (Seddon 2004). It is mostly formed of depositional material transported through the activities of wind, rivers and lakes or as a result of marine processes (McArthur and Bettenay 1960; McArthur and Bartle 1980; Seddon 2004). The geomorphic features of the plain include the Ridge Hill Shelf, the Pinjarra Plain and the Bassendean, Spearwood and Quindalup Dune Systems.

The Ridgehill Shelf is a narrow strip of laterite forming the foothills of the Darling Scarp (McArthur and Bettenay 1960). The Pinjarra Plain, an area which mostly consists of alluvial soils and older yellow podzolic soils (Bettenay 1983) extends from the foot of the Ridge Hill Shelf and is bordered on the western edge by the Bassendean Dune System. The Spearwood Dune System is located to the west of the Bassendean Dunes, followed by the Quindalup Dune System running along the present coastline (McArthur and Bettenay 1960).

The Bassendean Dune System is made up of low hills intersected by swampy flats (McArthur 1991). The soils in the wetter areas are generally humus podzols with bleached sands occurring on the dunes (Bettenay 1983). It is likely that the insoluble siliceous sediments were once calcareous sand which has been gradually leached of all carbonates (McArthur and Bettenay 1960). Conversely, the Spearwood Dune System is a younger system mostly containing siliceous sands overlying limestone. The youngest system, the Quindalup Dune system is comprised of calcareous sands (Bettenay 1983). Wetlands including Lakes Pinjar and Coogee, North Lake and Wellard Swamp separate the Bassendean and Spearwood Dune Systems, while waters such as Lake Cooloongup and Lake Clifton separate the Spearwood and Quindalup Dune Systems (Ministry of Education 1988).

The Perth Metropolitan Region accounts for approximately 20% of the Swan Coastal Plain (Mitchell *et al.* 2002) and encompasses many of the aforementioned geomorphic features. Subsequently, the soil types of the area range from the calcareous soils of the coast to the alluvial soils near the Darling Scarp. Wagerup is

located near the Ridge Hill Shelf in the area where podzols give way to sandy mottled yellow duplex soils. In contrast, the town of Collie lies over the ironstone gravelly siliceous sands common to the edge of the Darling Plateau (Bettenay *et al.* 1986).

2.1.3 Vegetation

The vegetation of the south-western region of the state ranges from heath and mallee to woodland and forest (Beard 1979). Karri (*Eucalyptus diversicolor*) forests, jarrah (*E. marginata*) and marri (*E. calophylla*) dominate the most humid areas of the region and the valleys often contain swamps of reeds or heath shrubs. In the areas receiving less rainfall (650 -1000mm per year) jarrah and marri grow on the lateritic plateau crusts, jarrah and tuart (*E. gomphocephala*) woodlands grow on the coastal sands over limestone and deep sands support *Banksia* sp. woodlands (Beard 1986).

Perth, Wagerup and Collie are all situated in the Darling Botanical District (Beard 1979, 1990). Collie is located in the southern Jarrah Forest subregion or Menzies Botanical subdistrict, near the border of the northern jarrah forest (Dale Botanical sub-region) (Beard 1990). Accordingly, the area includes jarrah (*Eucalyptus marginata*) forest and marri-wandoo (*E. calophylla and E. wandoo*) woodlands (Beard 1979). The river gum (*E. rudis*) is commonly found on streambanks in the region (Beard 1990).

Perth and Wagerup are located in the Swan Coastal Plain subregion or Drummond Botanical subdistrict of the Darling Botanical District (Beard 1990). *Banksia* woodlands are known to occupy the dry leached sands of the region and were used by the early settlers to indicate inferior soils. Jarrah and marri occur on the weakly leached sands of the Spearwood Dune System or on the margins of the swampy flats found within the Bassendean Dune System (Havel 1979). Tuart is also found in association with weakly leached soils in the region (Beard 1990). Sedgelands and shrublands are present on seasonally inundated acid peats of the area (Havel 1979) and *Melaleuca* is commonly found in association with poorly drained areas such as swamps (Beard 1990).

Much of the native vegetation in this region is under threat or has already been cleared (McKenzie *et al.* 2003). For example, wetlands account for over a quarter of the land on the plain and many of these areas have been at least partially cleared for purposes such as grazing (Hill *et al.* 1996). In the eastern parts of the coastal plain, land which previously supported marri woodlands has been cleared of most undergrowth to allow for agriculture (Erickson *et al.* 1973).

2.1.4 Land-use

Land-use in the south-west of Western Australia is varied. Urban and rural residential areas dominate the Swan Coastal Plain, (Mitchell *et al.* 2002) with approximately 80% of the state's population (1.2 million people) inhabiting this area (Davis and Froend 1999). Accordingly, roads and infrastructure are also common. Other dominant uses of the land in this region include conservation and crown reserves, agriculture and forestry plantations. Mining is also conducted in limited areas (Mitchell *et al.* 2002).

It has been estimated that approximately 70% of the original wetland area on the plain has been lost or undergone substantial modification (Halse 1989). Land clearing in the region has led to increased water levels in many of the remaining wetlands through factors such as increased surface run-off and higher water-tables (Davis and Froend 1999) and drainage waters. These additional inputs have also been associated with decreased water quality because of higher levels of nutrients and other contaminants (Chessman *et al.* 2002).

The Perth Metropolitan Region supports a number of land-uses, although residential suburbs and infrastructure and industrial development are particularly common (Jarvis 1986). The substantially smaller area of Wagerup displays less diversity in terms of land-use. Agriculture is one of the major uses in the area and mostly includes the production of milk and beef (Alcoa 1978). The mining industry also utilises the area with Alcoa operating the Wagerup Alumina Refinery in the vicinity (ENVIRON Australia 2005).

On the Darling Plateau, land-uses include agriculture, mining, timber production, water collection and recreation (McArthur 1991). In the Collie region of the plateau agriculture, power production, wildflower harvesting, pine plantations and tourism are among the common industries (EPA 1992). The power generating activities of the region are linked with the Collie Power station, a coal-fired power station located in the Collie Basin, approximately 10 km east north east of the town. Pine plantations are found in the Collie River Valley (McArthur 1991) but are not a major industry as approximately 75% of the Collie Basin is characterized as state forest. The area also has extensive groundwater resources and contains two dams: the Wellington Reservoir located on the Collie River approximately 25 km downstream of Collie and the Harris Dam north of the town (CWAG 1996). The primary land-use in the Collie Basin is coal-mining (EPA 1992). During the last hundred years of mining in the basin, a total of 25 underground and 14 open-cut coal mines have operated, the majority of which are not currently operational (Johnson and Wright 2003). Some of the abandoned open-cut voids were left to fill with water and become lakes with a few now used for recreational purposes (Stedman 1988).

2.2   Wetland classification

A wetland classification system based on pH was employed for the seasonal study. Three groups of wetlands were identified from the 20 study sites using pH ranges adapted from Foged's (1978) work on eastern Australian water bodies. Group 1 sites recorded pH values of < 6.5 and were classified as acidic. Group 2 sites ranged in pH from 6.5-7.5 and were classified as circumneutral. Group 3 sites displayed pH readings of > 7.5 and were described as alkaline.

Given the seasonal nature of the study, the pH groupings were flexible, with the number of wetlands assigned to each group varying in accordance with seasonal fluctuations in pH. These changes in the classification of wetlands were most apparent for the circumneutral grouping, probably as a result of the smaller pH range of this category. The following section provides information about the study sites representative of each pH category. The sites are described in reference to the pH group in which they were generally classified. Plates of selected sites from the three groupings are presented in Appendix 2.2.

2.2.1 Group 1 – acidic sites

Group 1 comprises acidic wetlands (pH < 6.5) from each of the three study regions. The water bodies range from mine-void lakes located in the Collie Basin through to wetlands situated in Wagerup and Perth. The Collie sites are typically deep, for example Ewington 2 (Appendix 2.2a) reaches a depth of 11 m (John *et al.* 2000). However sampling was conducted in the shallow areas near the lake edges. The Wagerup wetland of Blind Roo A (Appendix 2.2b) and the acidic Perth sites are relatively shallow in comparison. The land surrounding the Collie and Wagerup sites is mostly pastoral while the sites in the metropolitan region are surrounding by residential, pastoral and some forestry plantations. Littoral vegetation ranges from very sparse at water bodies such as the Blue Waters void in Collie (Appendix 2.2c) to relatively dense at the Collie site of Stockton Tailings Pond (Appendix 2.2d) and Lakelands in the Perth Metropolitan Area (Appendix 2.2e). A similar range is evident for submerged flora with some of the sites displaying abundant macroscopic algal growth. For example, the filamentous green alga *Mougeotia* sp. has been commonly identified from the Perth site Gnangara Lake (Appendix 2.2f) and from Stockton Tailings Pond in Collie (John *et al.* 2000).

The low pH of the sites can be linked to factors including mining processes (Commander *et al.* 1994) and the oxidation of acid-sulfate soils resulting from decreased groundwater (McHugh 2004). Organic acids were probably also a contributing factor in the low pH of certain wetlands, having been previously associated with low pH waters in the south-west of Western Australia (Schmidt and Rosich 1993; Kinnear and Garnett 1999). For example, the pH of Blind Roo A wetland in Wagerup is linked to humic acid derived from high levels of vegetative material deposited in the site (J. John pers. comm.).

2.2.2 Group 2 – circumneutral sites

The circumneutral sites include wetlands from the Perth Metropolitan Region, Wagerup and Collie. Land-use varied among these sites and included urban development and agriculture. For example, the Perth wetland, Kurrajong Village Lake (Appendix 2.2g), is located on the grounds of Curtin University of Technology, an area surrounded by residential development. Another of the circumneutral sites,

Exelby wetland in Wagerup, has become a permanent water body through the drainage of excess irrigation waters from surrounding farmland (ENVIRON Australia 2005). However, Exelby and the circumneutral Perth wetlands are still relatively shallow in comparison to the Collie site of Wallsend (Appendix 2h). Probably as a result of varying land-uses, the littoral vegetation of the circumneutral sites ranges from a sparse to moderate density at the Perth site of Tuscan Park and Exelby Wetland in Wagerup through to a relatively dense growth pattern at the Wallsend site.

The circumneutral pH of these wetlands is likely be related to a number of factors. Wallsend is one of the oldest mine voids in the Collie region and while the below neutral pH of the site is probably attributable to past mining procedures, the values were higher than the pH levels of younger mine voids. Differences in the pH and other chemical variables of older void lakes in comparison with newer voids have been previously documented by Thomas and John (2006) in a study on mine-void lakes in the Collie Basin. Other factors contributing to the circumneutral pH of the Group 2 wetlands may include similar processes to those related to the acidic Group 1 wetlands including oxidation of acid sulfate soils (DoE 2005) and the presence of organic acids.

2.2.3   Group 3 – alkaline sites

Group 3 encompassed alkaline wetlands from Wagerup and the Perth Metropolitan Region. The surrounding land-uses of these areas include residential, pastoral and water collection. For example, the metropolitan site of Herdsman Lake (Appendix 2.2i) is used for conservation and educational purposes and is surrounded by residential land and light industry (Jaensch and Lane 1993). Blue Gum Lake (Appendix 2.2j), one of the wetlands that form the East Beeliar wetland chain, is another site located within a residential area (Fox and MacShane 2004). In contrast, Lake Moyanup near Wagerup is a water resource created by the Drakesbrook Dam (CALM and WAWA 1988b) while the site of Blind Roo B is situated on pastoral land (Appendix 2.2k).

The alkaline sites are generally shallow, for example the Perth site of Lake Monger has a depth of < 2 m (Lund and Davis 2000). An exception is Herdsman Lake which

reaches depths of approximately 19 m in some areas as a result of artificial dredging (Schmidt and Rosich 1993). The vegetation at the alkaline sites ranges from sparse to moderate at waterbodies such as Blind Roo B and Perth's Neil McDougall Park (Appendix 2.2l) in contrast with the moderately high densities present at sites such as Bibra Lake.

The alkaline pH of these wetlands may be partly related to substrate type. For example, the Wagerup site of Blind Roo B was originally created through clay extraction (J. John pers. comm.) and sites such as Lake Monger are located on the limestone rich Spearwood Dune System (Lund and Davis 2000). Additionally, many of the wetlands on the plain are nutrient enriched (Arnold 1990). The level of photosynthesis that occurs in eutrophic waters can increase the pH by removing aqueous CO_2, and subsequently converting an HCO_3 ion to a CO_3 ion. This ion then reacts with Ca^{2+} in water to precipitate $CaCO_3$ (Manahan 2005).

2.3   Diatom-based inference model sites

The seasonal data-set used to examine the effects of pH on the biotic communities included 60 samples (20 sites sampled during summer, winter and spring). The autumn data was incomplete and was therefore excluded. The diatom-based inference model data-set included only the spring diatom samples for each of the 20 seasonally sampled sites along with samples from an additional 20 wetlands. The additional sites were sampled on one occasion between 2000 and 2004, mostly in spring. Each of the sites included in the model data-set has been assigned a code beginning with M to distinguish between the seasonal and model data-sets (Appendix 2.3). The 40 model sites are each located in the south-west of Western Australia within 250 km of the Perth Metropolitan Region (Figure 2.2; Appendix 2.3). Detailed descriptions of the geology, climate, vegetation and land-uses of the south-west region have been provided earlier in the chapter. As information about the 20 seasonal sites has been outlined previously, the following section will focus on the additional 20 sites included in the inference model. Plates of selected model sites are presented in Appendix 2.4.

Lake Brockman, the first of the additional sites, is located in the Logue Brook Catchment between Wagerup and Harvey. The lake was created when Logue Brook which forms part of the Harvey River Basin, was dammed. The area is situated on the Darling Plateau and approximately 93% of the catchment is state forest. Subsequently, the predominant land-uses in the area include water collection and conservation (CALM and WAWA 1988a). The pH of the site is approximately neutral. Thirteen of the 20 additional sites are located in the Perth Metropolitan Region of the Swan Coastal Plain. The surrounding land-uses of the sites are mostly residential.

Vegetation for the model sites ranges from sparse to relatively dense. For example wetlands such as Sheldrake Park and Dog Swamp had little fringing vegetation whereas Booragoon Lake possesses extensive low-closed forest and scrub (Jaensch and Lane 1993). Acidic pH values were recorded from sites such as Lake Gillon, a shallow wetland in the suburb of Karrawarra. Circumneutral sites include the neighbouring Piney Lakes and Booragoon Lake, both of which have been described as coloured waters (Cheal *et al.* 1993; Jaensch and Lane 1993). North Lake, a surface expression of the Jandakot Mound groundwater flow system (Balla and Davis 1993) was one of the alkaline sites included in the inference model data-set.

The final six model sites were located in Gwindinup, approximately 25 km south south-east of Bunbury and 10 km south of Moyanup (EPA 2005). Farming is one of the major land-uses of the area although the regional centre of Bunbury does support relatively high residential and retail land-use (Gentilli 1979). The sites were shallow in nature and contain coloured, acidic waters due to tannins and humic acid (J. John pers. comm.).
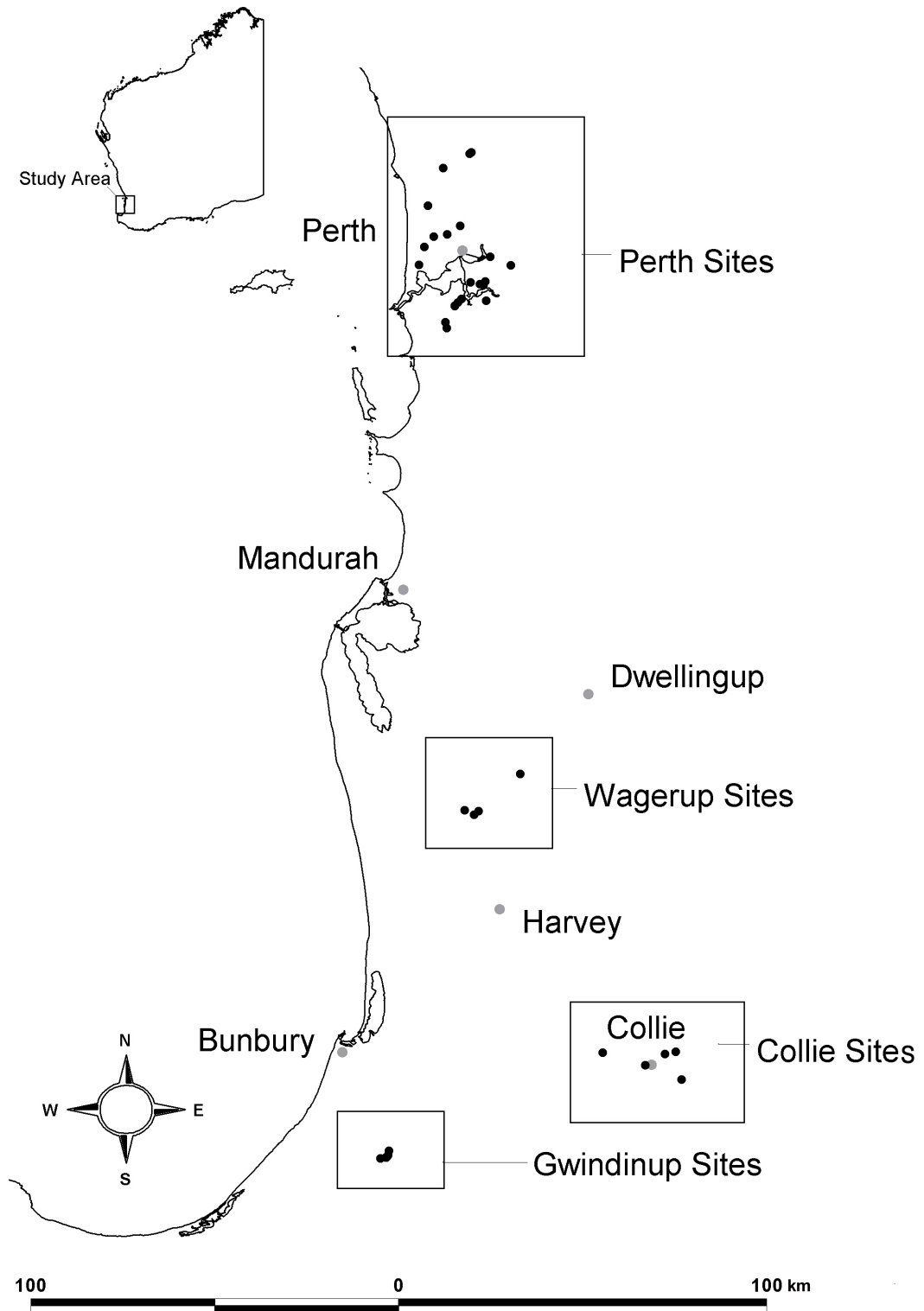
Figure 2.2: Location of the 40 sites in the south-west of Western Australia selected for use in the diatom-based transfer function for pH. Black points indicate the study sites located in the Perth Metropolitan Region. Grey points represent the nearest neighbouring towns.

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Appendix 2.1: Table of the 20 seasonal sampling locations displaying site code, location, GPS co-ordinates seasons sampled and pH groupings.

Site Name	Site Code	Site Location	GPS	Season	pH Group
Bibra Lake	1	Perth Metropolitan	32º05.19s 115º49.38e	Summer	3
				Winter	3
				Spring	3
Black Diamond	2	Collie Basin	33º20.33s 116º05.58e	Summer	1
				Winter	1
				Spring	1
Blind Roo A	3	Wagerup	32º 55.20s 115º 51.23e	Summer	1
				Winter	2
				Spring	1
Blind Roo B	4	Wagerup	32º 55.21s 115º 51.25e	Summer	3
				Winter	3
				Spring	3
Blue Gum Lake	5	Perth Metropolitan	32º02.20s 115º50.90e	Summer	3
				Winter	3
				Spring	3
Blue Waters	6	Collie Basin	33º20.24s 116º13.16e	Summer	1
				Winter	1
				Spring	1
Ewington 2	7	Collie Basin	33º20.48s 116º12.02e	Summer	1
				Winter	1
				Spring	1
Exelby Wetland	8	Wagerup	32º 55.64s 115º 52.23e	Summer	3
				Winter	2
				Spring	2
Gnangara Lake	9	Perth Metropolitan	31º46.97s 115º51.96e	Summer	1
				Winter	1
				Spring	1
Herdsman Lake	10	Perth Metropolitan	31º55.71s 115º48.03e	Summer	3
				Winter	3
				Spring	3
Knapping Wetland	11	Wagerup	32º 55.27s 115º 52.69e	Summer	3
				Winter	2
				Spring	3
Kurrajong Village Lake	12	Perth Metropolitan	32º00.76s 115º53.19e	Summer	2
				Winter	1
				Spring	2
Lake Monger	13	Perth Metropolitan	31º55.50s 115º49.45e	Summer	3
				Winter	3
				Spring	3
Lake Moyanup	14	Wagerup	32º51.41s 115º56.99e	Summer	3
				Winter	2
				Spring	3
Lakelands	15	Perth Metropolitan	31º50.55s 115º47.29e	Summer	1
				Winter	2
				Spring	1
Neil McDougall Park	16	Perth Metropolitan	32º00.45s 115º51.83e	Summer	3
				Winter	3
				Spring	3
Stockton Lake	17	Collie Basin	33º23.13s 116º13.75e	Summer	1
				Winter	1
				Spring	1
Stockton Tailings Pond	18	Collie Basin	33º23.13s 116º13.74e	Summer	1
				Winter	1
				Spring	1
Tuscan Park	19	Perth Metropolitan	31º47.12s 115º51.75e	Summer	1
				Winter	2
				Spring	2
Wallsend Lake	20	Collie Basin	33º21.65s 116º09.99e	Summer	1
				Winter	2
				Spring	2

Appendix 2.2: Selected seasonal sampling sites from the three pH groupings. Plates (a) – (f) represent sites from the acidic pH group (Group 1).(a) Ewington 2 (b) Blind Roo A (c) Blue Waters (d) Lakelands (e) Stockton Tailings Pond (f) Gnangara Lake.

Appendix 2.2 continued. Selected seasonal sampling sites from the three pH groupings. Plates (g) – (h) represent sites from the circumneutral pH group (Group 2). (g) – Kurrajong Village Lake (h) Wallsend. Plates (i) – (l) represent sites from the alkaline pH grouping. (i) Herdsman Lake (j) Blue Gum Lake (k) Blind Roo B (l) Neil McDougall Park.
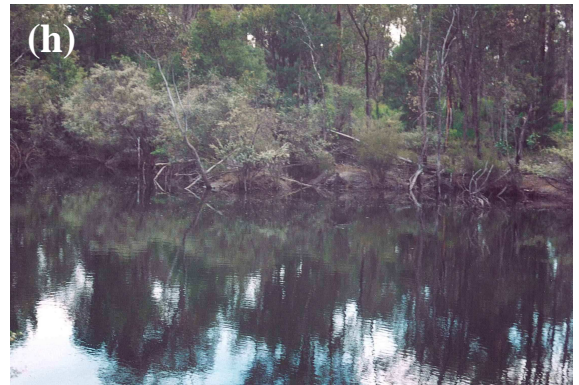
Appendix 2.3: Table of the 40 sampling locations used for the diatom-based predictive model displaying the site codes and GPS co-ordinates.

Site Name	Site Code	Site Location	GPS	Season
Bibra Lake	M1	Perth Metropolitan Region	32º05.19s 115º49.38e	Spring
Black Diamond Lake	M2	Collie Basin	33º20.33s 116º05.58e	Spring
Blind Roo A	M3	Wagerup	32º 55.20s 115º 51.23e	Spring
Blind Roo B	M4	Wagerup	32º 55.21s 115º 51.25e	Spring
Blue Gum Lake	M5	Perth Metropolitan Region	32º02.20s 115º50.90e	Spring
Blue Waters	M6	Collie Basin	33º20.24s 116º13.16e	Spring
Booragoon Lake	M7	Perth Metropolitan Region	32º02.58s 115º50.57e	Spring
Dog Swamp	M8	Perth Metropolitan Region	31º54.63s 115º50.77e	Winter
Ewington 2 Lake	M9	Collie Basin	33º20.48s 116º12.02e	Spring
Exelby Wetland	M10	Wagerup	32º 55.64s 115º 52.23e	Spring
Gnangara Lake	M11	Perth Metropolitan Region	31º46.97s 115º51.96e	Spring
G.O. Edwards Park	M12	Perth Metropolitan Region	31º57.82s 115.53.89e	Winter
Gwindinup 1	M13	Gwindinup	33º30.51s 115º43.44e	Spring
Gwindinup 2	M14	Gwindinup	33º31.00s 115º43.34e	Spring
Gwindinup 3	M15	Gwindinup	33º31.11s 115º43.25e	Spring
Gwindinup 4	M16	Gwindinup	33º31.20s 115º43.15e	Spring
Gwindinup 5	M17	Gwindinup	33º31.23s 115º43.13e	Spring
Gwindinup 6	M18	Gwindinup	33º31.30s 115º42.54e	Spring
Herdsman Lake	M19	Perth Metropolitan Region	31º55.71s 115º48.03e	Spring
Jack Finney Lake	M20	Perth Metropolitan Region	32º00.38s 115º53.38e	Spring
Knapping Wetland	M21	Wagerup	32º 55.27s 115º 52.69e	Spring
Kurrajong Village Lake	M22	Perth Metropolitan Region	32º00.76s 115º53.19e	Spring

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Appendix 2.3 continued. Table of the 40 sampling locations displaying the site codes and GPS co-ordinates.

Site Name	Site Code	Site Location	GPS	Season
Lake Brockman	M23	Harvey	32º02.93s 115º50.23e	Spring
Lake Claremont	M24	Perth Metropolitan Region	31º58.66s 115.46.55e	Winter
Lake Gillon	M25	Perth Metropolitan Region	32º00.67s 115º52.82e	Winter
Lake Goollelal	M26	Perth Metropolitan Region	31º48.63s 115º49.00e	Spring
Lake Gwelup	M27	Perth Metropolitan Region	31º52.53s 115º47.50e	Summer
Lake Monger	M28	Perth Metropolitan Region	31º55.50s 115º49.45e	Spring
Lake Moyanup	M29	Wagerup	32º51.41s 115º56.99e	Spring
Lakelands	M30	Perth Metropolitan Region	31º20.53s 115º05.58e	Spring
Neil McDougall Park	M31	Perth Metropolitan Region	32º00.45s 115º51.83e	Spring
North Lake	M32	Perth Metropolitan Region	32º04.61s 115º49.26e	Summer
Perry Lakes	M33	Perth Metropolitan Region	31º56.78 115º47.10e	Summer
Piney Lakes	M34	Perth Metropolitan Region	32º02.90s 115º50.22e	Summer
Sheldrake Park	M35	Perth Metropolitan Region	32º02.37s 115.53.46e	Winter
Stockton Lake	M36	Collie Basin	33º23.13s 116º13.75e	Spring
Stockton Tailings Pond	M37	Collie Basin	33º23.13s 116º13.74e	Spring
Tomato Lake	M38	Perth Metropolitan Region	31º58.68s 115.56.05e	Winter
Tuscan Park	M39	Perth Metropolitan Region	31º47.12s 115º51.75e	Spring
Wallsend Lake	M40	Collie Basin	33º21.65s 116º09.99e	Spring

Appendix 2.4: Selected water bodies from the 40 model sites. (a) Booragoon Lake (b) Lake Brockman (c) Lake Goollelal (d) Lake Gwelup (e) North Lake (f) Piney Lakes.
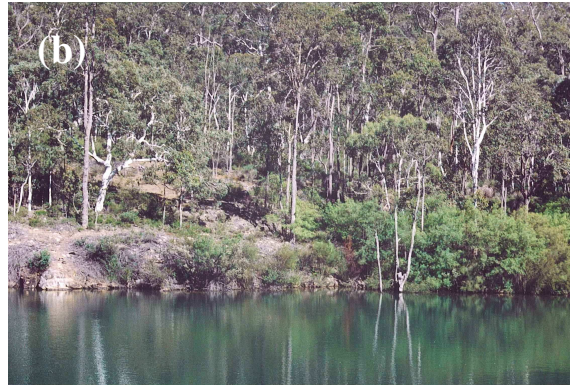
Chapter 3: Environmental variables of wetlands of the south-west of Western Australia with special reference to pH

3.1 Abstract

The environmental variables of 20 sites from the south-west of Western Australia were investigated over three seasons, corresponding with invertebrate and diatom sampling. The pH of the sites ranged from 3.01 to 10.00. The low pH values were generally attributed to the oxidation of sulphidic materials, while substrate type was commonly associated with the presence of high pH values. Electrical conductivity ranged from 124.80 μS cm^{-1} to 5230.00 μS cm^{-1} and was influenced by factors such as evaporative water loss during summer. The wide range of temperature was also related to seasonal variation. Examination of the environmental variables in relation to pH groups revealed few trends, possibly as a result of the heterogeneous nature of the wetlands within the groups. Electrical conductivity was the only exception, with higher mean and maximum values in the acidic Group 1 wetlands, probably as a result of mining impacts and the oxidation of acid sulphate soils. While there was some seasonal variation evident within the individual pH groups, temperature was the only variable that displayed a strong seasonal difference across the three pH groups. Multivariate analyses revealed that the variables explaining the largest amount of variation between the sites varied between seasons.

3.2 Introduction

The variable of pH affects many chemical and biological processes within a water body, making it an important component of water quality assessment (Chapman and Kimstach 1992). It is however, likely to be of particular relevance in areas impacted or threatened by surface water acidification.

Acidification or pH decline is a growing problem in the south-west of Western Australia which has been linked to the degradation of waters in areas of the Swan and Scott Coastal Plains, parts of the Wheatbelt region and in close proximity to towns such as Mandurah and Albany (EPA 2006). The northern area of the Perth Metropolitan Region has been particularly affected, with a number of wetlands permanently or periodically acidified (Sommer and Horwitz 2001; Appleyard *et al.* 2004; McHugh 2004). The problem, which is mostly caused by the exposure of acid sulphate soils, has been partly attributed to poor planning and inadequate management of land-use development (EPA 2006).

Despite these concerns, the pH of wetlands in the south-west region currently ranges from acidic to alkaline with the majority of systems having near neutral pH values (EPA 2006). A broad range is also evident for other environmental variables with many of the parameters including pH being influenced by a number of different factors. Variables such as electrical conductivity and salinity differ not only between wetlands but also seasonally. For example, evaporation during the warmer months generally leads to an increase in the salt concentration (Williams 1983; Boulton and Brock 1999). Evaporation is also known to increase the hydrogen ion concentration, thereby depressing the pH (Boulton and Brock 1999). Surface water temperature is another variable that displays seasonal variation and is also subject to temporal changes (Chapman and Kimstach 1992). Uptake of heat from solar radiation and subsequent cooling through convection loss of heat can result in major changes in the water column (Thomas *et al.* 1992). In deep lakes, vertical temperature stratification may develop causing the water body to stratify into two layers during the warmer periods of the year (Williams 1980). Lakes of a very shallow nature rarely thermally stratify due to constant mixing from wind energy (Thomas *et al.* 1992).

Dissolved oxygen concentration varies due to a number of factors including the presence of organic wastes (Tebbutt 1992), the nature of the water body (flowing or still) and depth (Baird 1999). Temperature is also important in relation to dissolved oxygen concentration as the solubility of oxygen in water decreases with increasing temperature (Connell 2005; Manahan 2005). Increases in salinity have a similar effect (Schmidt and Rosich 1993).

The variable of pH is also temperature dependent (Chapman and Kimstach 1992). Other factors known to influence both pH and dissolved oxygen are biological activities such as photosynthesis and respiration. During the day, primary productivity exceeds the bacterial decomposition of detritus and oxygen concentrations may reach levels of over-saturation. Concomitantly, pH values can exceed 10. During evenings, both dissolved oxygen and pH levels are likely to decline (Meybeck *et al.* 1992).

The underlying substrate or bottom sediment of a wetland is another factor that plays an important role in the water chemistry of wetlands in the region (Townley *et al.* 1993). Through its direct link with a wetland's hydrology, the sediment can act as a determinant of variables such as pH and electrical conductivity (Hammer 1992). For example, the high pH of many wetlands on the Swan Coastal Plain has been attributed to bicarbonate buffering derived from the surrounding limestone of the Spearwood and Quindalup Dune systems. Conversely, the comparatively lower pH of many wetlands that lie on Bassendean sands has been associated with the lack of carbonate in the highly leached substrate (Wrigley *et al.* 1991).

Surrounding land-uses are also known to influence the environmental parameters of wetlands. In the non-urban catchments of Australia, changes in physico-chemical parameters are likely to be caused by mining or agricultural processes. The removal of forest vegetation for crops and pastures can indirectly affect the pH of nearby water bodies. The pH balance of the soil changes following the removal of the native vegetation, becoming progressively more acidic. If these acidic materials are transported in drainage waters, a decline in the pH of receiving waters may result (Archer 2001). Increases in salinity and electrical conductivity caused by land-uses such as agriculture are another threat to surface waters. It has been estimated that

approximately 5.7 million hectares of Australian agricultural and pastoral land is at risk of dryland salinisation as a result of shallow watertables. The largest area of impacted land occurs in the south-west of Western Australia, particularly in the Wheatbelt region. A major off-site risk associated with this increased salinity is the salinisation of previously fresh water bodies (NLWRA 2001). Additionally, some waters in this region face the combined threat of acidification and salinisation, a problem likely to result in severe degradation (EPA 2006).

Fringing vegetation is a further environmental variable impacted by land-use. Practises such as vegetation clearing and grazing contribute to the degradation of the fringing vegetation in both urban and rural regions of the south-west (McKenzie *et al.* 2003). Moreover, the removal or destruction of this vegetation can in turn affect other environmental parameters. For example, salinity is frequently elevated in wetlands that have been stripped of surrounding vegetation (McComb and Lake 1990). In addition, the presence of fringing vegetation is known to moderate water temperatures through the provision of shade (Brearley 2005; EPA 2006). The pH of a wetland may also be influenced by the presence or absence of vegetation. Allochthanous plant material entering a water body is degraded by micro-organisms and other processes, leaving dark coloured humic substances (humic and fulvic acids). The low pH of dune lakes in eastern Australia and the wetlands on the Swan Coastal Plain have both been attributed to the presence of organic acids, with the lowest pH values generally associated with the highest colouration (Bayly 1964; Cheal *et al.* 1993). Conversely, the removal of fringing vegetation may result in a wetland's source of colour being lost (Davis and Froend 1999).

The wetlands of the south-west of Western Australia vary in chemical and physical composition. Monitoring of various parameters provides insight into the functioning of the systems and is of growing importance as water quality issues such as acidification become more prevalent. Previous studies in the south-west including the Swan Coastal Plain (Growns *et al.* 1992; Schmidt and Rosich 1993; Balla and Davis 1995; Kinnear and Garnett 1999) and the Wheatbelt region (Kay *et al.* 2001; Halse *et al.* 2003; Blinn *et al.* 2004; Cale *et al.* 2004; Pinder *et al.* 2005) have investigated various environmental parameters. While most studies have included pH measurements as part of their sampling regimes, few have focused on the variable

specifically. Subsequently, the objectives of the current study were to investigate the environmental variables of sampled wetlands in the south-west of Western Australia and elucidate their relationship to pH. A secondary objective of the chapter was to examine seasonal variation in the environmental variables. This chapter will provide valuable additional data on sites that have been previously sampled and baseline data for sites that have not. Moreover, the extensive range of pH sampled and the inclusion of several highly acidic sites separate this study from most other research in the south-west of Western Australia.

3.3 Methods

3.3.1 Environmental variables

The location of the 20 sites included in the study is presented in Figure 3.1. The physico-chemical parameters of each site were measured seasonally in the littoral zone of the sites (depth < 1 m) to coincide with the collection of biota. A TPS WP-81 hand held meter was employed to measure pH (pH units), electrical conductivity (μS cm^{-1}), salinity (ppm) and temperature (ºC) while dissolved oxygen (mg L^{-1}) was recorded with a YSI 550 DO meter. The meters were calibrated using standard techniques. The fringing vegetation of each wetland was observed and scored on a scale of 1 – 5; a score of 1 for the lowest density of fringing vegetation and a score of 5 for the most densely vegetated sites.
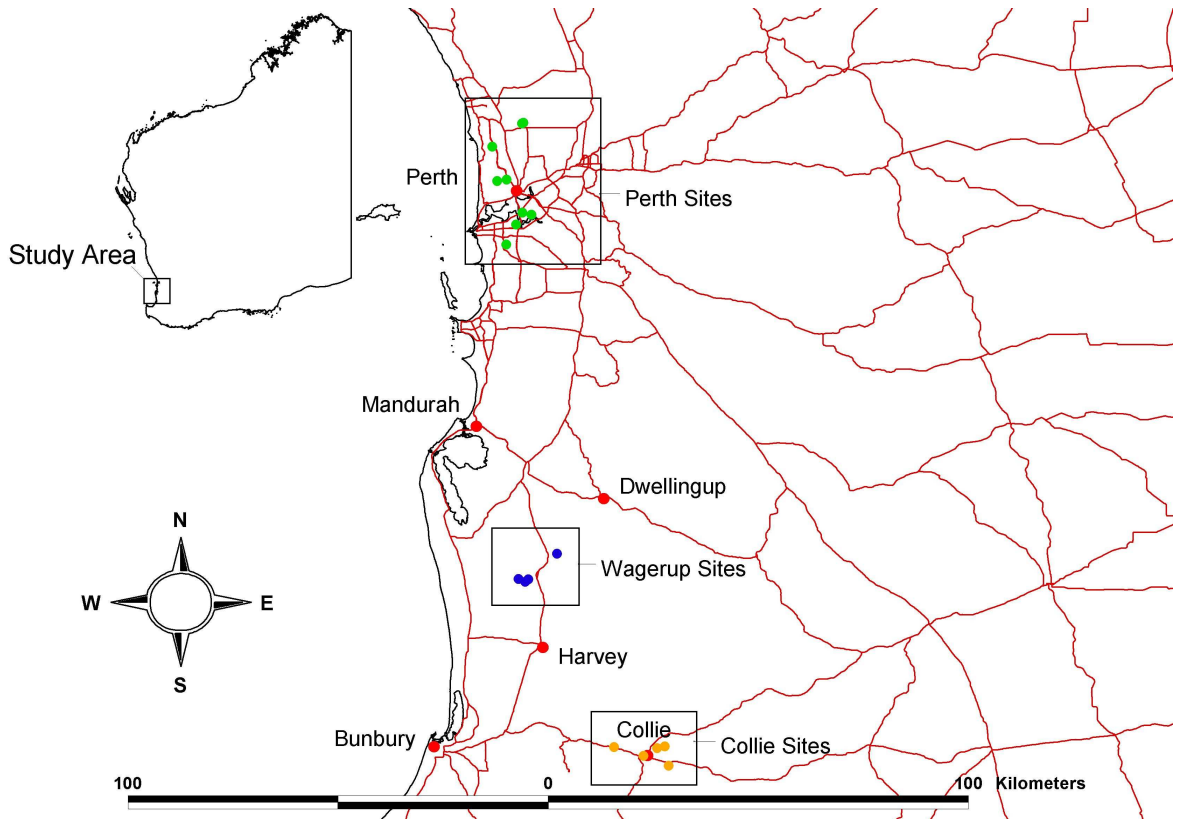
Figure 3.1: Location of the 20 sampling sites in the south-west of Western Australia selected for the seasonal study. Green points indicate the sites located in the Perth Metropolitan Region. Blue points indicate study sites located in the Wagerup area. Yellow points represent sites located in the Collie Basin. Red points represent the nearest neighbouring towns.

3.3.2 Data analyses

The environmental data presented in this chapter were not appropriate for univariate analyses and were therefore subjected to multivariate methods. Ordinations of the data were generated using correlation based principal components analysis (PCA) to determine the major patterns of variation. The environmental variables were standardized prior to analyses and included pH, electrical conductivity, temperature, dissolved oxygen and vegetation. Salinity, being collinear with electrical conductivity, was excluded from the data-set. Electrical conductivity and dissolved oxygen concentration were fourth root transformed to reduce skewness. All analyses were conducted using the statistical package PRIMER 5 for Windows Version 5.2.9 (PRIMER-E Ltd 2002). The three pH groupings used were adapted from Foged (1978).

3.4 Results

3.4.1 Variable range

A summary of the environmental variables measured during the study is displayed in Table 3.1. The complete set of environmental data is presented in Appendix 3.1. The pH of the wetlands ranged from highly acidic (pH = 3.01) to strongly alkaline (pH = 10). Electrical conductivity also displayed a wide range from fresh water (124.80 μS cm^{-1}) through to 5230.00 μS cm^{-1}. Temperature and dissolved oxygen were both highly variable and a range of peripheral vegetation densities were identified through the assignment of vegetation scores (Table 3.1).

Table 3.1: The range of environmental variables from the 20 sites sampled in the south-west of Western Australia over the duration of the study.

Variable	Minimum	Maximum
pH (pH unit)	3.01	10.00
Electrical Conductivity (μS cm^{-1})	124.80	5230.00
Temperature (˚C)	12.70	32.00
Dissolved Oxygen (mg L^{-1})	3.81	13.50
Vegetation Score	1.00	4.00

3.4.2 Environmental variables in relation to pH groups

The means and ranges of environmental parameters from each of the three pH groupings are presented in Figure 3.2. Little variation was evident in the pH range of the Group 1 wetlands between seasons (Figure 3.2a). The lowest values recorded were all strongly acidic (pH < 3.5) and the highest approached circumneutral. Additionally, while the geometric mean pH for the summer data appeared slightly higher than in other seasons, the mean pH readings for each season remained below pH 5. The pH range of the Group 2 wetlands was also relatively stable during most of the study with the winter and spring sampling events producing similar pH values (Figure 3.2 b). During summer however, only one of the study sites was identified as circumneutral (pH = 6.54).

The greatest seasonal fluctuations in pH were present in the Group 3 wetlands (Figure 3.2c). The mean pH value of 9.20 recorded in the summer sampling period was higher than the values for either winter or spring with a similar pattern evident for the minimum and maximum readings. The winter sample displayed the lowest mean and minimum pH for Group 3 with values of 8.33 and 7.66 respectively.

Electrical conductivity was reasonably variable and differences were evident between both seasons and pH groups (Figure 3.2 d-f). The Group 1 wetlands consistently displayed the highest mean and maximum values while Group 2 displayed the lowest. Overall, the highest electrical conductivities were generally recorded in summer with Groups 1 and 3 displaying their highest maximums and means during this season. Group 2 was the exception with the highest maximum measured during winter. The lowest minimum conductivity was recorded from the Group 1 wetlands during summer but varied over the remaining seasons.

Temperature fluctuated between seasons with the highest mean, minimum and maximum values for each group in summer and the lowest in winter (Figure 3.2g-i). Mean temperatures calculated for the summer sample were comparable for each of the groups and Group 3 had the largest range (20.4 – 34.6 °C). Winter temperatures were low with a maximum of 16.4 °C. The Group 3 wetlands displayed a slightly higher mean temperature than the other groups while Group 1 had the highest

maximum. During spring both the highest mean and maximum temperature were recorded from Group 3.

Dissolved oxygen concentrations were variable with no strong trends evident for either season or wetland group (Figure 3.2j-l). During summer sampling Group 3 possessed a mean concentration of 8.27 mg L^{-1} in contrast with the relatively low mean of Group 1 (5.59 mg L^{-1}). However, during winter sampling the Group 1 wetlands displayed the highest mean (7.12 mg L^{-1}) with Group 2 recording the lowest mean of 5.92 mg L^{-1}. Groups 1 and 3 were very similar with mean concentrations of 8.27 and 8.25 mg L^{-1} respectively during spring. The range for each group was generally small with the exception of the Group 3 in summer which displayed a minimum of 4.64 and a relatively high maximum of 13.50 mg L^{-1}.

The vegetation scores of the three groups displayed limited variation between the groups and between seasons. During the summer sample the Group 1 wetlands displayed the highest mean vegetation score (2.90) while in winter the Group 2 wetlands had the highest mean (3.00). During spring Group 2 had the lowest mean (2.50) with Groups 1 and 3 displaying the same mean vegetation score of 2.88.
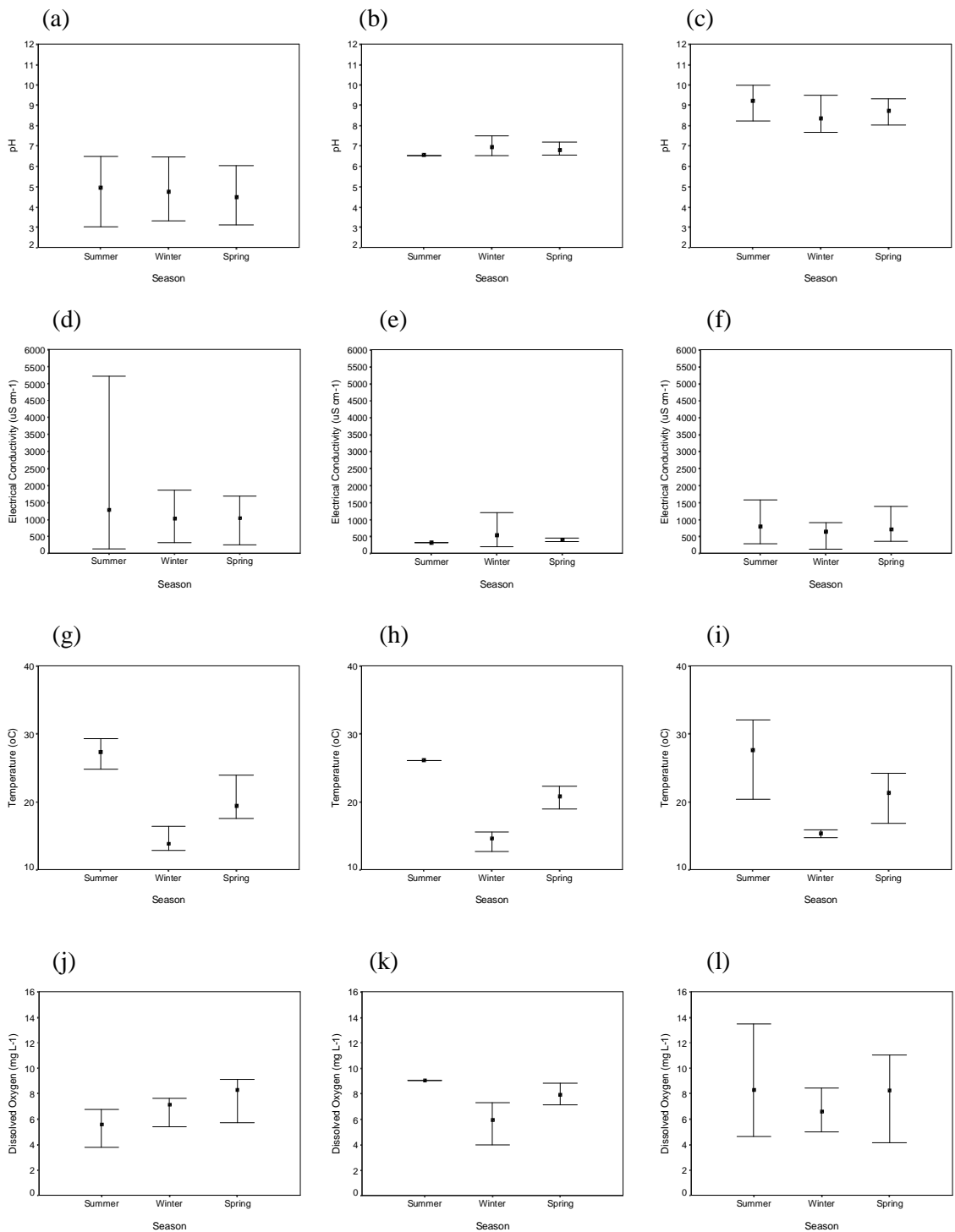
Figure 3.2: Minimum, mean and maximum readings of environmental variables over the three seasons with pH Groups 1 to 3 displayed from left to right. (a) – (c) pH (d) – (f) Electrical Conductivity (g) – (i) Temperature (j) – (l) Dissolved Oxygen. Means displayed are arithmetic with the exception of pH where geometric means have been calculated.

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3.4.3 Principal components analysis

Principal components analysis was used to group wetlands based on similarities in environmental variables (Figure 3.3a-c). Highly correlated variables have been represented by superimposed circles proportional in size to the variable gradient (Figure 3.4a-f). Ordination of the data from the summer sample determined that 35.10 % of the variance was explained by the first principal component (PC1) while 25.60 % of the variance was explained by the second principal component (PC2) (Figure 3a). Dissolved oxygen (Figure 4a) and pH (eigenvalues = -0.63 and -0.62 respectively) were the variables that displayed the strongest relationships with PC axis 1. Temperature was the variable most closely associated with PC axis 2 (eigenvalue = 0.75) (Figure 3.4b).

The first two principal components accounted for 70.30 % of the total variability in the winter data (Figure 3.3b). While temperature displayed the highest eigenvalue (-0.54) for PC axis 1 (Figure 3.4c), several other variables had relatively similar values. Vegetation score displayed a strong positive correlation along axis 2 with an eigenvalue of 0.824 (Figure 3.4d).

Principal component 1 explained 37.60 % of the total variability in the spring data while the second principal component accounted for 26.00 % of the variance (Figure 3.3c). With an eigenvalue of -0.566, temperature (Figure 3.4e) was the variable most closely related to axis 1 while pH was the parameter mostly closely correlated with axis 2 (eigenvalue = -0.580) (Figure 3.4f).

(a)

(b)

(c)
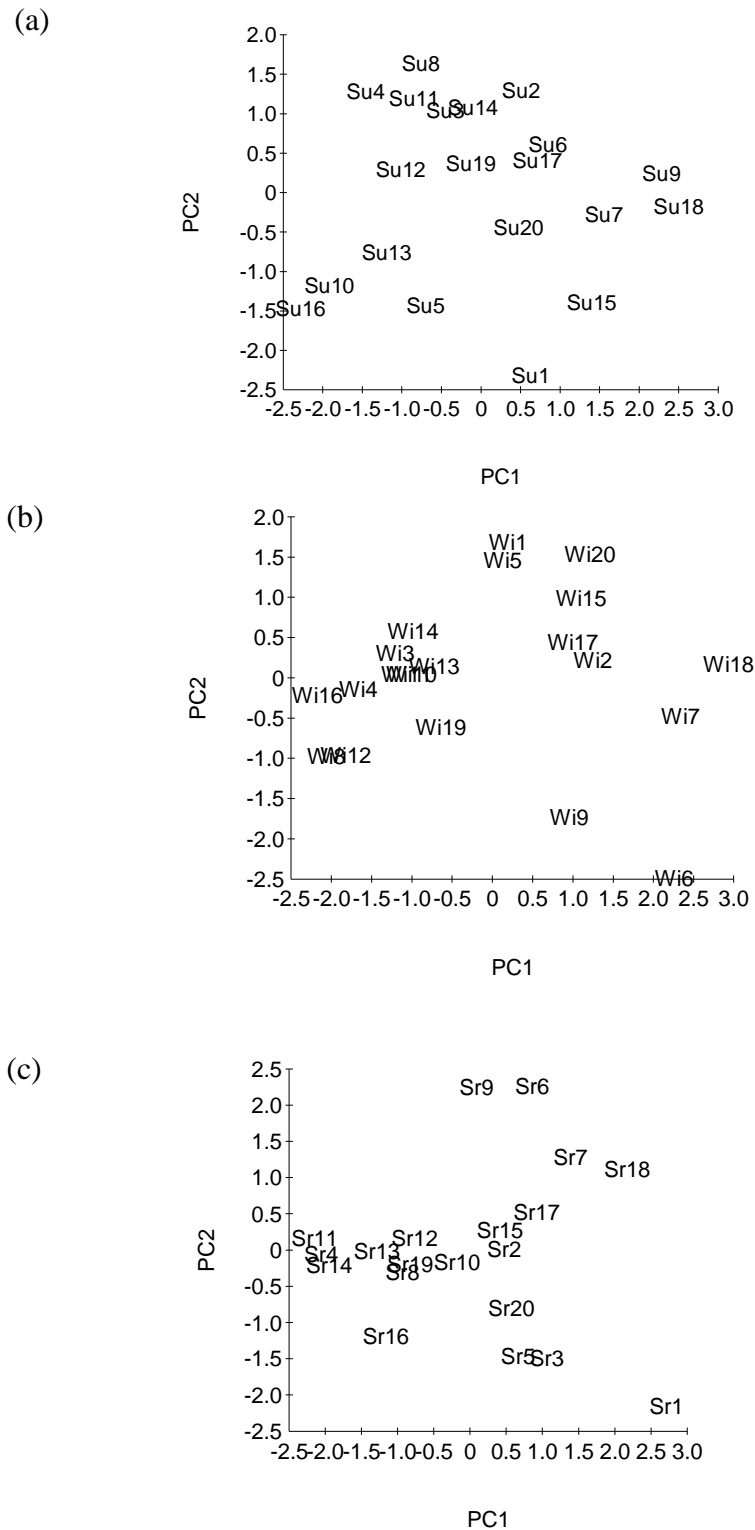
Figure 3.3: Two dimensional principal components analysis ordination of environmental parameters from the seasonal samples of the 20 sites sampled Electrical conductivity and dissolved oxygen concentration have been fourth root transformed. (a) summer sample (b) winter sample (c) spring sample. Site codes are preceded by the seasonal prefix Su to represent the summer sample, Wi to represent the winter sample and Sr to represent the spring sample

(a)

(b)

(c)

(d)

(e)

(f)
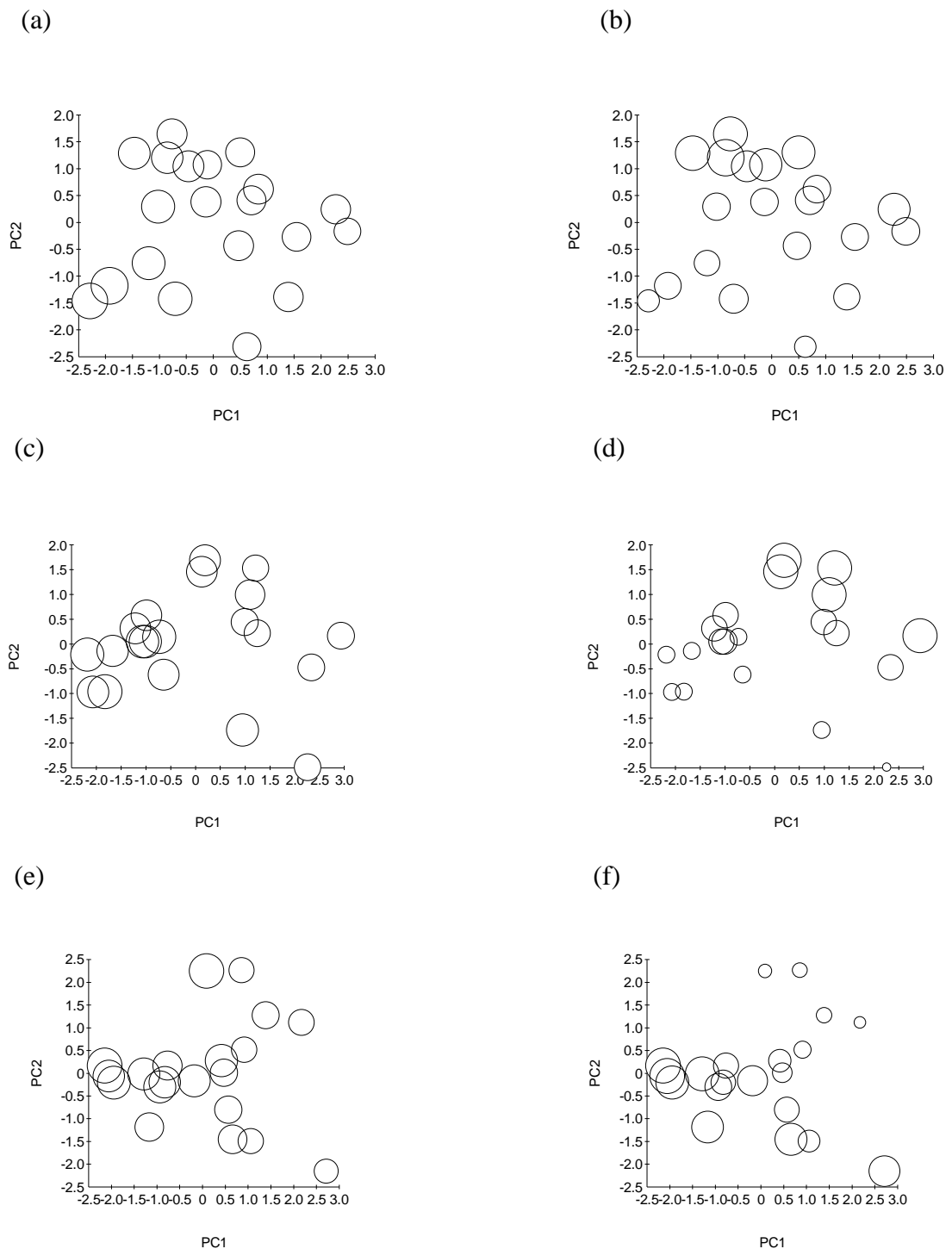
Figure 3.4: Principal components analysis ordination of environmental variables with superimposed circles representing the gradients of environmental parameters. Only variables with the highest eigenvectors for each axis have been displayed. (a) – (b) Summer data. (a) Dissolved oxygen overlay (b) Temperature overlay (c) - (d) Winter data (c) Temperature overlay (d) Vegetation score overlay (e) – (f) Spring data. (e) Temperature overlay (f) pH overlay.

3.5 Discussion

3.5.1 The range of environmental variables of the study sites

A wide range of pH values were recorded from the study sites with both the minimum and maximum pH measured falling outside the pH range of 6.5 - 9 most frequently recorded from natural wetlands (Boyd 1996). The lowest pH (3.01) was recorded from the Perth Metropolitan Region wetland of Gnangara Lake while a pH of 10.00 was measured from the Wagerup site of Blind Roo B. Gnangara Lake is a shallow water body (Davis *et al.* 1991) which has been permanently acidified since 1976 (Arnold 1990a). The pH decline has been attributed to the oxidation of acid sulphate soils, exposed as a result of decreasing water levels (McHugh 2004). Factors which have contributed to these water reductions include the positioning of a pine plantation to the east of the lake during the 1960s and low annual rainfall (Arnold 1990a). Disturbance caused by diatomite mining has also been implicated in the acidification of the lake and the remnants of the diatomaceous earth are still visible (John and Kupfer 1994). In contrast, the high pH of Blind Roo B is likely to be related to substrate type, with the site originally used for clay extraction.

A large range of electrical conductivities were also recorded from the wetlands, with a minimum of 125 uS cm^{-1} recorded at the Wagerup site of Blind Roo A and a maximum of 5230 uS cm^{-1} recorded at Gnangara Lake. A value of 1500 uS cm^{-1} is the recommended guideline trigger value for wetlands in the south-west of Western Australia (ANZECC and ARMCANZ 2000b) and in that context, the maximum of 5230 uS cm^{-1} could be considered relatively high. However, it is important to note that the value is not excessive when compared with the electrical conductivity of waters in the lower rainfall regions of the south-west (< 600 mm per year) where studies have recorded mean values in excess of 17 mS cm^{-1} (Kay *et al.* 2001).

The range of temperature displayed during the study was largely due to the seasonal changes with the lowest reading (12.70 °C) recorded during winter and the highest in summer (32.00 °C). Dissolved oxygen concentration also displayed a wide range of 3.81 – 13.5 mg L^{-1} with the minimum falling outside the range of 6 – 14 mg L^{-1} commonly recorded in wetlands (Connell 2005). The minimum value may be of concern as low dissolved oxygen concentrations are known to adversely affect

aquatic organisms such as fish, invertebrates and microorganisms. However, certain taxa are likely to be more sensitive to low levels of oxygen than others. For example, stonefly nymphs (Plecoptera) have higher oxygen demands than caddisfly (Trichoptera) larvae or Tubificid oligochaetes, which are generally tolerant of low dissolved oxygen concentrations (Abel 1989). Additionally, dissolved oxygen concentrations may undergo substantial diurnal fluctuations under natural conditions (ANZECC and ARMCANZ 2000b), possibly explaining the low minimum and relatively high maximum recorded.

The wetlands sampled during the study ranged from sparsely vegetated sites such as the mine-void lake Blue Waters (Site 6) (vegetation score = 1) through to moderately vegetated sites such as Bibra Lake (Site1) (vegetation score = 4). None of the wetlands included in the seasonal sampling regime were classified as densely vegetated (score = 5). This is probably a reflection of the increased urban development in wetland areas which is often coupled with the removal or destruction of fringing vegetation (Davis and Froend 1999).

3.5.2 Environmental variables in relation to the pH groups

The study revealed that the Group 1 wetlands maintained relatively stable pH values throughout the sampling periods. The minimum and maximum pH showed little variation between the seasons and although the mean pH was slightly higher in summer than in the other seasons, it remained below pH 5. Furthermore, the slightly higher mean pH of the summer sample is probably attributable to the larger number of wetlands classified as acidic during summer and the subsequent inclusion of sites displaying pH values near the upper limit of the category.

The mean pH of the Group 2 wetlands was relatively similar during winter and spring and this is likely to be partly related to the limited pH range defined as circumneutral (6.5-7.5). The limited pH range classified as circumneutral also that means that the circumneutral group is relatively unstable in terms of site numbers. For example, small pH fluctuations can result in wetlands moving into either the acidic or alkaline groups, as evidenced by the low number of sites in the circumneutral group during summer. These variations in the pH of the Group 2

wetlands between summer and the other seasons may have been related to pH changes associated with factors such as evapoconcentration (Boulton and Brock 1999).

In contrast to Group 2, the pH of the Group 3 wetlands peaked in summer before reaching the lowest mean and minimum levels in winter. Primary productivity is known to influence pH and the apparent seasonal trend in the Group 3 wetlands is likely to be related to the level of photosynthetic activity that occurs on sunny days (Boulton and Brock 1999). This would particularly affect Group 3, which contained eutrophic wetlands such as Lake Monger (Lund and Davis 2000), Blue Gum Lake (Fox and MacShane 2004) and Bibra Lake (Arnold 1990c).

The acidic wetlands (Group 1) displayed the highest electrical conductivities with the mean and maximum value for each season exceeding 1000 uS cm^{-1}. The conductivity of freshwaters generally ranges from 10 to 1000 uS cm^{-1} but can exceed 1000 uS cm^{-1} in polluted waters or those receiving large amounts of run-off (Chapman and Kimstach 1992). The inclusion of water bodies such as Stockton Lake and Ewington 2 that have been created by mining was probably reflected in the comparatively higher electrical conductivity of Group 1, as high values are characteristic of acid mine drainage (Kelly 1988). The inclusion of coloured sites may also have been a factor as organic acids are known to contribute to the conductance of water bodies (Boulton and Brock 1999). The presence of shallow seasonal wetlands would also have contributed to the higher mean electrical conductivity of the acidic sites (Group 1). For example, Schmidt and Rosich (1993) suggested that in the years when seasonal wetlands on the Swan Coastal Plain dried out, substantially greater fluctuations in solute concentration were experienced in comparison with permanent wetlands.. The high electrical conductivity of Gnangara Lake in particular, was probably a combination of the lake's mining history in conjunction with the presence of acid sulphate soils and low water levels. In contrast to the mostly high electrical conductivity of the Group 1 sites, Groups 2 and 3 displayed means within the range generally recorded for freshwaters (Chapman and Kimstach 1992).

Increases in conductivity commonly occur during the drying phase of a water body (Boulton and Brock 1999) and in the wetlands of south-western Australia larger values (> 3000 uS cm^{-1}) are often observed during summer (ANZECC and ARMCANZ 2000a). This pattern of increase was supported by the current study in which the highest conductivities were generally recorded during summer. For example, Gnangara Lake and Lakelands had electrical conductivities of 5230 and 2086 uS cm^{-1} respectively during summer sampling in contrast to the 1172 and 1219 uS cm^{-1} recorded in winter. The shallow nature of these wetlands coupled with high levels of groundwater abstraction and low rainfall are likely to have exacerbated the concentration of salts during summer.

There were no consistent differences in the temperature of the three pH groups and seasonal fluctuations followed normal climatic variation (Chapman and Kimstach 1992). Concomitantly, there were no obvious seasonal trends in dissolved oxygen concentration across the groups despite the influence of temperature on the solubility of oxygen. This lack of clear relationship is probably related to the wide range of environmental variables that influence or interact with the parameter. For example, dissolved oxygen concentration is largely influenced by factors such as salinity (Cale *et al.* 2004) and biological processes such as photosynthesis and respiration (Chapman and Kimstach 1992). It may also be affected by turbulence created through water movement or rainfall (Boulton and Brock 1999).

There was little difference in the mean and maximum vegetation scores of the three pH groupings. The littoral vegetation of many water bodies in the south-west region has been degraded or removed through activities such as agriculture and urban development (EPA 2006). Subsequently the similarities between the average vegetation scores of the three groups are probably partly attributable to the degradation of wetland vegetation throughout much of the south-west. For example, inflows from irrigation have increased water levels at the Wagerup site of Exelby Wetland (ENVIRON Australia 2005) and have been implicated in the death of fringing trees. A similar problem has occurred at the Perth Metropolitan Region site of Blue Gum Lake. Increased water levels in the lake related to urban development have resulted in the death of surrounding vegetation including *Eucalyptus rudis* and *Melaleuca rhaphiophylla* (Arnold 1990b). Additionally, the inclusion of mining

lakes, some of which were poorly vegetated, may have contributed to the relatively low vegetation scores. It is also important to note that any differences in the vegetation scores between the seasons could be attributed to the movement of some sites between pH groupings in response to seasonal pH variation.

3.5.3 Multivariate analyses

Principal components analyses demonstrated that the factors influencing the separation of wetlands varied according to season. Temperature was commonly among the major determinants of site separation while pH, dissolved oxygen concentration and vegetation score were also closely correlated to the distribution of the sites in various sampling periods. These findings are not surprising considering the generally complex relationships and interactions that occur between environmental variables. The results highlight the importance of various parameters in explaining the variance in the environmental data of the wetlands. The findings also suggest a level of seasonal variability for individual environmental parameters and consequently, the importance of seasonal sampling for the collection of water quality data in the region. It should be noted that these results are based on one-off measurements of the sites during each season and any differences may be a result of the separate sampling occasions rather than actual seasonal variation. In addition, the small number of sites included in the study, particularly in relation to the number of variables measured, may have influenced the reliability of the results and should be considered in future studies. Lastly, certain parameters such as dissolved oxygen should always be considered with caution because of high levels of variability. For example, dissolved oxygen is known to vary from early morning to night in response to changes in factors such as temperature and photosynthetic activity.

3.6 Conclusion

The environmental parameters of the selected wetlands generally displayed a wide range in terms of minimum and maximum values. The wide range of pH reflected the deliberate selection of acidic, circumneutral and alkaline wetlands. The range displayed by the other variables was probably attributable to the seasonal nature of the study in conjunction with factors such as geology, hydrology, surrounding land-uses and site origin.

The findings of the study suggest that aside from pH, electrical conductivity was the only parameter that displayed variation between pH groups, with higher means and maximums consistently recorded from Group 1. There were no obvious trends between the pH groupings and the remaining environmental variables. It is probable that the general lack of trend in the water quality parameters of the three pH groups partly reflected the heterogeneity of the wetlands within each group. The wetlands were grouped based on pH alone and subsequently each pH category contains sites which varied in terms of geography, surrounding land-use, substrate type, depth, vegetation density and composition. For example, the acidic Group 1 sites comprised shallow seasonal wetlands such as Lakelands and Gnangara Lake in the Perth Metropolitan Region through to deeper mine-void water bodies in the Collie Basin. Although there were some seasonal differences within individual pH groups, with the exception of temperature, there were no obvious seasonal trends in environmental variables observable across the groupings. This is probably also partly attributable to the heterogeneity of the wetlands and the movement of sites between pH groups in the various seasons.

Multivariate analyses showed that the factors explaining the most variance in the environmental data differed between seasons. As previously noted, this variation was also probably related to the heterogeneous nature of the study sites and highlights the potential influence of seasonal sampling on environmental variables.

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Appendix 3.1: The environmental parameters of the 20 study sites. pH (pH units), salinity (ppm), electrical conductivity (μS cm^{-1}), temperature (°C), dissolved oxygen (mg L^{-1}), vegetation score (1-5).

Site Code	Season	pH Group	pH	Salinity	Electrical Conductivity	Temperature	Dissolved Oxygen	Vegetation Score
Su1	Summer	3	9.84	896.00	1588.00	20.40	4.64	4
Su2	Summer	1	5.63	132.00	325.00	31.00	5.35	3
Su3	Summer	1	6.45	60.30	124.80	29.30	6.76	3
Su4	Summer	3	10.00	242.00	473.00	32.60	7.56	2
Su5	Summer	3	9.65	944.00	1666.00	27.40	9.54	4
Su6	Summer	1	4.03	594.00	1366.00	26.20	5.94	1
Su7	Summer	1	4.25	555.00	1282.00	25.60	5.15	3
Su8	Summer	3	8.22	151.00	302.00	32.00	6.19	2
Su9	Summer	1	3.01	3140.00	5230.00	30.20	5.68	2
Su10	Summer	3	8.88	397.00	729.00	25.40	13.50	3
Su11	Summer	3	9.46	337.00	637.00	34.60	7.26	3
Su12	Summer	2	6.54	165.00	316.00	26.10	9.03	2
Su13	Summer	3	8.93	506.00	917.00	24.20	8.92	2
Su14	Summer	3	8.24	142.00	284.00	30.50	4.95	3
Su15	Summer	1	6.15	1209.00	2086.00	24.80	5.67	4
Su16	Summer	3	9.78	270.00	503.00	20.90	11.87	2
Su17	Summer	1	5.53	185.00	447.00	27.10	5.21	3
Su18	Summer	1	3.31	451.00	1053.00	26.40	3.81	4
Su19	Summer	1	6.38	262.00	479.00	26.00	6.38	2
Su20	Summer	1	6.49	177.00	430.00	25.90	5.93	4
Wi1	Winter	3	8.48	324.00	764.00	14.90	7.50	4
Wi2	Winter	1	5.73	193.00	538.00	12.80	7.21	3
Wi3	Winter	2	6.64	73.80	202.50	14.80	5.42	3
Wi4	Winter	3	9.08	187.00	495.00	15.10	5.49	2
Wi5	Winter	3	7.79	246.00	683.00	14.80	6.92	4
Wi6	Winter	1	4.17	706.00	1866.00	12.90	7.52	1
Wi7	Winter	1	4.44	516.00	1381.00	12.90	7.32	3
Wi8	Winter	2	7.01	154.00	410.00	15.30	4.01	2
Wi9	Winter	1	3.86	461.00	1172.00	15.50	7.30	2
Wi10	Winter	3	7.66	361.00	923.00	15.60	5.02	3
Wi11	Winter	2	7.28	267.00	697.00	15.60	5.14	3
Wi12	Winter	1	6.30	111.00	315.00	16.40	5.40	2
Wi13	Winter	3	9.48	301.00	781.00	16.00	8.43	2
Wi14	Winter	2	7.50	104.00	281.00	14.80	6.04	3
Wi15	Winter	2	6.73	481.00	1219.00	14.30	6.93	4
Wi16	Winter	3	7.66	44.10	130.80	15.90	6.04	2
Wi17	Winter	1	6.45	187.00	523.00	13.10	7.47	3
Wi18	Winter	1	3.34	504.00	1350.00	13.00	7.64	4
Wi19	Winter	2	6.54	134.00	361.00	14.90	6.60	2
Wi20	Winter	2	6.92	179.00	503.00	12.70	7.32	4
Sr1	Spring	3	8.23	663.00	1386.00	16.90	4.17	4
Sr2	Spring	1	5.26	195.00	447.00	19.00	8.27	3
Sr3	Spring	1	5.86	115.00	249.00	17.70	5.72	3
Sr4	Spring	3	9.29	187.00	395.00	21.90	10.18	2
Sr5	Spring	3	8.57	363.00	780.00	20.00	6.60	4
Sr6	Spring	1	3.98	686.00	1485.00	17.60	9.11	1
Sr7	Spring	1	4.16	637.00	1380.00	18.80	8.69	3
Sr8	Spring	2	7.19	201.00	423.00	22.30	7.15	2
Sr9	Spring	1	3.59	802.00	1689.00	23.90	8.28	2
Sr10	Spring	3	8.04	523.00	1124.00	22.30	7.42	3
Sr11	Spring	3	9.30	289.00	603.00	24.20	11.03	3
Sr12	Spring	2	6.73	177.00	392.00	20.20	8.81	2
Sr13	Spring	3	9.03	360.00	785.00	22.30	8.35	2
Sr14	Spring	3	8.81	168.00	355.00	22.90	10.70	3
Sr15	Spring	1	6.04	526.00	1125.00	22.50	8.27	4
Sr16	Spring	3	8.50	78.00	179.50	19.90	7.57	2
Sr17	Spring	1	4.59	258.00	582.00	17.70	9.02	3
Sr18	Spring	1	3.12	618.00	1332.00	18.00	8.83	4
Sr19	Spring	2	6.56	156.00	351.00	21.80	7.36	2
Sr20	Spring	2	6.66	195.00	447.00	19.00	8.27	4

Chapter 4: Invertebrates in wetlands in the south-west of Western Australia: community structure with reference to pH.

4.1 Abstract

Wetlands in the south-west of Western Australia were sampled over three seasons with the intention of examining the influence of pH and other environmental variables on invertebrate community structure. Multivariate analyses revealed differences in the invertebrate community composition based on geographical region and pH groupings. The largest differences in regions were evident between the Wagerup and Collie sites and were partly attributable to pH. The wetlands were separated into three pH groups – Group 1 (acidic), Group 2 (circumneutral) and Group 3 (alkaline) and differences in the invertebrate assemblages of the three pH groups were greatest between Group 1 and Group 3 wetlands. Differences were also detected between the Group 2 and Group 3 sites, whereas the community structure of the Group 1 and Group 2 wetlands was similar. The variable of pH was identified as the single factor most closely associated with invertebrate community composition, although a combination of variables including pH generally produced stronger correlations between environmental variables and biotic patterns. This suggests that while invertebrates are sensitive to pH, they are also likely to be influenced by other variables. While many of the dominant invertebrate taxa were shown to have reasonably broad pH tolerances, a limited number of potential indicator taxa were identified from each of the three pH groupings. The sampling regime revealed an inconsistent pattern of seasonal variation at a community level, although there was some evidence of differences in individual taxa. This generally suggests that the influence of pH on overall invertebrate community structure should be evident regardless of season.

4.2 Introduction

The previous chapter reported the relationship between pH and other environmental variables in 20 wetlands in the south-west of Western Australia. This chapter explores the relationship between the environmental parameters, especially pH, and the distribution pattern of invertebrate communities.

Changes in surrounding land-use practices including groundwater abstraction, urban development and drainage in agricultural areas have lead to an increased risk of acidification in water bodies in south-western Australia (Sommer and Horwitz 2001; WRC and DEP 2002; Smith *et al.* 2004). This process has the potential to adversely impact on the aquatic biota of the region with depressions in pH often resulting in shifts in community composition (Webster *et al.* 1992) or loss of sensitive species (Harvey 1989).

Invertebrates are among the organisms used as biological indicators of acidification (Fjellheim and Raddum 1990; Sandin *et al.* 2004). However, while extensive research into the influence of pH on invertebrate communities has been conducted throughout Europe (Dangles and Guérold 2000), the United States (Bradford *et al.* 1998; Madarish and Kimmel 2000) and Canada (McNicol *et al.* 1995) published work in this area in Australia has been limited. Previous Australian studies have concentrated on a specific invertebrate group (Cranston *et al.* 1997) or have focused on wetlands over a restricted pH range (Bayly 1964; Bayly *et al.* 1975).

In Western Australia specifically, studies have tended to focus on relatively small geographical areas (Pusey and Edward 1990; Bayly 1992b; Sommer and Horwitz 2001; Woodhouse 2004; McKay and Horwitz 2006; Thomas and John 2006). Furthermore, while a number of larger studies conducted on aquatic invertebrate communities have measured pH as part of their sampling regime (Growns *et al.* 1993; Kay *et al.* 2001; Pinder *et al.* 2004), it has rarely been the specific focus of the research. The present study incorporates wetlands from a relatively wide region of the south-west of Western Australia that range from acidic to alkaline. The sites

selected include acidic mining lakes, shallow seasonal wetlands and anthropogenically modified alkaline lakes.

The main objective of the present chapter was to investigate invertebrate assemblages along a pH gradient and identify differences between the community structures of wetlands with varying pH. The chapter aimed to assess the sensitivity of invertebrates to pH and identify potential indicator taxa for different pH categories. A further objective of the chapter was to examine any seasonal differences in invertebrate communities and assess the impact of season on their effectiveness as pH biomonitors.

4.3 Methods

4.3.1 Sampling

A total of 20 sites were included in the study (Figure 4.1) and their water chemistry and aquatic invertebrates were sampled on three occasions: summer (December – February), winter (June – August) and spring (September - November) of 2001. The environmental variables of pH, electrical conductivity (μS cm^{-1}), salinity (ppm), temperature (ºC), dissolved oxygen (mg L^{-1}) and vegetation score were recorded during each sampling event.

Invertebrate sampling involved the use of a D-framed 250 μm mesh net which was swept through the sediment along a 10 m transect, agitating the substrate and vegetated areas within the littoral zone to dislodge invertebrates. Depth of the sampling sites did not exceed 1 m. Samples were preserved in the field using 4% formalin. While the original intention of the sampling was to collect macroinvertebrates, the technique also resulted in the collection of zooplankton and these specimens were therefore included in the study.

The collected material was sub-sampled in the laboratory using a box sub-sampler based on Marchant (1989). The sub-sampler was sealed and agitated to evenly distribute the material. A number was randomly selected and the contents of the corresponding cell removed using a vacuum pump. Sub-sampling continued until

200 invertebrates had been collected or the entire sample had been sorted. Specimens were preserved in 70% ethanol with the frequency of each taxa being recorded on a dedicated tally sheet.

Identification was carried out to species level where possible using specialised literature including Edward (1964), Watts (1978); Williams (1980); Benzie (1988), Bayly (1992a) Lansbury (1995), Shiel (1995), Dean and Suter (1996), Davis and Christidis (1999), Hawking and Smith (1997), Hawking and Theischinger (1999), Suter (1999), St Clair (2000), Watts (2002), Andersen and Weir (2004) and Dean *et al.* (2004). The sub-family Ceratopogoninae was separated into morphotypes and the groups Oligochaeta, Nematoda and Cyclopoida were not identified further. Voucher specimens were placed in a voucher collection located at the Department of Environmental Biology, Curtin University of Technology. The complete list of taxa and relevant authorities has been included in Appendix 4.1.
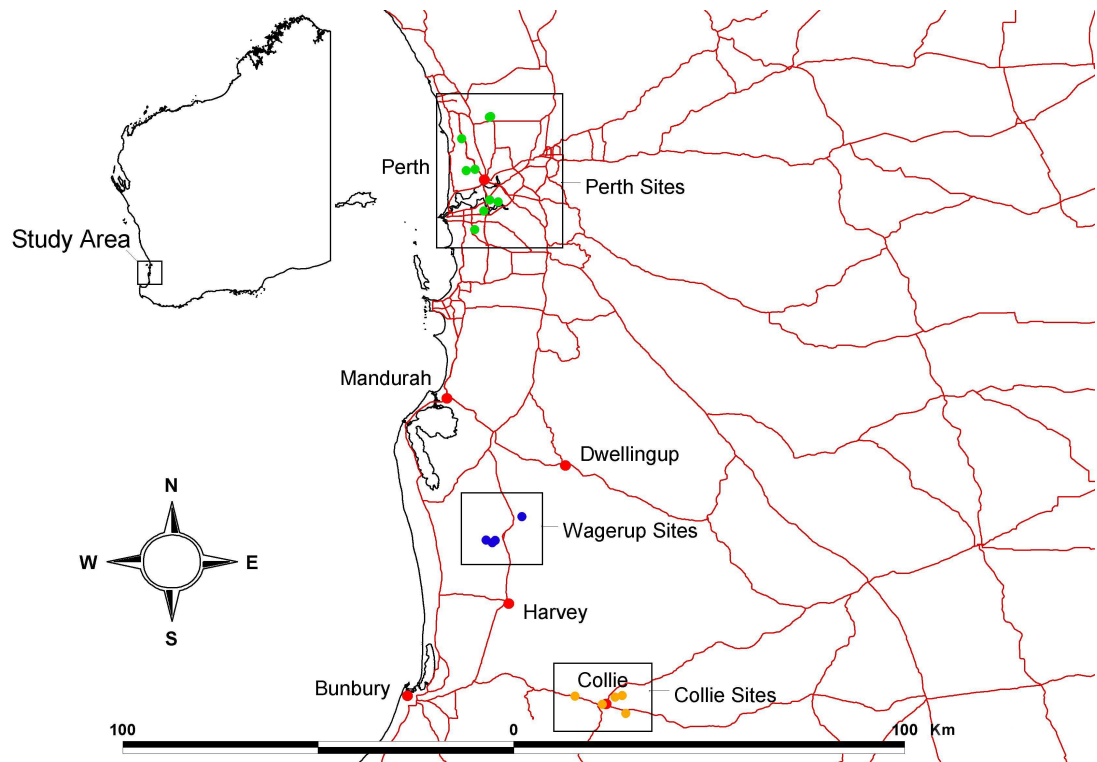
Figure 4.1: Location of the 20 sites in the south-west of Western Australia selected for the seasonal study. Green points indicate the sites located in the Perth Metropolitan Region. Blue points indicate study sites located in the Wagerup area. Yellow points represent sites located in the Collie Basin.

4.3.2 Data analysis

Multivariate analyses were employed to investigate invertebrate community structure. Taxa abundance data were fourth root transformed to increase the importance of moderately (intermediate) abundant taxa (Clarke and Warwick 2001) and similarity matrices were constructed using the Bray Curtis similarity measure (Bray and Curtis 1957). Ordinations produced by non-metric multi-dimensional scaling (MDS) (Kruskal and Wish 1978) were used to identify groups of wetlands with similar invertebrate assemblages. One way analysis of similarity (ANOSIM) (Clarke and Green 1988) was performed to determine the level of separation and significance of trends in invertebrate distribution patterns ($p < 0.05$). A maximum of 999 permutations were used to calculate the probability of the observed values for each analysis. R statistics generated from the ANOSIM analyses have values of approximately zero if there are no differences between the groups. R = 1 indicates that differences among the sites in a group are less than the differences between groups (Clarke and Warwick 2001). BIO-ENV (Clarke and Ainsworth 1993) was employed to elucidate the relationship between the environmental variables and biotic patterns. The procedure uses the Spearman rank correlation coefficient (p_s) to identify the combinations of environmental variables which produce matrices most highly correlated with the matrices based on the biotic data. Significance was accepted at $p < 0.05$ and $p < 0.01$. All analyses were conducted using the software package PRIMER 5.0 for Windows Version 5.2.9 (PRIMER-E Ltd 2002).

4.4 Results

4.4.1 Invertebrate taxa

A total of 165 invertebrate taxa were recorded during the study, although this number would be likely to increase if all groups were identified to species level. Ninety nine taxa were collected during summer sampling with 76% belonging to the class Insecta. The orders Diptera and Coleoptera were the most diverse groups of insects, contributing 20 and 23 taxa respectively (Appendix 4.1). Chironomids dominated the dipteran fauna with 14 species while the family Dytiscidae was the most speciose group of coleopterans (15 taxa). The class Crustacea accounted for a further 16% of

invertebrates collected during summer with ostracods making up almost half of that number (Appendix 4.1).

A small decrease in taxa richness was evident in winter with a total of 92 invertebrate groups collected. Insects remained the most diverse group accounting for 61% of the taxa (56) and the proportion of crustaceans increased to 29%. Ten of the 27 crustacean taxa belonged to the subclass Ostracoda, followed by the suborder Cladocera with nine (Appendix 4.1). Calanoid copepods contributed a further four species to the taxa list. Dipterans were the most abundant group of insects with 25 taxa and were dominated by chironomids (16 taxa) and ceratopogonids (7 taxa). A total of 20 coleopteran taxa were recorded during the winter sample with Dytiscidae remaining the dominant family.

The highest taxa richness was recorded in spring with a total of 111 taxa (Appendix 4.1). Insects represented more than 65% of the taxa (73 taxa) and were dominated by dipterans (25 taxa) and coleopterans (24 taxa). Chironomids accounted for the majority of dipterans with greater than 60% of the taxa and dytiscids accounted for a similar percentage of the coleopteran taxa. An additional 25 taxa were recorded from the class Crustacea, in which cladocerans and ostracods were the most common groups (8 and 10 taxa respectively).

4.4.2 Dominant invertebrates of the wetland pH groups

Taxa that were recorded from at least two sites in a pH group with an abundance of $\geq$ 10% were deemed common (Table 4.1). Crustaceans were a major component of the invertebrate fauna in the Group 1 wetlands with abundant taxa including *Calamoecia tasmanica subattenuata*, Cyclopoida and *Macrothrix indistincta*. However, few of these taxa were restricted to the acidic wetlands. *Calamoecia tasmanica subattenuata* was also abundant in the circumneutral wetlands of Group 2 during winter sampling and cyclopoid copepods were recorded in high numbers from Group 3 wetlands during the same season. In contrast, *Macrothrix indistincta* was an abundant taxon in the acidic wetlands only and was collected during all three seasons.

Dipteran larvae were the dominant insects of the Group 1 wetlands with ceratopogonids and the chironomid *Tanytarsus fuscithorax/semibarbitarsus* both seasonally abundant in the acidic waters. Larvae of the dytiscid beetle *Sternopriscus* sp were the only other insects classified as frequently occurring in Group 1. Other taxonomic groups commonly collected from Group 1 included Nematoda and Oligochaeta although neither taxon was restricted to acidic waters. Nematodes were also found commonly in the spring sample of the Group 2 wetlands and oligochaetes were abundant in the winter samples of Group 2 and the summer samples of Group 3 wetlands (Table 4.1).

Similarly to the Group 1 wetlands, crustaceans were among the dominant invertebrates of the circumneutral Group 2 wetlands. *Calamoecia tasmanica subattenuata* and the cladocerans *Daphnia carinata* and *Alona quadrangularis* were all frequently recorded during the winter sample. However while *Calamoecia tasmanica subattenuata* and *Daphnia carinata* were also abundant in other pH groups (Group 1 sites and Group 3 respectively), *Alona quadrangularis* was only commonly recorded from Group 2. Nematodes and oligochaetes were also frequently collected from the Group 2 wetlands but, as previously established, were not restricted to these conditions (Table 4.1).

The dominant invertebrates of the alkaline Group 3 wetlands represented a shift from those in Groups 1 and 2. Ostracods were the most numerous crustaceans in Group 3 with *Candonocypris novaezelandiae* common in spring and *Sarscypridopsis aculeata* abundant in all three seasons. Cyclopoid copepods were commonly found in winter but were also abundant in the spring sample of the Group 1 wetlands. Frequently recorded insects in Group 3 waters included juvenile hemipterans from the family Corixidae, the chironomid *Polypedilum nubifer* and the ephemeropteran *Tasmanocoenis tillyardi*. Oligochaetes completed the list of common Group 3 taxa but were not exclusively abundant in alkaline wetlands (Table 4.1).

Table 4.1: Summary of the dominant invertebrate taxa of each pH group of wetlands sampled in the south-west of Western Australia over the three sampling occasions. Taxa included were present in at least two sites within a group with an abundance of $\geq$ 10% .

Taxa	Summer Group 1	Summer Group 3	Winter Group 1	Winter Group 2	Winter Group 3	Spring Group 1	Spring Group 2	Spring Group 3
Alona quadrangularis				X				
Calamoecia tasmanica subattenuata	X		X	X		X		
Candonocypris novaezelandiae								X
Corixidae (juvenile)								X
Ceratopogoninae 2						X		
Ceratopogoninae 7	X							
Cyclopoida					X	X		
Daphnia carinata				X	X			
Macrothrix indistincta	X		X			X		
Nematoda	X		X				X	
Oligochaeta	X	X	X	X				
Polypedilum nubifer		X						
Sarscypridopsis aculeata		X			X			X
Sternopriscus sp. larvae						X		
Tanytarsus fuscithorax/semibarbitarsus	X					X		
Tasmanocoenis tillyardi		X						X

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4.4.3 Multivariate analyses

Multi-dimensional scaling based on invertebrate abundance data revealed a moderate level of grouping among sites when overlaid with symbols for the three regions (Figure 4.2a-c). Wagerup sites generally grouped together and were mostly well separated from the Collie wetlands. The Perth Metropolitan Region wetlands occupied an intermediate position in the ordinations, displaying overlap with both the Wagerup and Collie sites (Figure 4.2a-c). One-way ANOSIM conducted on the invertebrate assemblages of the regions detected significant differences during each season ($p < 0.01$) (Table 4.2). Pairwise comparisons showed that the Wagerup sites displayed significantly different invertebrate assemblages to the other regions during summer ($p < 0.01$). However, the community structure of the Wagerup wetlands was similar to that of the Perth sites during the remaining seasons. The Perth wetlands exhibited similar assemblages to the Collie wetlands during summer, supporting the less defined grouping of the Collie sites (Figure 4.2a) but were significantly different in the other seasons. The R statistics for the significant pairwise comparisons were generally low ($R < 0.50$), indicating that the differences in community structure were not substantial. The differences between the Collie and Wagerup sites were an exception, with a relatively high R value of > 0.70 recorded for spring and R values exceeding 0.50 for the other seasons.

The ordinations of the sites overlaid with the pH groups displayed relatively similar patterns for the Group 1 and Group 3 wetlands during the seasons (Figures 4.2d-f). The acidic Group 1 wetlands were generally positioned at opposite sides of the axes to the alkaline Group 3 wetlands, although the separation was not as clearly defined during summer. The summer data displayed some overlap with acidic sites including Blind Roo A (Su3), Lakelands (Su15), Stockton Lake (Su17) and Wallsend (Su20) situated in close proximity to alkaline sites such as Blue Gum Lake (Su5) and Blind Roo B (Su4) (Figure 4.3a). The separation of the acidic sites was stronger during winter and spring (Figure 4.2e-f) with only Kurrajong Village Lake (Wi12) and Lakelands (Sr15) displaying overlap in the respective ordinations (Figure 4.3b-c). Little pattern was evident for Group 2 during summer as only one site was classified as circumneutral but during the remaining seasons Group 2 wetlands maintained an intermediate position (Figure 4.2d-f).
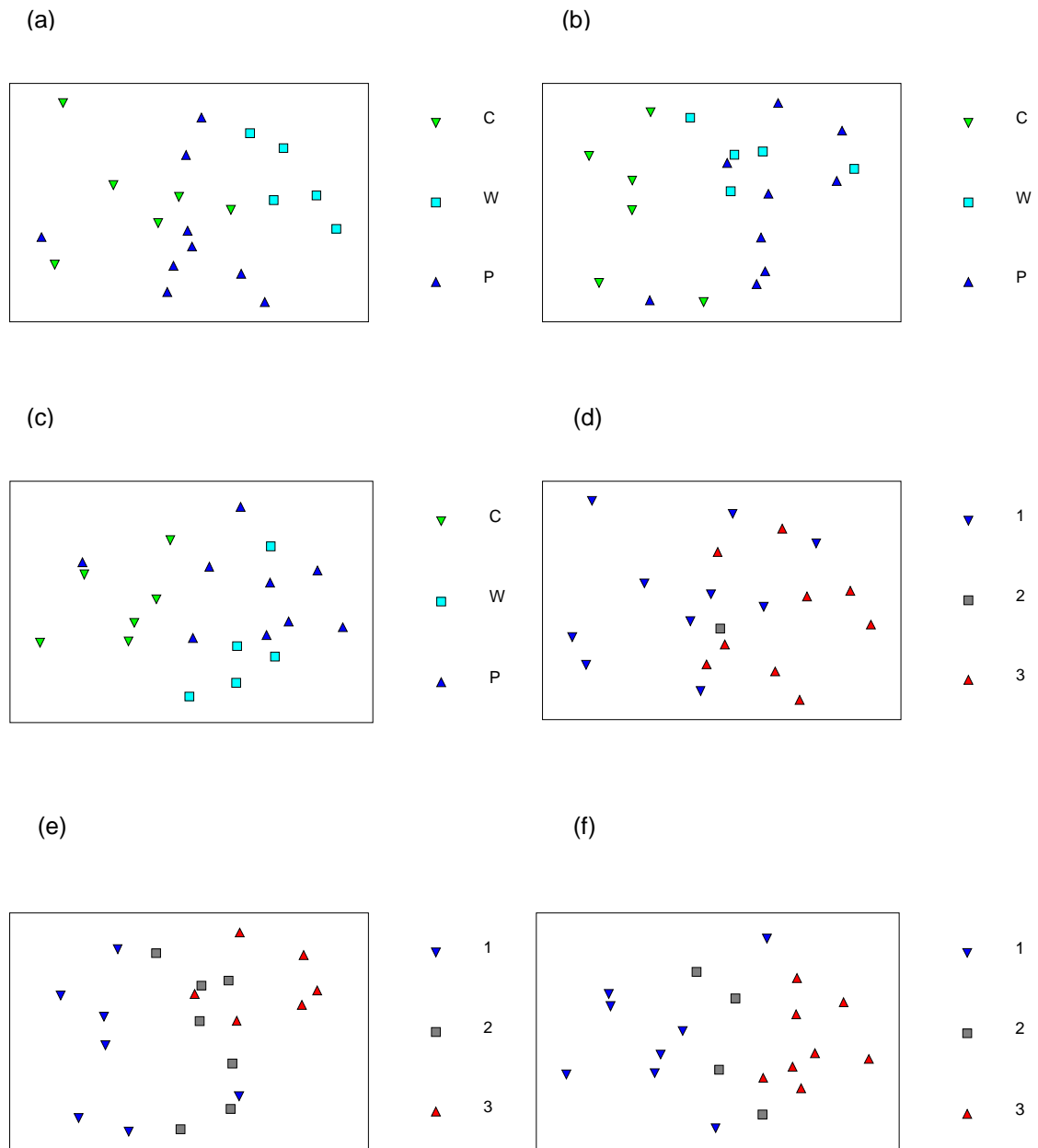
Figure 4.2: Two dimensional MDS of fourth root transformed invertebrate abundance data with superimposed symbols representing the three regional groups and three pH groups. (a) Regional overlay of summer sample, stress = 0.17. (b) Regional overlay of winter sample, stress = 0.20. (c) Regional overlay of spring sample, stress = 0.17. C represents Collie sites, W represents Wagerup sites and P represents Perth Metropolitan sites. (d) – (f) Overlays of the three pH groups for the summer, winter and spring data respectively.
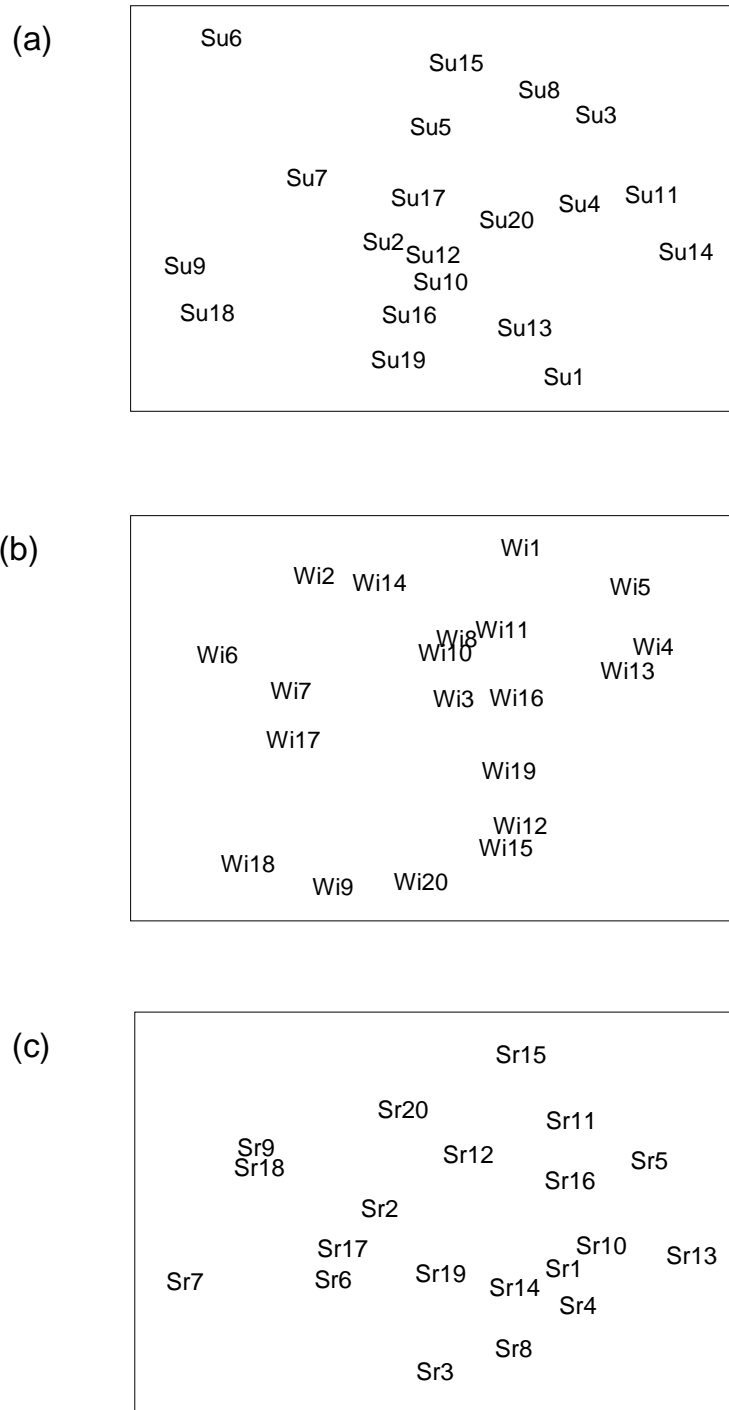
Figure 4.3: Two dimensional Multi-dimensional scaling ordination of fourth root transformed invertebrate abundance data. (a) summer sample, stress = 0.17. (b) Winter sample, stress = 0.20. (c) Spring sample, stress = 0.17. Site codes are preceded by the seasonal prefix Su to represent the summer sample, Wi to represent the winter sample and Sr to represent the spring sample.

Table 4.2: Results of global and pairwise tests from one-way analyses of similarities (ANOSIM) based on Bray-Curtis similarities in fourth root transformed invertebrate abundance data from the three geographical regions sampled in the south-west of Western Australia. Bold type indicates significant difference ($p < 0.05$).

Season	Location	R	Probability
Summer	All Regions	0.32	**< 0.01**
	Perth Metropolitan, Collie	0.09	> 0.05
	Perth Metropolitan, Wagerup	0.43	**< 0.01**
	Collie, Wagerup	0.54	**< 0.01**
Winter	All Regions	0.39	**< 0.01**
	Perth Metropolitan, Collie	0.49	**< 0.01**
	Perth Metropolitan, Wagerup	0.19	> 0.05
	Collie, Wagerup	0.54	**< 0.01**
Spring	All Regions	0.39	**< 0.01**
	Perth Metropolitan, Collie	0.42	**< 0.01**
	Perth Metropolitan, Wagerup	0.15	> 0.05
	Collie, Wagerup	0.74	**< 0.01**

Results of the ANOSIM analyses based on invertebrate community structure and pH groups detected significant differences in each season (Table 4.3). The largest differences in community structure were evident between the Group 1 and Group 3 wetlands ($p < 0.05$) with R statistics of > 0.50 generated during the winter and spring sampling period. Given that only one wetland in the summer sample was identified as circumneutral, Group 2 was excluded from the summer ANOSIM analysis. Inclusion in the winter and spring ANOSIM tests determined that Groups 2 and 3 contained differing invertebrate assemblages ($p < 0.05$). In contrast, the community structure of Groups 1 and 2 were similar during both the winter and spring sampling periods (Table 4.3).

Table 4.3: Results from global and pairwise tests from one-way analyses of similarities (ANOSIM) based on Bray-Curtis similarities in fourth root transformed invertebrate abundance data from the three pH groups of wetlands sampled in the south-west of Western Australia. Bold type indicates significant difference ($p < 0.05$).

Season	pH Groups	R	Probability
Summer	Group 1, Group 3	0.24	**< 0.01**
Winter	All Groups	0.37	**< 0.01**
	Group 1, Group 2	0.22	> 0.05
	Group 1, Group 3	0.58	**< 0.01**
	Group 2, Group 3	0.30	**< 0.08**
Spring	All Groups	0.50	**< 0.01**
	Group 1, Group 2	0.09	> 0.05
	Group 1, Group 3	0.68	**< 0.01**
	Group 2, Group 3	0.48	**<0.01**

The BIO-ENV analyses between environmental variables and invertebrate abundance data indicated that pH was the single variable most closely correlated with the invertebrate distribution patterns for each season (Table 4.4). The combination of variables that produced the strongest correlation varied between sampling periods. The strongest correlation ($\rho_s = 0.46$) for the summer BIO-ENV was produced by a combination of vegetation score, pH, electrical conductivity and temperature. The biotic patterns observed in the winter data were best explained by the variable combination of pH and temperature ($\rho_s = 0.59$). The BIO-ENV conducted on the spring data revealed that pH produced the strongest correlation with the faunal distribution ($\rho_s = 0.68$) (Table 4.4).

Table 4.4: BIO-ENV results giving the combinations of environmental variables with the highest rank correlations between the abiotic and the fourth root transformed invertebrate similarity matrices as measured by Spearman rank correlation (ρ_s). A correlation cut-off of $p_s < 0.40$ was applied. The strongest correlation is presented in bold type. Veg = vegetation score, EC = electrical conductivity, Temp = Temperature, DO = Dissolved oxygen.

Season	n	Variables	p_s
Summer	4	**Veg, pH, EC, Temp**	**0.46**
	2	pH, EC	0.45
	3	pH, EC, Temp	0.43
	3	Veg, pH, EC	0.43
	5	Veg, pH, EC, Temp, DO	0.41
	1	pH	0.41
Winter	2	**pH, Temp**	**0.59**
	3	Veg, pH, Temp	0.52
	1	pH	0.52
	3	pH, EC, Temp	0.50
	4	Veg, pH, EC, Temp	0.46
	3	pH, Temp, DO	0.43
	2	Veg, pH	0.42
	4	Veg, pH, Temp, DO	0.41
Spring	1	**pH**	**0.68**
	2	pH, EC	0.52
	2	pH, Temp	0.48
	3	pH, EC, Temp	0.44
	2	pH, Veg	0.44
	2	pH, DO	0.41
	3	pH, EC, Veg	0.41

4.5 Discussion

4.5.1 Invertebrate taxa

The majority of taxa collected over the three seasons belonged to the class Insecta, supporting similar findings by previous Western Australian studies (Davis and Rolls 1987; Pusey and Edward 1990; Kay *et al.* 1999). Coleoptera and Diptera were the most diverse orders within the class, although the proportions changed according to season. Coleopterans were the most speciose group in the summer sample, whereas a higher number of dipteran taxa were present during the winter and spring sampling events. These orders are both well represented in aquatic systems (Gullan and Cranston 2005) and studies on Western Australian waters have frequently identified them among the most diverse insects. For example, high diversities of coleopterans and dipterans have been recorded from wetlands on the Swan Coastal Plain (Davis and Rolls 1987; Growns *et al.* 1992; Balla and Davis 1995). The two groups were also identified as the most diverse orders in a study of water bodies in the north-west of the state (Kay *et al.* 1999).

A large proportion of the coleopterans recorded during the present study belonged to the Dytiscidae, a family noted to be particularly abundant in the littoral zones of small freshwater ponds Watts (1978). The family Chironomidae, which is widely distributed and commonly abundant in the wetlands of south-western Australia (Davis and Christidis 1999), was the most speciose dipteran family. Chironomids were also found to be the dominant dipterans during a survey of wetlands and rivers in the southern Carnarvon Basin (Halse *et al.* 2000).

Following the class Insecta, crustaceans displayed the next highest diversity. A high proportion of the crustacean taxa collected in the study belonged to the subclass Ostracoda, a group considered to be reasonably diverse in the waters of temperate Australia (Gooderham and Tsyrlin 2002). The taxon was similarly identified as an important component of the invertebrate fauna in a previous study in south-western Australia (Davis and Rolls 1987).

The overall taxa richness varied seasonally, with the highest number recorded in spring and the lowest in winter. These seasonal changes in taxa numbers corresponded with the findings of previous research in south-western Australia. Balla and Davis (1993) noted that the highest species richness generally occurred in spring and early summer, coinciding with the highest water levels. A later study by Sommer and Horwitz (2001) on Lake Jandabup also attributed the higher number of invertebrate families recorded during spring to the higher water levels and the subsequent increase of submerged habitat.

4.5.2 Invertebrate taxa in relation to the pH groups

Separation of the wetlands into the three pH groupings revealed sixteen dominant taxa across the three wetland groups. Plates of these taxa or their distinguishing body parts have been presented in Appendix 4.2. The abundant organisms comprised insects, crustaceans, nematodes and oligochaetes, all of which are commonly recorded groups in wetlands of south-western Australia (Growns *et al.* 1993).

Despite the sensitivity of some crustaceans to low pH (Schindler 1987; Harvey 1989; Stokes *et al.* 1989; Johnson *et al.* 1993; Bradford *et al.* 1998; Vrba *et al.* 2003) crustacean taxa formed an important component of the acidic Group 1 wetland assemblages. For example, the cladoceran *Macrothrix indistincta* was recorded exclusively from the Group 1 wetlands and was the only invertebrate to display a dominant presence in acidic waters during each season. The genus *Macrothrix* is commonly associated with acidic waters (Timms 1986; Branco *et al.* 2000) but information on the distribution of *Macrothrix indistincta* specifically is currently limited. Aside from the current study, the taxon has been collected from two gnammas or rock pools on granite outcrops in Western Australia (Bayly 1997). One of these sites was acidic with a pH of 5.7 while the other displayed a pH of 8.7 (Bayly 1997). This record implies that the taxon may be tolerant over a broad range of pH, which would thereby limit its ability to act as an effective indicator of pH. Consequently, while the results of the present study suggest that *Macrothrix indistincta* is acidophilic, further research on the taxon's distribution and abundance is required before an assessment of its potential as an indicator can be made.

Calamoecia tasmanica subattenuata was also recorded frequently from acidic wetlands. The taxon, which is a commonly recorded Western Australian endemic species (Maly *et al.* 1997), was described by Bayly (1992a) as generally preferring acid-humic conditions. During the summer sample, the taxon was recorded from acidic wetlands with pHs ranging from 4.03 – 6.49. Conversely, during winter *Calamoecia tasmanica subattenuata* occurred commonly in both 1 and Group 2 wetlands. Growns *et al.* (1993) similarly recorded the taxon from both acidic and circumneutral sites during their study on wetlands of the Swan Coastal Plain, generally reporting high numbers from the more coloured waters.

The results of the present study, in conjunction with past studies, suggest that while *Calamoecia tasmanica subattenuata* is not representative of either acidic or circumneutral conditions exclusively, the taxon appears to favour waters in these pH ranges. Subsequently while the species cannot be used as a specific indicator of either pH category it may still have applications for the biomonitoring of pH decrease.

The dominance of the taxon in both Group 1 and Group 2 wetlands during winter is probably related to the increased number of sites with higher water levels rather than any seasonal pH changes. Many calanoid copepods produce diapause eggs which remain dormant for extended periods and may be resistant to stresses such as desiccation (Couch *et al.* 2001). In the current study, the wetlands containing high numbers of *C. tasmanica subattenuata* during summer were among the deeper sites sampled. In contrast, during winter the sites containing *C. tasmanica subattenuata* expanded to include shallow waters such as Lakelands and Knapping Wetland, which experience drying during summer and autumn. These findings suggest that the species may persist in seasonal systems through the production of diapause eggs or other dormant life stages. Adaptation to seasonal drying in Western Australian wetlands has previously been demonstrated by Balla and Davis (1993) who recorded calanoid copepods after reflooding a dried wetland substrate in the laboratory.

Cyclopoid copepods were the other crustaceans identified as abundant in the Group 1 wetlands, occurring frequently in the spring samples. However despite their frequency in the Group 1 wetland sites they appeared indifferent to pH, with high

numbers also recorded from the alkaline Group 3 wetlands. Further taxonomic resolution is necessary before an accurate assessment on their potential as indicators can be made as their response to pH may differ at the lower taxonomic levels.

The dominant insects of the Group 1 wetlands belonged to the orders Diptera and Coleoptera, both of which are known to contain families tolerant of low pH (Welch 1936; Roback 1974; Berezina 2001; Rodrigues and Scharf 2001). Among the abundant taxa were Ceratopogoninae larvae, a subfamily of the Ceratopogonidae or biting midges (Gullan and Cranston 2005). Ceratopogonids have been previously documented from acidic waters in regions such as Australia (Trayler and Davis 1998; Sommer and Horwitz 2001; Pinder *et al.* 2004) and Japan (Sasaki *et al.* 2005). These findings coupled with the results of the current study suggest that certain taxa within the group are acidophilic and could potentially be used as biological indicators of acidification. Greater taxonomic resolution of the ceratopogonids in future studies would provide greater insight into the potential of the group to act in this capacity.

The chironomid larvae *Tanytarsus fuscithorax/semibarbitarsus* was also well represented in the acidic Group 1 wetlands. The taxon has generally been referred to as *Tanytarsus fuscithorax* in Western Australian literature (Edward 1964; Growns *et al.* 1993; Davis and Christidis 1999). In the present study however, the taxon has been referred to as *Tanytarsus fuscithorax/semibarbitarsus* because Ekrem (2001) suggested that the immatures of T. *fuscithorax* can easily be confused with those of T. *semibarbitarsus* and that the two are probably closely related. The species is common in permanent lentic waters throughout Australia (Edward 1986) and is known to inhabit shallow pools in the south-west of Western Australia (Edward 1964). While *Tanytarsus fuscithorax/semibarbitarsus* has been documented from both acidic and alkaline wetlands (Davis and Rolls 1987), the results of the present research suggest that the species is likely to be acidophilic. This is supported by studies such as the Office of Minerals and Energy Resources (2002) which recorded an abundance of the taxon in low pH waters associated with acid mine drainage. The study also noted that *Tanytarsus* was the only taxon found breeding in the low pH waters of the site, suggesting that it is able to thrive in acidic conditions. However, the ability of T. *fuscithorax/semibarbitarsus* to exist over a range of pH implies that its presence alone cannot be used to indicate low pH. Instead, the frequency of the

taxon needs to be taken into account, with high numbers possibly indicating acidification.

Additionally, while certain dipteran larvae appear to be potential indicators of pH change, it must be noted that dipterans such as Chironomidae may be tolerant to different types of ecosystem degradation such as pollution (Hardwick *et al.* 1995). As a result it may be difficult to separate pH effects from other forms of disturbance using diptera as indicators.

Dytiscid larvae from the genus *Sternopriscus* sp. were the only coleopterans classified as abundant in the Group 1 wetlands. The genus is endemic to Australia (Watts 2002) and both adult and larval forms have been commonly recorded from coastal wetlands, over a range of pH (Timms 1973; Bayly *et al.* 1975; Balla and Davis 1993; Growns *et al.* 1993). The species of larvae recorded during the present study is unknown and may actually represent a number of different taxa. Despite this, the wide ranging tolerance displayed by various species within the genus mean that the likelihood of these larvae being suitable indicators of specific pH levels is low.

The phylum Nematoda was another group frequently identified from the low pH waters of Group 1 wetlands. Members of this phylum are ubiquitous and widespread in aquatic environments, maintaining abundant populations in most types of inland waters. This suggests that as a phylum they are unlikely to be effective indicators of acidic conditions. The present study supports this suggestion as the taxon was also identified from Group 2 and Group 3 wetland sites. Moreover, the suitability of nematodes as indicators is diminished by the diversity and poorly known taxonomy of the taxon in Australia (Gooderham and Tsyrlin 2002). Similar findings were evident for the Oligochaeta, a class of the Phylum Annelida. The presence of oligochaetes in low pH waters has been documented by authors including Koryak *et al.* (1972); Trayler and Davis (1998) and Nijboer *et al.* (2004). However while oligochaetes were common inhabitants of the low pH wetlands in the current study, they were also seasonally abundant in Group 2 and Group 3 wetlands. This is supported by studies including Davis and Rolls (1987) and Madarish and Kimmel (2000) which have previously recorded the taxon in waters ranging from acidic to alkaline (mean pH 5.6 to 8.53 and < 4 -7.5 respectively). However, despite the

apparent indifference of oligochaetes and nematodes to pH, it is feasible that higher taxonomic resolution could reveal species within the groups that exhibit specific preferences.

The circumneutral Group 2 wetlands were dominated by crustaceans. For example, the calanoid copepod *Calamoecia tasmanica subattenuata* was collected frequently from the Group 2 wetlands although as discussed previously the taxon was also recorded in high numbers from Group 1 wetlands. The cladoceran *Daphnia carinata* was another common crustacean inhabitant of the Group 2 wetlands, occurring frequently during winter. However the species, which is known to be widespread in both south-western and eastern Australia (Benzie 1988), was also common in the alkaline Group 3 wetlands. Mitchell and Williams (1982) similarly recorded the species over a pH range from circumneutral to alkaline (pH 7.1-10.5) in waste stabilization ponds in South Australia. In addition, the taxon was collected from more than 25 wetlands ranging from acidic to alkaline, during a study on the Swan Coastal Plain (Growns *et al.* 1993).

Another cladoceran associated with circumneutral wetlands in the present study was *Alona quadrangularis*. Aside from Smirnov and Timms (1983) who described the species as sparsely distributed in south-western Australia and southern Victoria, there is little published literature on the occurrence and preferences of this taxon in Australian waters. Research from the United States suggested that the species is generally found near the benthos of vegetated littoral areas (Pennak 1978). Literature from other regions of the world tends to support the pH preference suggested by the present study. For example, Rundle and Ramsay (1997) found the highest numbers of the species in circumneutral lowland sites during a study in the United Kingdom. The species has also been identified from circumneutral ponds in New Zealand (pH 6.7-7) (Burns *et al.* 1984). Nematodes and oligochaetes were the other abundant groups in the Group 2 wetlands during the present study but given their frequent occurrence in the other pH groupings, could not be considered characteristic of circumneutral conditions.

The present study suggests that while some invertebrates appear to prefer circumneutral waters, few are restricted to them. This finding may be partly

attributable to unbalanced pH ranges of the three wetland pH groups. The circumneutral wetland group had a smaller pH range (6.5-7.5) in comparison to the other wetland pH groupings (< 6.5 for the acidic Group 1 wetlands and > 7.5 for the alkaline Group 3 sites) and was therefore subject to greater change in wetland numbers in the various seasons. The relative instability of the circumneutral wetland group may have been a factor rather than a lack of invertebrates that favour circumneutral conditions. Conversely, it may be a reflection of the wide pH tolerances or adaptability of various invertebrates. Additional investigation into circumneutral wetlands is required before a more definitive conclusion can be reached.

In contrast to the Group 1 and 2 wetlands which were dominated by copepods and cladocerans, ostracods were among the abundant crustaceans in the alkaline Group 3 sites. The ostracod *Sarscypridopsis aculeata* was abundant during the three seasons while *Candonocypris novaezelandiae* was collected in high numbers during the spring sample. *Sarscypridopsis aculeata* is a cosmopolitan species (De Deckker 1983) which has been recorded during various studies in Australia (De Deckker and Williams 1982; Robson *et al.* 1999; Pinder *et al.* 2000) and other regions of the world (Malmqvist *et al.* 1997; Boix *et al.* 2005). The taxon appears to have a broad tolerance to pH, having been collected from both acidic and alkaline waterbodies. For example, Davis and Rolls (1987) recorded *Sarscypridopsis aculeata* from sites including the acidic Lake Jandabup (yearly mean pH of 5.6$\pm$ SE 0.27) and more commonly from North Lake (yearly mean pH of 7.07 $\pm$ 0.13) and the alkaline sites of Lake Monger (yearly mean of 7.91 $\pm$ 0.19) and Thomsons Lake (yearly mean pH of 8.47 $\pm$ 0.16). The species was also recorded from gnammas ranging from pH 6.2 – 7.9 (Bayly 1997). *Candonocypris novaezelandiae* seems to display a similarly broad pH tolerance, having been collected from the same sites as *Sarscypridopsis aculeata* during the investigations by Davis and Rolls (1987) and from an acidic site (pH = 5.2) during Bayly's study (1997).

Although the findings of the present study in conjunction with other studies indicate that *Sarscypridopsis aculeata* and *Candonocypris novaezelandiae* favour circumneutral and alkaline waters, the tolerance of the two species to a range of pH suggests that they may not be suitable indicators of pH. Furthermore, Growns *et al.*

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(1992) identified large numbers of the two species from wetlands with high levels of nutrient enrichment, implying that the distribution of these taxa may be strongly influenced by variables other than pH.

The cladoceran *Daphnia carinata* was commonly collected from both the Group 2 and Group 3 wetlands during winter. While these results and those of previous studies tend to suggest that the taxon favours circumneutral to alkaline waters, the use of the *Daphnia carinata* as a pH indicator is problematic. Firstly, the taxon has been recorded from wetlands with a wide range of pH (Growns *et al.* 1993). Secondly, similarly to the ostracods *Sarscpridopsis aculeata* and *Candonocypris novaezelandiae*, *Daphnia carinata* is known to occur abundantly in eutrophic waters (Cheal *et al.* 1993). Therefore, its dominant presence in circumneutral and alkaline wetlands in the current study may be more closely related to factors such as nutrient levels than pH.

Copepods from the order Cyclopoida were another common group of crustaceans in the Group 3 wetlands, occurring frequently during winter sampling. The value of this taxon as an indicator of pH is questionable at this taxonomic level, with the spring sample recording an abundance of cyclopoids in the Group 1wetlands. As discussed previously, greater taxonomic resolution may reveal differing responses to pH and identify valuable indicator species. For example, it appears likely that the shift in the abundance of the cyclopoids between the acidic and alkaline wetlands during the two seasons may be related to species differences.

Juveniles from the family Corixidae were among the dominant insects collected from the Group 3 wetlands, occurring commonly during the spring sampling period. Corixids are found in most still and slow flowing freshwaters (Williams 1980), decreasing their usefulness as indicators of pH at the family level. Furthermore, the Group 3 wetlands included sites such as Herdsman Lake which are known to contain high levels of nutrients (Arnold 1990). Consequently it is possible that the high numbers of juvenile corixids in the alkaline sites were at least partially related to nutrient enrichment rather than pH.

Another insect occurring abundantly in the alkaline Group 3 wetlands was the chironomid *Polypedilum nubifer*. This species is one of the common nuisance species in the wetlands around the Perth Metropolitan Region (Edward 1986) and is known to be tolerant of a large range of environmental conditions (Growns *et al.* 1993). For example, while the species has been identified in large numbers from alkaline wetlands such as Lake Joondalup (Lund *et al.* 2000), Lake Monger and Thomsons Lake, it has also been recorded from acidic waters such as Lake Jandabup (Davis and Rolls 1987). These finding imply that the species is unlikely to be useful as a specific indicator of pH change.

The ephemeropteran larvae *Tasmanocoenis tillyardi* was also commonly collected from the Group 3 wetlands. Ephemeropterans are considered to be reasonably sensitive to low pH (Bell 1971) and while various species are able to tolerate different pH ranges, most cannot tolerate pH < 5.0 (Courtney and Clements 1998). During the current study, the lowest pH value recorded from waters containing *Tasmanocoenis tillyardi* was 6.49 at Wallsend (Su20) and the majority of specimens were collected from the alkaline Group 3 wetlands. Other records of the taxon from low pH waters include Storey *et al.* (1993) who collected the species from a slightly acidic river (pH = 6.29 and 6.31 for summer and winter) and a circumneutral lake (pH = 6.78) in the south of the state and Bunn *et al.* (1986) who collected the taxon from streams of similar pH in the northern Jarrah Forest. The findings of those studies in conjunction with the current research suggest that *Tasmanocoenis tillyardi* displays a broader pH tolerance than some other species of ephemeropteran larvae. Nonetheless, it may still have applications in the biological monitoring of pH decline in circumneutral wetlands. Oligochaetes were the other invertebrates listed among the dominant taxa of Group 3 but the taxon cannot be considered characteristic of alkaline waters, having also been frequently recorded from Group 1 and 2 sites.

4.5.3   Patterns of community structure

Multivariate analyses of the sites based on the similarities in invertebrate assemblages and geographical region revealed differences in community structure during each season. The differences were generally not strong however, as demonstrated by the low R statistics. Furthermore, the regions identified as

significantly different from each other changed seasonally, which suggests that factors other than geographical region were at least partly responsible for the distributional patterns. Collie and Wagerup were the only regions to consistently display significantly different invertebrate assemblages during each of the seasonal comparisons, a finding likely to be related to differences in the water chemistry. For example, the pH of the Wagerup wetlands was generally comparatively higher than the Collie sites. Considering the documented influence of pH on invertebrate community composition (Hall *et al.* 1980; Simpson *et al.* 1985; Mason 1991), it is likely that the variable was a contributing factor to the differences between these regions. The pH of the sites also appeared to influence the distribution of the Perth Metropolitan wetlands in ordination space, with the acidic and circumneutral wetlands such as Gnangara Lake, Lakelands and Tuscan Park generally positioned near the Collie sites and alkaline sites such as Herdsman Lake (Site 10) and Neil McDougall Park (Site 16) mostly grouped near the Wagerup wetlands.

However, while it is probable that water chemistry variables such as pH influenced the invertebrate community structure in these regions, the influence of other factors cannot be discounted. For example, Rundle and Ramsay (1997) found large differences in the overall community composition of acidic streams in different regions and suggested that the differences may related to physical factors.

The multivariate analyses of the sites based on invertebrate assemblages and pH groupings also detected differences in invertebrate community composition. These differences were most obvious between the acidic Group 1 and alkaline Group 3 wetlands, particularly in winter and spring, suggesting that most taxa are unable to exist over such a wide pH range. The smaller differences apparent between the two groups during summer were probably attributable to the low number of sites classified as circumneutral during this season. This is supported by the ordination of the summer data, with Group 1 sites such as Tuscan Park (Su19), and Wallsend (Su20) which were generally circumneutral in other seasons being situated relatively close to alkaline sites such as Neil McDougall Park (Su16), Blue Gum Lake (Su5) and Herdsman Lake (Su10). The invertebrate community structure of Blind Roo A (Su3) was atypical of the acidic wetlands and was instead located in close proximity to alkaline sites such as Exelby (Su8) in ordination space. This was probably related

to similarities in unmeasured physical or chemical factors. Differences in invertebrate community structure were also detected between the circumneutral Group 2 and the alkaline Group 3 sites, although the dissimilarities were not as large as those found between Groups 1 and 3.

The only comparisons that did not reveal statistically different invertebrate abundance data were those between the Group 1 and Group 2 sites. These results suggest that at least some of the taxa recorded from wetlands within these pH groupings have a relatively broad pH tolerance. This corresponds with the findings of Kay *et al.* (2000) who suggested that macroinvertebrate families in Western Australia had wide-ranging environmental tolerances and were probably tolerant of low pH.

According to BIO-ENV analyses, pH was the single variable that best explained the faunal patterns of the sites during each season. Additionally, pH was the only factor consistently included in the strongest variable combinations. The strongest correlation for the summer data was achieved by a combination of vegetation, pH, electrical conductivity and temperature, all of which are factors known to influence invertebrate community structure (Mitchell and Williams 1982; Simpson *et al.* 1985; Kefford 1998; Trayler and Davis 1998; Brittain *et al.* 2001; Kay *et al.* 2001; Hudson *et al.* 2003). However, the relatively low correlation recorded for this season ($p_s <$ 0.50) tend to imply that factors other than those measured probably contributed to the biotic patterns. In contrast, the highest correlations for the winter and spring data were generated by variable combinations of pH and temperature and pH respectively.

The findings suggest that although invertebrate composition was not necessarily constrained by pH, the variable was relatively important in comparison to the other parameters measured. The results also highlight the complex relationship between organisms and environmental variables, demonstrating that biota are commonly influenced by a combination of factors rather than a single over-riding variable. It is important to note however that the relatively low number of sites included in the study, particularly in relation to the number of variables selected, may have influenced the reliability of the results. Ideally, analyses should include approximately fifteen sites per variable (Stevens 1992) to provide a robust analysis.

It should also be noted that BIO-ENV does not provide a formal statistical inference and instead should be considered an exploratory technique for comparing biotic and environmental patterns (Clarke and Warwick 2001).

4.5.4 Seasonal variability

Seasonal variation in invertebrate community structure has been documented by a number of studies in south-western Australia (Bunn *et al.* 1986; Pusey and Edward 1990; Storey *et al.* 1993). The results of the current study also suggested that there was some variation in individual taxa between sampling periods, although there did not appear to be any consistent patterns of seasonal change at the community level. Additionally, it is possible that any apparent differences were a result of heterogeneity in the data-set rather than substantial seasonal variation. It is important to take into account the fact that the current study used a single sample from each of the 20 sites to represent each season. An increase in the number of samples taken would probably provide a more comprehensive assessment of seasonal variability. Nonetheless, it seems unlikely that season would have a major impact on the usefulness of invertebrates as biological monitors of pH.

4.5.5 Other possible influences on invertebrate community structure

Factors other than those measured during the current study might have contributed to differences in invertebrate community composition. In particular, variables such as nutrient enrichment (Growns *et al.* 1992), the presence of predators and competition between taxa (Nielson *et al.* 1999) may have influenced the invertebrate distribution and therefore community composition. The sampling technique used may also have impacted upon the composition of the samples. While the original intention of the research was to investigate macroinvertebrate community structure, the use of the D-frame or sweep net resulted in the collection of both plankton and macroinvertebrates. This corresponds with the findings of Cheal *et al.* (1993) which found that because the sweep net effectively sampled both the water column and the benthos, it generally collected a greater number of species then other techniques tested.

4.6 Conclusion

The single variable most closely correlated with invertebrate community structure during the study was pH. Accordingly, some differences in invertebrate community composition were obvious, based on pH groupings and geographical regions. Regional differences were particularly evident between the mostly alkaline Wagerup wetlands and the acidic to circumneutral Collie wetlands. Differences among the pH groupings were strongest between the acidic Group 1 and alkaline Group 3 wetlands. These differences suggest that many of the invertebrates collected were either unable to exist over a wide range of pH (acidic to alkaline) or occurred in differing proportions in the various categories, thereby exhibiting at least a coarse level of sensitivity to pH. In contrast, the acidic Group 1 and circumneutral Group 2 wetlands had similar invertebrate assemblages. This implies that some of the taxa collected from these sites are not sensitive to the differences in pH over this lower range and are instead able to tolerate waters of both low and intermediate pH. Additionally, seasonal fluctuations in pH and the changing of sites between the acidic and circumneutral groups as a result of seasonal changes may have been reflected in the similarities between the two pH groupings.

Taxa such as *Macrothrix indistincta* from the acidic wetlands and *Alona quadrangularis* from the circumneutral wetlands displayed relatively narrow pH tolerances and were identified as potential indicator species. In contrast, the majority of taxa that dominated the pH groups did not appear highly specific in their pH preferences. It is however important to consider what level of sensitivity is needed to indicate pH changes such as acidification. Taxa that are able to distinguish between two of the three pH groups could still be potentially useful, even if they may lack further sensitivity to distinguish between each of the three pH classifications. For example the calanoid copepod *Calamoecia tasmanica subattenuata* which commonly occurred in both acidic and circumneutral waters during this study was not present in any alkaline samples and could potentially be used to indicate a decrease in the pH of alkaline waters. Other taxa identified as possible indicator species included *Tanytarsus fuscithorax/semibarbitarsus*, a species which favoured acidic sites, juvenile corixids and the mayfly larvae *Tasmanocoenis tillyardi,* both of which commonly occurred in the alkaline wetlands.

The results of the study also highlighted the influence of factors other than pH in shaping invertebrate community structure. Despite this, most of the invertebrates collected displayed some sensitivity to pH. Furthermore, the findings suggest that season probably did not have a substantial impact on community structure, implying that the biological monitoring of pH using invertebrates can be implemented regularly throughout the year, increasing the sensitivity of monitoring.

4.7 References

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Appendix 4.1: Invertebrate taxa collected during the summer (Su), winter (Wi) and spring (Sr) 2001 sampling period

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
CNIDARIA																				
HYDROZOA																				
Hydridae																				
Hydra sp.	Sr			Sr							Sr	Sr		Sr		Wi,Sr			Sr	
PLATYHELMINTHES																				
TURBELLARIA					Su					Su		Su								
NEMATODA	Sr	Su,Wi,Sr	Wi				Wi	Wi,Sr	Wi,Sr	Su,Wi,Sr	Wi,Sr	Su,Wi,Sr	Su	Wi	Wi,Sr	Su,Wi,Sr	Su,Wi,Sr		Su,Wi,Sr	Wi,Sr
ANNELIDA																				
OLIGOCHAETA	Sr	Su,Sr	Su,Wi,Sr	Su,Sr	Su,Sr	Wi,Sr	Su	Su,Wi,Sr	Wi	Su,Wi	Wi	Su,Wi,Sr	Wi	Wi,Sr	Su	Su	Su,Sr	Wi,Sr	Sr	Su
HIRUDINEA																				
Erpobdellidae	Su																			
Glossiphonidae					Su		Su						Su	Su	Sr					
ARTHROPODA																				
ARACHNIDA																				
ACARINA																				
MESOSIGMATA								Wi				Wi		Wi						
ORIBATIDA					Su,Sr					Su,Wi		Sr				Su,Wi,Sr			Wi	
Malaconothridae		Wi				Su,Wi	Su,Wi										Sr			Sr
PROSTIGMATA																				
Hydracarina																				
Hygrobatidae																				
Coaustraliobatis sp.																				Su,Wi,Sr
Hydrachnidae																				
Hydrachna sp.				Sr				Sr												
Limnesiidae																				
Limnesia sp.																				Sr
Pionidae																				
Piona sp.	Sr				Wi															
Mideopsidae																				
Gretacarus sp.		Sr																		
ARANEAE																				
NEOCRIBELLATAE																				
Pisauridae						Sr														
CRUSTACEA																				
CLADOCERA																				
Chydoridae																				
Alona kendallensis Henry																			Wi	
Alona quadrangularis (OF Müller)																Wi, Sr	Su,Wi		Wi,Sr	
Alona setigera Brehm																	Wi			
Alona sp. 1															Su,Sr					
Chydorus sp.														Wi,Sr						Sr
Macrothricidae																				
Macrothrix breviseta Smirnov									Sr											
Macrothrix indistincta Smirnov						Su,Wi,Sr	Su,Wi,Sr										Wi,Sr			
Neothrix armata Gurney																				Wi
Daphniidae																				
Ceriodaphnia laticaudata Mueller					Wi									Wi,Sr						
Daphnia carinata King	Wi		Wi		Wi			Wi		Wi	Wi		Wi			Wi			Wi	
Daphnia lumholtzi Sars																	Wi			
Simocephalus sp. 1			Sr																	
Simocephalus sp. 2												Sr		Sr						

Appendix 4.1 continued: Invertebrate taxa collected during the summer (Su), winter (Wi) and spring (Sr) 2001 sampling period.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
OSTRACODA																				
Cypridopsidae																				
Sarscypridopsis aculeata (Costa)	Su,Wi,Sr			Wi,Sr	Wi,Sr					Su,Sr	Wi,Sr	Su,Wi,Sr	Su,Wi,Sr		Wi,Sr	Su,Wi,Sr			Wi	Su,Wi,Sr
Cyprididae																				
Alboa worooa De Deckker															Wi					
Bennelongia australis (Brady)			Sr		Wi,Sr			Wi,Sr			Wi,Sr			Sr	Sr					
Bennelongia barangaroo de Deckker											Su									Su
Candonocypris novaezelandiae (Baird)	Su			Wi,Sr	Wi					Su,Sr	Wi		Su,Wi,Sr							
Cypretta baylyi McKenzie								Su,Wi							Su				Sr	
Cypretta sp. 1											Sr	Sr		Wi,Sr	Wi					Sr
Cypretta sp. 2			Sr																	
Ilyodromus aff. *candonites* De Deckker			Wi,Sr	Wi							Su,Sr	Su								Su,Wi
Ilyodromus amplicelis De Deckker															Su				Su,Wi,Sr	
Ilyodromus sp. 1		Su,Sr	Wi														Wi			Su,Wi,Sr
Ilyodromus sp. 2		Wi	Wi,Sr																	Wi
CALANOIDA																				
Centropagidae																				
Boeckella robusta maxima Bayly			Wi					Sr												
Boeckella triarticulata (Thomson)				Wi,Sr				Wi			Wi									
Calamoecia attenuata (Fairbridge)		Wi	Sr							Wi					Wi,Sr				Su,Wi,Sr	Sr
Calamoecia tasmanica subattenuata (Fairbridge)			Sr			Su	Su,Wi		Wi			Wi			Wi,Sr	Wi	Sr		Wi	Su,Wi
CYCLOPOIDA	Su, Wi,Sr	Su,Wi,Sr	Sr	Wi,Sr		Wi,Sr	Su,Wi	Wi,Sr		Su,Wi,Sr	Wi	Su		Wi,Sr		Su,Wi	Su,Wi,Sr	Su	Su,Wi,Sr	Sr
AMPHIPODA																				
Ceinidae																				
Austrochiltonia subtenuis (Sayce)	Su,Sr												Su,Wi,Sr	Sr					Su,Wi,Sr	
Perthidae																				
Perthia branchialis (Nicholls)								Wi,Sr									Su,Sr			
DECAPODA																				
Palaemonidae																				
Palaemonetes australis Dakin										Su				Su,Wi						
INSECTA																				
COLLEMBOLA																				
Hypogastruridae		Wi					Wi	Wi		Wi				Wi		Wi				
Isotomidae																				Sr
EPHEMEROPTERA																				
Baetidae					Su															Sr
Cloeon sp.															Su				Sr	
Caenidae																				
Tasmanocoenis tillyardi (Lestage)	Su,Sr			Su,Sr				Su		Su,Sr	Su	Su,Sr	Su		Sr				Su	Su
ODONATA																				
ZYGOPTERA																				
Zygoptera (early instar)																Su				
Lestidae																				
Austrolestes psyche (Hagen)							Wi													
Coenagrionidae																				
Austrogrion coerulum (Tillyard)											Su,Sr	Su,Wi								
Ischnura aurora (Brauer)			Su												Su					
Xanthagrion erythroneurum Selys	Su																			
ANISOPTERA																Su				
Aeshnidae																				
Aeshna brevistyla Rambur							Sr							Wi						

Appendix 4.1 continued: Invertebrate taxa collected during the summer (Su), winter (Wi) and spring (Sr) 2001 sampling period.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Gomphidae							Su										Su			
Hemicordulidae																	Su			
Hemicordulia tau Selys		Su										Su			Su,Sr					
Libellulidae													Su		Su	Su				
Diplacodes bipunctata (Burmeister)		Wi													Su				Sr	
Nannophya sp.						Su														
Orthetrum caledonicum Brauer									Wi		Su				Su		Su			Su
Oxygastridae																				
Hesperocordulia sp.																Sr				
HEMIPTERA																				
Corixidae																				
Agraptocorixa eurynome (Kirkaldy)											Sr				Su					
Agraptocorixa sp.	Su,Sr							Sr					Sr							
Corixidae (juvenile)	Sr		Wi,Sr	Wi,Sr		Su,Sr	Su	Su,Sr		Su,Sr		Sr	Su,Sr	Sr	Wi	Wi,Sr			Sr	
Micronecta robusta Hale				Wi	Sr			Sr		Wi,Sr	Sr		Su,Wi,Sr	Sr		Sr				
Micronecta sp.	Sr							Su,Wi						Su,Wi					Su	
Sigara mullaka Lansbury				Su	Sr			Su							Su					
Mesoveliidae																				
Mesovelia sp.				Sr																
Notonectidae			Su,Sr					Su												
Anisops sp.							Su,Wi,Sr	Wi,Sr			Sr	Su,Wi								
Anisops thienemanni Lundblad							Su	Sr			Sr									
Veliidae																				
Microvelia sp.								Sr				Sr					Su			
DIPTERA																				
Chironomidae																				
Ablabesmyia notabilis (Skuse)		Sr		Sr								Su		Sr		Sr	Wi			Su
Chironominae pupae		Sr																		
Chironomus alternans Walker *nomen dubium*			Sr							Su,Wi,Sr	Wi									
Chironomus occidentalis Skuse	Wi									Wi,Sr										
Cladopelma curtivalva (Kieffer)										Su				Su		Wi			Sr	Wi
Corynoneura sp.			Wi	Sr										Sr		Wi				
Cricotopus sp.	Su							Sr												
Cryptochironomus griseidorsum (Keiffer)	Su,Sr	Wi	Su	Su,Sr									Su	Su,Sr					Sr	Su
Dicrotendipes conjunctus (Walker)															Su				Wi	
Kiefferulus intertinctus (Skuse)	Wi							Su												
Kiefferulus martini (Freeman)			Su,Sr					Sr			Su				Su					
Orthocladiinae							Wi										Sr			
Orthocladiinae pupae	Wi																			
Paralimnophyes pullulus (Skuse)		Sr					Wi,Sr	Wi			Wi			Wi,Sr	Wi				Sr	Su,Wi
Paramerina levidensis (Skuse)																			Wi	
Paratanytarsus grimmii (Schneider)			Wi								Su,Wi	Su,Wi,Sr		Su,Wi,Sr	Sr					
Paratrichocladius sp.				Sr							Sr									
Polypedilum nubifer (Skuse)	Su,Wi			Su	Su		Wi	Su		Su	Sr		Sr	Sr	Su	Wi,Sr				
Procladius villosimanus Kieffer			Su	Su,Sr	Su			Sr			Su,Wi,Sr		Su,Sr			Sr	Su,Wi			Su,Wi
Tanytarsini sp. 1					Su															
Tanytarsus fuscithorax (Skuse)	Wi	Su,Sr		Su		Sr	Su,Wi,Sr		Su,Sr				Su,Sr		Su	Sr	Su,Wi,Sr	Wi,Sr	Su,Sr	Su,Wi,Sr
Culicidae																				
Anopheles sp.											Sr									

Appendix 4.1 continued: Invertebrate taxa collected during the summer (Su), winter (Wi) and spring (Sr) 2001 sampling period.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Ceratopogonidae																				
Ceratopogonidae pupae					Sr		Su		Wi			Su,Sr						Su		Su
Ceratopogoninae 1												Wi,Sr				Wi				
Ceratopogoninae 2						Wi,Sr	Su,Sr		Su,Wi,Sr									Wi,Sr		
Ceratopogoninae 3							Wi		Wi									Wi		
Ceratopogoninae 4		Sr																		Su
Ceratopogoninae 5	Su																			
Ceratopogoninae 6																Sr				
Ceratopogoninae 7							Su		Su,Sr									Su,Wi,Sr		
Ceratopogoninae 8											Wi									Su
Ceratopogoninae 9				Wi,Sr				Sr												
Muscidae																				
Muscidae pupae										Wi										
Psychodidae																				
Psychodidae larvae										Wi			Sr							
TRICHOPTERA																				
Hydroptilidae																				
Acritoptila globosa Wells			Sr											Sr	Sr	Sr	Su,Wi			Sr
Ecnomidae																				
Ecnomus sp.	Su,Sr										Su			Su						
Leptoceridae								Su	Su,Sr											Sr
Notalina sp. 1																				Wi
Notalina spira St Clair														Su						
Oecetis sp.	Su			Sr							Su,Sr			Su						
Triplectides australis Navas			Su	Su				Su			Su									
Triplectides sp.											Sr						Sr			
COLEOPTERA																				
Carabidae																				
Carabidae larvae											Wi									
Chrysomelidae																				
Chrysomelidae larvae									Wi											
Curlionidae																				
Erirhininae									Wi											
Dytiscidae																				
Allodessus sp.			Su					Su												
Antiporus femoralis (Boheman)														Su						
Antiporus gilberti (Clark)														Su						
Antiporus sp. larvae											Wi									
Bidessini larvae											Wi	Wi								
Gibbidessus chipi Watts				Sr																
Hyphydrus elegans (Montrouzier)					Su,Sr						Su									
Hyphydrus sp. larvae											Sr									
Liodessus dispar (Sharp)			Su					Su,Sr			Sr							Sr		
Liodessus inornatus (Sharp)		Su	Su	Su							Su			Su						
Megaporus howitti (Clark)				Su				Su,Sr			Su,Wi									
Megaporus solidus (Sharp)								Wi												
Megaporus sp.							Sr													
Megaporus sp. larvae			Wi								Sr									

Appendix 4.1 continued: Invertebrate taxa collected during the summer (Su), winter (Wi) and spring (Sr) 2001 sampling period.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Necterosoma darwini (Babington)																				Sr
Necterosoma penicillatus (Clark)															Su					
Necterosoma sp.						Sr			Sr						Su		Sr	Sr		Su,Sr
Necterosoma sp. larvae						Sr			Wi,Sr						Wi		Wi	Su,Wi,Sr		Wi,Sr
Rhantus sp.								Sr												
Rhantus sp. larvae															Wi			Wi		
Rhantus suturalis W.S MacLeay								Su												
Sternopriscus browni Sharp							Su,Wi							Sr	Su					
Sternopriscus multimaculatus (Clark)				Su,Wi,Sr				Su						Su	Su					
Sternopriscus sp.								Su,Sr									Sr	Su,Wi		
Sternopriscus sp. larvae	Sr	Sr	Sr				Wi	Sr			Sr			Sr			Su,Sr		Sr	
Uvarus pictipes (Lea)									Wi											
Gyrinidae																				
Aulonogyrus strigosus (Fabricius)		Sr						Sr												
Haliplidae																				
Haliplus sp. 1															Wi					
Hydraenidae																				
Ochthebiinae											Wi,Sr									
Hydrochidae																				
Hydrochus sp.				Su			Sr	Su			Su,Sr			Su						Su,Sr
Hydrophilidae																				
Hydrophilidae larvae					Su									Sr				Su		
Berosus approximans Fairmaire								Su,Sr												
Berosus sp.							Sr		Wi,Sr		Wi			Wi						
Berosus sp. 1																Sr				
Berosus sp. larvae											Su			Su						
Enochrus elongatus (W.S. MacLeay)								Su												
Enochrus maculiceps (W.S. MacLeay)			Su																	
Enochrus sp.								Sr	Wi											
Limnoxenus zelandicus (Broun)								Su												
Paracymus pygmaeus (W.S. MacLeay)		Su	Sr					Su,Sr												

Appendix 4.2: Dominant invertebrate taxa from the three pH groupings (a) *Alona quadrangularis* (b) Fifth legs of a male *Calamoecia tasmanica subattenuata* (c) *Candonocypris novaezelandiae* (d) Ceratopogoninae 2 (e) Ceratopogoninae 7 (f) Corixidae juvenile.
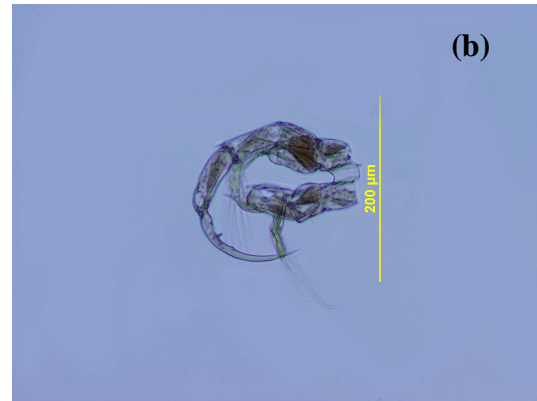
Appendix 4.2 continued: Dominant invertebrate taxa from the three pH groupings (g) Cyclopoida (h) *Daphnia carinata* (i) *Macrothrix indistincta* (j) Nematoda (k) Oligochaeta (l) Head of *Polypedilum nubifer*
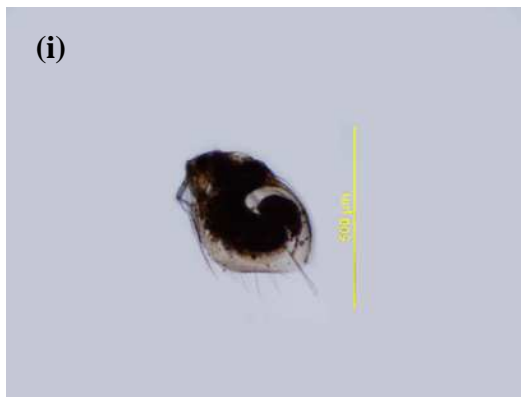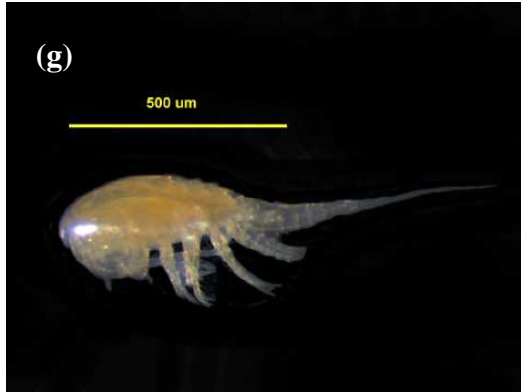
Appendix 4.2 continued: Dominant invertebrate taxa from the three pH groups (m) *Sarscypridopsis aculeata* (n) *Sternopriscus* sp. larvae (o) *Tanytarsus fuscithorax* (p) *Tasmanocoenis tillyardi*
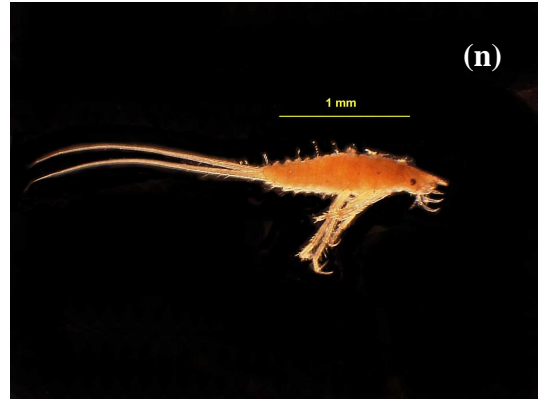
Chapter 5: Diatoms in wetlands in the south-west of Western Australia: community structure with reference to pH

5.1 Abstract

A total of 20 wetlands were sampled over three seasons to examine the relationship between pH and diatom community structure in the south-west of Western Australia. Multi-dimensional scaling and analyses of similarities were used to identify differences in the diatom community structure according to geographical region and pH groupings. Regional differences in diatom assemblages were largest between Collie and Wagerup and were probably linked to varying pH. The largest differences among pH groups were evident between the acidic Group 1 and alkaline Group 3 sites. Differences were less defined in the comparisons with the circumneutral Group 2 wetlands. Analyses determined that pH was the variable most closely correlated with diatom distribution patterns during each season and potential indicator species were identified for each pH group. The results also indicated that diatoms are likely to be useful biological monitors of pH in various seasons and can therefore be incorporated into a regular sampling program for monitoring pH in the wetlands of the south-west of Western Australia.

5.2 Introduction

In the previous chapter the structure of invertebrate communities of the 20 wetlands was examined in relation to environmental variables, particularly pH. The present chapter focuses on the relationship between diatom distribution and pH.

The acidification of surface waters has been shown to negatively impact aquatic organisms, with decreases in biodiversity and shifts in community structure commonly reported from various regions of the world (Mason 1991). A further concern for the south-west of Western Australia is the potential contamination of groundwater resources as a result of acidification (McHugh 2004). The use of an effective biological monitoring tool could form an integral part of the management strategy for threatened and acidified waters in the region. A biological monitor such the micro-algae diatoms could provide both early detection of the impacts of pH decline and instigate the implementation of mitigation procedures.

The sensitivity of diatoms to pH has been clearly demonstrated by authors including Hustedt (1938-1939), Cholnoky (1968); Charles (1985); Stokes & Yung (1986); Round (1990); Watanabe and Asai (2001). However, while they are widely used as pH indicators (van Dam *et al.* 1981) only limited work has been carried out in Western Australia. Studies conducted by John (1993) and Thomas & John (2006) in the south-west of the state both investigated diatom community structure in relation to pH but were restricted in scope. Diatom assemblages from 10 sand-mining lakes in Capel were examined by John (1993) while Thomas and John (2006) analysed the diatom community structure of five coal mine lakes in Collie (pH < 6). Helleran (1993) investigated diatoms as indicators of water quality in wetlands of the Swan Coastal Plain and from the results suggested several species that could potentially be used to indicate acidic waters. However, the majority of sites included in the study were alkaline and only one site was considered to be permanently acidic at the time of sampling. In contrast, the present study encompasses a larger region of the south-west. Additionally, the project incorporates wetlands with a greater range of pH and includes waters that have been acidified as a result of mining, disturbance of acid sulphate soils and organic acids.

Given that an expanding number of wetlands in Western Australia are under threat of acidification (DoE 2004; McKay and Horwitz 2006) increasing knowledge on the relationship between pH and diatom communities is of growing importance. Therefore, the main objective of this chapter was to investigate the relationship between environmental variables and diatom community composition in wetlands of the south-west of Western Australia with particular reference to pH. A further aim was to identify potential indicator assemblages or species for the different pH classifications. The study also attempted to examine seasonal variability within the distribution patterns of diatoms and assess the impact of seasonality on their effectiveness of diatoms as biological monitors of pH.

5.3   Methods

5.3.1   Periphytic diatom sampling

Periphytic diatom samples were collected from each of the 20 study sites (Figure 5.1). Sampling was undertaken during summer (December –February), winter (June – August) and spring (September - November) of 2001. Each of the sites was sampled using an artificial substrate collector known as the JJ Periphytometer (John 1998). The periphytometers, fitted with 10 glass microscope slides, exposed to colonizers vertically, were employed to ensure uniform collection of diatoms. This method also avoids the problem of collecting dead cells and allows the diatom assemblages to be related to the ambient environmental conditions. Wire or 20 lb fishing line was used to secure the periphytometers to submerged structures such as a stake or tree root, ensuring the devices were well immersed. The periphytometers were retrieved after approximately 14 days of immersion, allowing sufficient time for the development of a climax community (John 1998). The slides removed from each periphytometer were placed in vials containing deionised water and preserved with the addition of 5 – 10mls of Transeau's Algal Preservative (6:3:1 H_2O, ethyl alcohol and formalin).

5.3.2   Permanent slide preparation of diatom samples

Permanent slide preparation followed the techniques outlined in John (1983). The film of periphyton on both sides of each of the 10 slides was scraped into a solution

of deonised water and Transeau's Algal Preservative using a single-edge blade. Between 10-20 ml of the samples were placed in 100ml Pyrex beakers with equal amounts of concentrated (70%) Nitric Acid and deionised water. The samples were digested within a fume cupboard on a hotplate set at 80ºC. After cooling, the suspensions were diluted with deionised water and centrifuged for five minutes at 3500 rpm using a BHG Roto Uni II Centrifuge. The supernatant was decanted off to remove the acid, leaving a pellet of diatom frustules, and deionised water was added to resuspend the frustules. The centrifugation process was repeated further four times to remove all traces of acid.

Aliquots of between 100 to 1000 µl of resuspended sample were pippetted onto cleaned glass coverslips (22 x 22mm) positioned on a hot plate (60° C). The concentration of solution placed on the coverslips varied according to the density of diatoms in the sample. Deionised water was added to the coverslips when aliquots of less than 1000 µl were used. The even distribution of diatom frustules on the coverslip was achieved through gentle stirring of the sample. Upon evaporation, the coverslips with the dried diatom samples were inverted and gently pressed down onto clean labelled glass slides prepared with 4 –5 drops of the mounting medium NBS Naphrax (refractive index 1.74). The slides were placed on the hotplate to boil until the solvent present in the Naphrax had evaporated. The slides were subsequently removed, allowing the medium to cool and solidify. Three permanent slides were prepared for each sample.
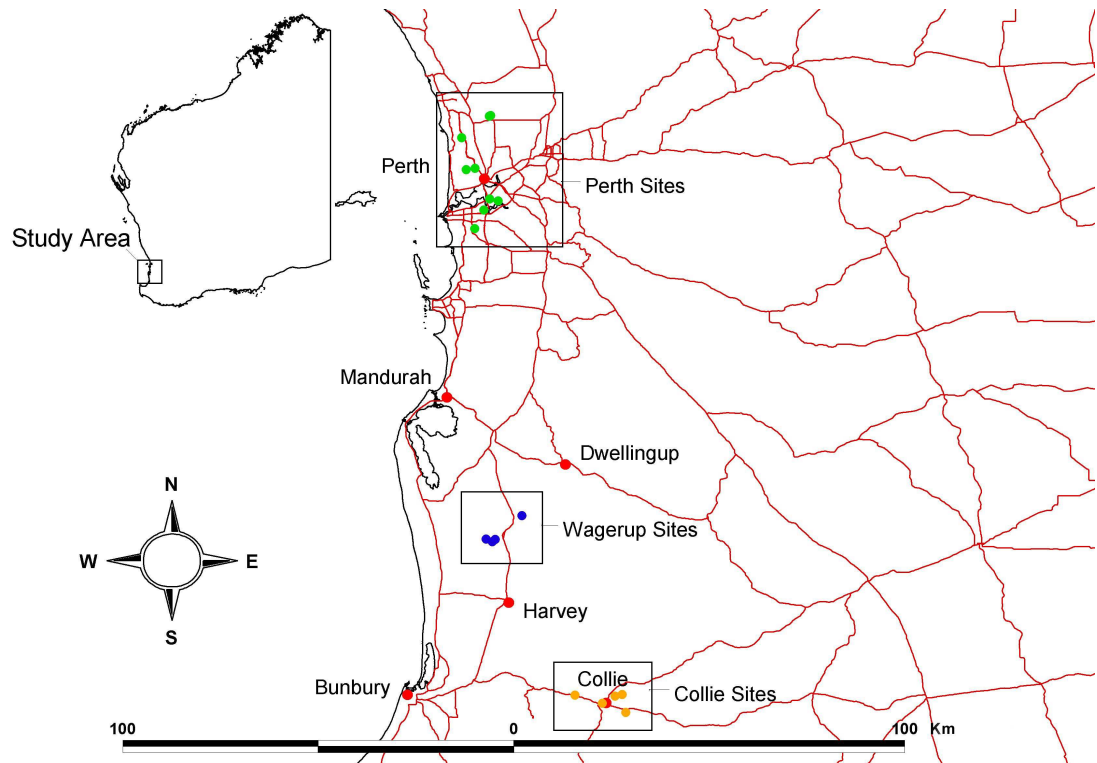
Figure 5.1: Location of the 20 sites in the south-west of Western Australia selected for the seasonal study. Green points indicate the sites located in the Perth Metropolitan Region. Blue points indicate study sites located in the Wagerup area. Yellow points represent sites located in the Collie Basin. Red points represent the nearest neighbouring towns.

5.3.3 Diatom enumeration

The slides were examined using a ORION BM-LUX-4 compound microscope under oil immersion at 1000x magnification. Depending on the density of diatoms on the slide between 100 - 350 frustules were counted and identified from each sample. Identification was to species level where possible and the relative frequencies of each taxon were determined. Diatoms were identified using specialised literature (Patrick and Reimer 1966; Foged 1974; Patrick and Reimer 1975; Foged 1978, 1979; John 1983; Hustedt and Jensen 1985; Gasse 1986; Krammer and Lange-Bertalot 1986; Holland and Clarke 1989; Lange-Bertalot and Moser 1994; Ehrlich 1995; John 1998; Snoeijs and Balashova 1998; Camburn and Charles 2000; John 2000b; Siver *et al.* 2005). Diatom nomenclature generally conformed to Fourtanier and Kociolek (1999). The taxonomic authorities for each species have been presented in Appendix 5.1. Photomicrographs of diatoms were taken under oil immersion using a VANOX photomicroscope at 1000x magnification to assist with identification of the diatoms. The diatom slides have been deposited in the International Diatom Herbarium at the Department of Environmental Biology, Curtin University of Technology.

5.3.4 Data analysis

Diatom community composition was investigated using multivariate analyses from the software package PRIMER 5.0 for Windows Version 5.2.9 (PRIMER-E Ltd 2002). Non-metric multi-dimensional scaling (MDS) ordinations (Kruskal and Wish 1978) were employed to identify groups of sites with similar diatom community structure. Species abundance data was square-root transformed and the Bray Curtis similarity measure (Bray and Curtis 1957) was used to construct similarity matrices. The significance of trends in diatom distribution was determined using one way analysis of similarity or ANOSIM (Clarke and Green 1988). A maximum of 999 permutations were used to calculate the probability of the observed values for each analysis. The BIO-ENV procedure (Clarke and Ainsworth 1993) identified the combinations of environmental variables which provided the best matches of biotic and environmental matrices. This was achieved using the Spearman rank correlation coefficient (p_s). Significance was accepted at $p < 0.05$ and $p < 0.01$. A more detailed explanation of the statistical methods employed in this section was provided in the previous chapter.

5.4 Results

5.4.1 Diatom taxa

A total of 154 diatom taxa from 44 genera were recorded from the 60 samples (Appendix 5.1; Table 5.1). Over 110 taxa were identified from the summer and winter samples (115 and 113 respectively) with 106 present in the spring collection. The genus *Nitzschia* displayed the highest species richness in each sampling period. *Achnanthidium*, *Navicula* and *Gomphonema* were also prominent although abundance varied. During the spring collection *Pinnularia* was among the common genera.

Table 5.1: List of genera identified from the 20 study sites in the south-west of Western Australia over the three sampling periods and the number of species present within each genus.

Genus	Species Number	Genus	Species Number
Achnanthes	1	*Gomphonema*	13
Achnanthidium	11	*Gyrosigma*	1
Amphora	6	*Hantzschia*	2
Aulacoseira	1	*Hippodonta*	1
Bacillaria	1	*Luticola*	1
Brachysira	5	*Mastogloia*	2
Caloneis	1	*Navicula*	13
Cocconeis	3	*Nedium*	3
Craticula	2	*Nitzschia*	18
Ctenophora	1	*Pinnularia*	9
Cyclotella	3	*Placoneis*	1
Cylindrotheca	1	*Planothidium*	1
Cymbella	2	*Pseudostaurosira*	1
Diadesmis	1	*Rhopalodia*	2
Encyonema	5	*Sellaphora*	2
Encyonopsis	1	*Stauroneis*	6
Epithemia	2	*Staurosira*	2
Eunotia	8	*Surirella*	3
Fallacia	1	*Synedra*	3
Fragilaria	6	*Tabellaria*	1
Fragilariforma	1	*Tabularia*	1
Frustulia	5	*Tryblionella*	1

5.4.2 Dominant taxa of the pH groups

Diatom taxa that were observed in at least two samples per pH group with an abundance of at least 10% were classified as dominant (Table 5.2). Photomicrographs of the dominant taxa are presented in Appendix 5.2. The species *Brachysira brebissonii*, *Frustulia magaliesmontana, Navicula* aff. *cari* and *Nitzschia paleaeformis* were commonly recorded from the acidic Group 1 wetlands throughout the study. *Brachysira styriaca*, *Eunotia bilunaris* and *Eunotia pectinalis* var. *minor* also displayed a dominant presence in the group although the seasons varied.

The dominant species of the Group 2 wetlands were excluded from the summer results due to the limited sample size. During winter the dominant taxa of the Group 2 displayed some overlap with Group 3. While *Achnanthidium minutissimum* and *Gomphonema parvulum* were classified as abundant in the Group 2 winter sample, both species displayed a dominant presence in the Group 3 wetlands throughout the study. *Brachysira vitrea* was the only taxon that occurred abundantly in the circumneutral wetlands alone. As none of the taxa recorded from the Group 2 wetlands during spring exceeded 10% abundance in at least 2 sites, species dominance was not established.

Nine diatom taxa were classified as abundant in the alkaline Group 3 wetlands, the majority of which were rarely recorded from the other wetland groups.
Gomphonema parvulum and *Staurosira construens* var. *venter* were commonly identified from the wetlands of Group 3 throughout the study while species such as *Cocconeis placentula, Pseudostaurosira brevistriata, Encyonopsis microcephala* and *Nitzschia palea* were seasonally abundant (Table 5.2).

5.4.3 Multivariate analyses

The ordinations of the sites based on the similarities in species assemblages displayed moderate levels of separation for each of the seasonal data-sets. (Figure 5.2a – c). Ordination of the sites according to region demonstrated that Collie wetlands generally clustered together, although the groupings were not discrete. In

contrast, the Perth Metropolitan Region wetlands displayed two separate groupings. Five of the sites tended to cluster near the Wagerup wetlands while the remaining four contained diatom assemblages similar to the Collie sites. The Wagerup sites grouped to the right of the axis during each season, displaying a reasonably strong separation from the Collie sites (Figure 5.3a - c).

An overlay of symbols representing the three pH groupings demonstrated that the acidic Group 1 wetlands were generally well separated from the alkaline Group 3 wetlands in each season (Figure 5.3d-f). The Group 1 wetland of Blind Roo A (Site 3) was the only exception, clustering with the Group 3 wetlands during summer and appearing relatively close in ordination space during spring. The circumneutral Group 2 wetlands mostly occupied an intermediate position and displayed less defined clustering than either the Group 1 or Group 3 wetlands (Figures 5.3).

One way analyses of similarities conducted on the seasonal diatom data detected significant differences in the community structure of the regional groups during each sampling period ($p < 0.05$). The diatom assemblages of the Collie and Wagerup regions were significantly different to each other in each season ($p < 0.05$) whereas Collie and the Perth Metropolitan Region were significantly different during spring only (Table 5.3).

ANOSIM tests on the summer and winter data also established significant differences in the diatom community structure of each pH group ($p < 0.05$) (Table 5.4). Comparisons of the acidic Group 1 wetlands and the alkaline Group 3 wetlands revealed the greatest differences during each season. Other groups were significantly different during winter and spring ($p < 0.01$) but the low R values ($R < 0.50$) suggest that the separation of these groups was not as strong. The spring ANOSIM generally displayed similar findings to the other seasons. The comparison between Group 1and Group 2 was the only exception, with no significant differences detected between the invertebrate communities of the two groups (Table 5.4).

Table 5.2: Summary of the dominant diatom taxa from each pH group of wetlands sampled in the south-west of Western Australia over the three sampling occasions. Taxa included were present in at least two sites within a group with an abundance of ≥ 10 %.

Taxa	Summer Group 1	Summer Group 3	Winter Group 1	Winter Group 2	Winter Group 3	Spring Group 1	Spring Group 2	Spring Group 3
Achnanthidium minutissimum		X		X				X
Brachysira brebissonii	X		X			X		
Brachysira styriaca						X		
Brachysira vitrea				X				
Cocconeis placentula								X
Encyonopsis microcephala		X						
Eunotia bilunaris	X							
Eunotia pectinalis var. minor	X		X					
Frustulia magaliesmontana	X		X			X		
Gomphonema parvulum		X		X	X			X
Navicula aff. c*ari*	X		X			X		
Navicula cryptocephala					X			X
Nitzschia palea								X
Nitzschia palea var. 1								X
Nitzschia paleaeformis	X		X			X		
Pseudostaurosira brevistriata		X						
Staurosira construens var. venter		X			X			X
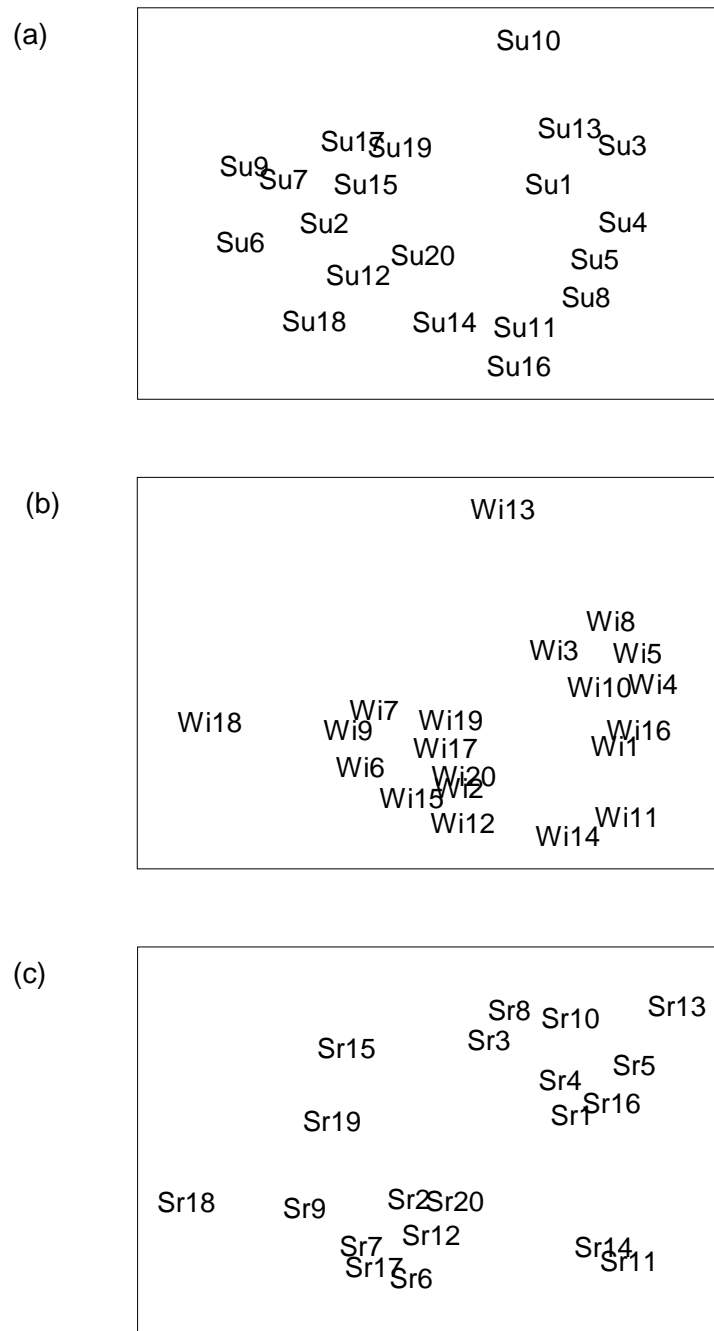
Figure 5.2: Two dimensional Multi-dimensional scaling ordination of square rooted transformed diatom abundance data (a) Summer sample. Stress = 0.14. (b) Winter. sample, stress = 0.11 (c) Spring sample, stress = 0.14. Site codes are preceded by the seasonal prefix Su to represent the summer sample, Wi to represent the winter sample and Sr to represent the spring sample.
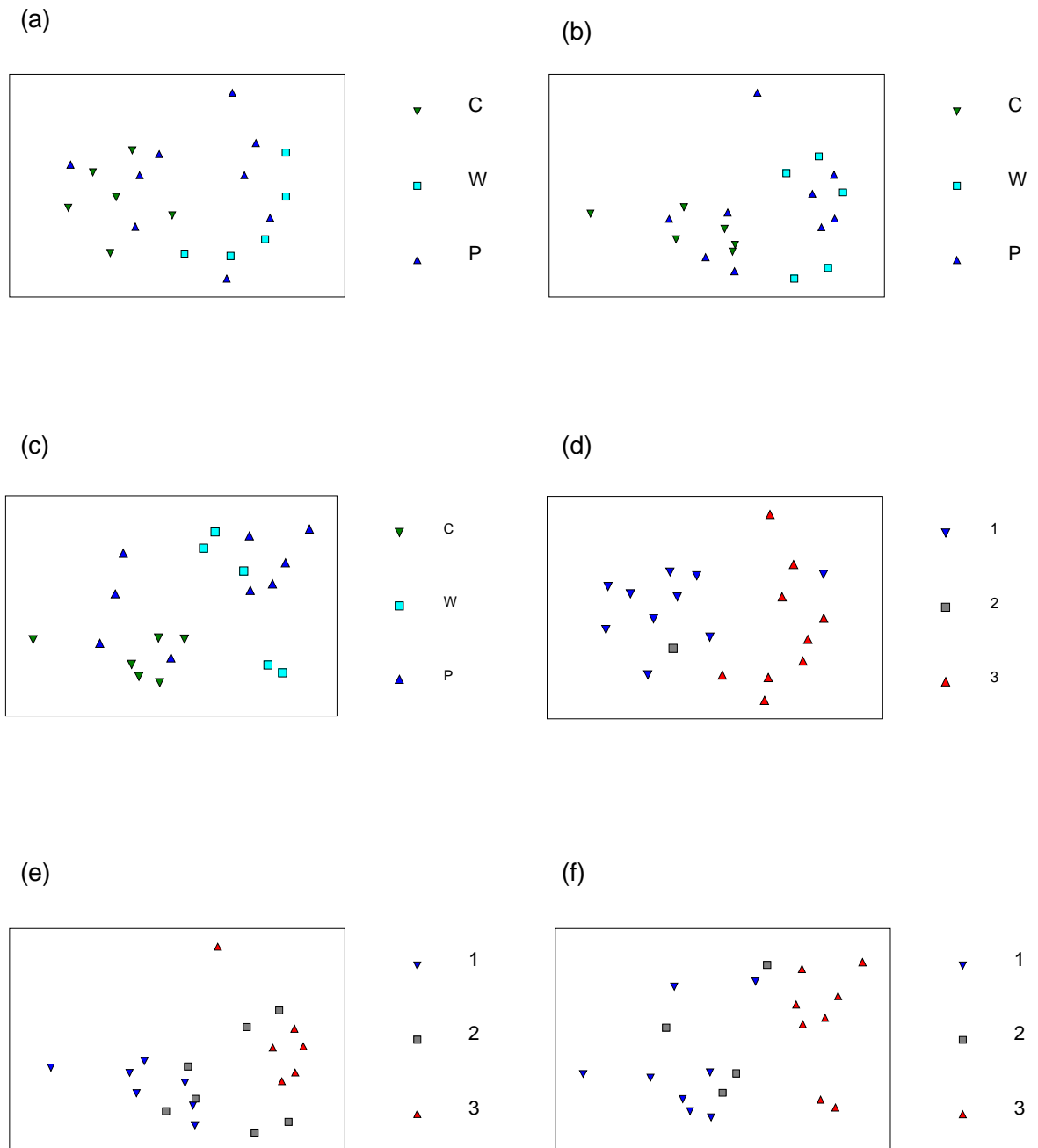
Figure 5.3: Two dimensional MDS of square root transformed diatom abundance data with superimposed symbols representing the three regional groups and three pH groups. (a) Regional overlay of summer data, stress = 0.14. (b) Regional overlay of winter sample, stress = 0.11. (c) Regional overlay of spring sample, stress = 0.14. C represents Collie sites, W represents Wagerup sites and P represents Perth Metropolitan sites. (d) – (f) Symbols representing the three pH groups have been superimposed for the summer, winter and spring data respectively.

Table 5.3: Results from one-way analysis of similarities and pairwise tests (ANOSIM) on Bray-Curtis similarities in square root transformed diatom abundance data from the regional groups of wetlands sampled in the south-west of Western Australia. Bold type indicates significant difference ($p < 0.05$).

Season	Region	R	Probability
Summer	All Regions	0.32	**< 0.01**
	Perth Metropolitan Region, Collie	0.19	> 0.05
	Perth Metropolitan Region, Wagerup	0.21	> 0.05
	Collie, Wagerup	0.78	**< 0.01**
Winter	All Regions	0.21	**< 0.05**
	Perth Metropolitan Region, Collie	0.15	> 0.05
	Perth Metropolitan Region, Wagerup	0.01	> 0.05
	Collie, Wagerup	0.72	**< 0.01**
Spring	All Regions	0.31	**< 0.01**
	Perth Metropolitan Region, Collie	0.26	**< 0.05**
	Swan Coastal Plain, Wagerup	0.12	> 0.05
	Collie, Wagerup	0.70	**< 0.01**

Table 5.4: Results from one-way analysis of similarities and pairwise tests (ANOSIM) on Bray-Curtis similarities in square root transformed diatom abundance data from the pH groups of wetlands sampled in the south-west of Western Australia. Bold type indicates significant difference ($p < 0.05$).

Season	pH Group	R	Probability
Summer	Group 1, Group 3	0.64	**< 0.01**
Winter	All Groups	0.46	**< 0.01**
	Group 1, Group 2	0.25	**< 0.05**
	Group 1, Group 3	0.82	**< 0.01**
	Group 2, Group 3	0.24	**< 0.05**
Spring	All Groups	0.51	**< 0.01**
	Group 1, Group 2	0.10	> 0.05
	Group 1, Group 3	0.76	**< 0.01**
	Group 2, Group 3	0.46	**< 0.05**

The BIO-ENV analyses between environmental variables and diatom abundance data determined that the strongest correlation in each of the seasons was produced by a single parameter. The variable of pH displayed the strongest correlation with community structure during summer and spring and achieved the same correlation as the two variable combination of pH and dissolved oxygen during winter ($\rho_s > 0.60$) (Table 5.5), supporting the relationship illustrated in Figure 5.3d-f.

Table 5.5: BIO-ENV results giving the combinations of environmental variables with the highest rank correlations between the abiotic and the square root transformed diatom similarity matrices as measured by Spearman rank correlation (ρ_s). A correlation cut-off of $\rho_s < 0.40$ was applied. The strongest correlation is presented in bold type. Veg = vegetation score, EC = electrical conductivity, Temp = temperature, DO = dissolved oxygen.

Season	n	Variables	ρ_s
Summer	1	**pH**	**0.62**
	2	pH, DO	0.58
	3	pH, Temp, DO	0.52
	2	pH, Temp	0.52
	3	pH, EC, DO	0.51
	4	pH, EC, Temp, DO	0.49
	2	pH, EC	0.48
	3	Veg, pH, DO	0.44
	4	Veg, pH, Temp, DO	0.43
	3	pH, EC, Temp	0.43
Winter	1	**pH**	**0.62**
	2	pH, DO	0.62
	3	pH, EC, DO	0.55
	3	pH, Temp, DO	0.55
	2	pH, Temp	0.52
	4	pH, EC, Temp, DO	0.52
	2	pH, EC	0.51
	3	Veg, pH, DO	0.49
	3	pH, EC, Temp	0.48
	4	Veg, pH, EC, DO	0.47
Spring	1	**pH**	**0.63**
	2	pH, DO	0.48
	2	pH, Temp	0.45
	2	pH, EC	0.44
	2	pH, Veg	0.41
	3	pH, Temp, DO	0.40

5.5   Discussion

5.5.1   Diatom flora

A total of 154 diatom taxa were recorded from the 20 sites during the study. The genera representing the most species were consistent with the findings of previous Western Australian studies and those from other areas of Australia. For example, *Achnanthidium, Gomphonema, Navicula* and *Nitzschia* were identified as some of the most species-rich taxa in a study on the classification of urban streams in Perth (John 2000a). Sonneman *et al.* (2001) also identified *Nitzschia, Navicula, Gomphonema* and *Achnanthes* as the taxa with the highest number of species during a study in the Melbourne region of Victoria. The aforementioned genera were also commonly recorded from streams in New South Wales and Victoria (Chessman *et al.* 1999).

Separation of the sites into the three pH groups identified 17 dominant taxa. The alkaline wetlands of Group 3 contained the highest number of abundant species followed by the acidic Group 1 wetlands. While the Group 2 wetlands had the lowest number of dominant taxa, this is may be indicative of the small sample sizes of the group rather than a lack of species favouring circumneutral conditions. The range of pH in the different groups is unbalanced with the circumneutral or Group 2 classification having a very limited range (pH 6.5 – 7.5) in comparison to the acidic and alkaline wetland groups (< 6.5 and > 7.5 respectively). This may influence the number of species that display a clear preference for circumneutral waters.

The acidic Group 1 wetlands were consistently dominated by diatoms including *Brachysira brebissonii*, *Frustulia magaliesmontana* and *Nitzschia paleaeformis*.
The occurrence of *Brachysira brebissonii* in acidic waters has been well documented (van Dam *et al.* 1981; DeNicola 1986; Vinebrooke *et al.* 2003). Cholnoky (1968) reported that the optimum pH for the species (presented as *Anomoeneis brachysira*) was 5.2 and Gasse (1986) noted that a low pH appeared to be the most important ecological variable for the species. *Frustulia magaliesmontana* has previously been reported from dystrophic acidic lakes in the western region of Tasmania (Vyverman *et al.* 1996) and from acidic sand mine lakes in Capel, southwestern Australia (pH < 4) (John 1993). *Nitzschia paleaeformis* was another taxon found commonly in

Capel's sand mine lakes (John 1993). Further records of the species include a study on Japanese water bodies (pH 2.6-3.9) (Watanabe and Asai 2004) and a study on lentic heathland waters in Belgium (Denys and van Straaten 1992).

Brachysira styriaca and *Eunotia bilunaris* were among the taxa that commonly contributed to the community structure of Group 1 wetlands in at least one season. *Brachysira styriaca* has been recorded from countries including Iceland and the United States of America and appears to favour waters with pH readings of < 7 (Foged 1974; Siver *et al.* 2005). *Eunotia bilunaris* has been described by Patrick and Reimer (1966) as a frequent inhabitant of acidic waters.

Brachysira vitrea was abundant in the Group 2 wetlands only and this apparent preference for circumneutral waters is supported by the findings of other studies. Round (1990) highlighted the association of *Brachysira vitrea* with the higher pH readings (mean 6.6) in a study of Welsh lakes ranging from pH 4.4 -6.8. While a study of mostly acidic lakes in Florida reported that the only lake *Brachysira vitrea* was recorded from was significantly less acidic than the other sites (mean pH = 7) (Shayler and Siver 2002). Additionally, Coring (1996) lists the taxon (presented as *Anomoeneis vitrea*) as being more indicative of dystrophic (humic acid) circumstances rather than anthropogenic acidification.

Achnanthidium minutissimum was one of the two dominant taxa that overlapped between the circumneutral wetlands and the alkaline sites of Group 3. The species is cosmopolitan in distribution (Foged 1979) and the pH preference of this species appears to vary between study and region. Foged (1978; 1979) classified the taxon as alkaliphilous while Gasse (1986) commonly recorded the species in weakly acidic to weakly alkaline waters (pH = 6-8.5). Cholnoky (1968) stated that the optimum pH for *Achnanthidium minutissimum* (presented as *Achnanthes minutissima*) lay between 7.5-7.8 and Patrick and Reimer (1966) found that the species occurred most frequently between pH levels of approximately 6.5 - 9. Similarly, in the current study the species was most abundant in wetlands with a pH > 6.5.

Gomphonema parvulum was the second taxon to occur frequently in both Group 2 and Group 3 wetlands. Similar to *Achnanthidium minutissum*, the pH preference of

the former appears less specific than many other species. Schoeman (1973) reported that *Gomphonema parvulum* had the ability to tolerate large pH fluctuations, commonly finding it in strongly alkaline and neutral waters and recording low frequencies from some acidic waters in Lesotho, Africa. Foged (1974; 1979) and Gasse (1986) noted that the taxon generally occurs in circumneutral to alkaline waters, thus supporting the dominant presence of the species in both the Group 2 and Group 3 wetlands of the current study.

Aside from *Gomphonema parvulum, Staurosira construens* var. *venter* was the only taxon to maintain a dominant presence in the Group 3 wetlands during the entire study. The species is known to be widely distributed (Patrick and Reimer 1966) and has been identified from regions including Russia (Laing and Smol 2000) and the United States of America (Camburn and Charles 2000). Previous records in Australia include streams and wetlands in Victoria (as *Fragilaria construens* f. *venter*) (Blinn and Bailey 2001; Gell *et al.* 2002), wetlands on the Swan Coastal Plain (Helleran 1993) and the Swan and Canning Rivers in Perth (John 1983). The species appears to prefer waters of medium pH and alkalinity, although it is able to tolerate more extreme conditions (Gasse 1986).

Cocconeis placentula, Pseudostaurosira brevistriata, Encyonopsis microcephala and *Nitzschia palea* were among the taxa abundant in a particular season. *Cocconeis placentula* was described by Cholnoky (1968) as a good indicator of moderately alkaline conditions (pH optimum of about 8) and *Nitzschia palea* was calculated to have an optimum pH of 8.4. *Encyonopsis microcephala* and *Pseudostaurosira brevistriata* have both been recorded from waters of varying pH conditions (Schoeman 1973; Hustedt and Jensen 1985) although they tend to occur most commonly in circumneutral to alkaline waters (Foged 1974; Gasse 1986).

5.5.2 Diatom community structure

Multi-dimensional scaling and analyses of similarities established a significant difference in the community composition of diatoms in the different regions sampled. The Collie wetlands, while not discretely grouped, tended to cluster together in ordination space and were generally well separated from the Wagerup

wetlands. This was supported by analyses of similarities which detected significant differences in the diatom assemblages of the two regions during each season. The differences in community structure are likely to be at least partly attributable to the water chemistry variable such as pH, with each of Collie lakes exhibiting lower pH values than most of the Wagerup wetlands. The Perth Metropolitan wetlands tended to separate into two groups. Acidic and circumneutral sites (Gnangara Lake, Lakelands, Tuscan Park and Kurrajong Village Lake) grouped near the Collie sites while alkaline sites such as Bibra and Blue Gum Lakes clustered near the Wagerup sites. This was generally supported by the analyses of similarities which detected a significant difference between the Collie and the Perth Metropolitan Region wetlands during spring only.

MDS and analyses of similarity also detected significant differences in the community structure of diatoms in the three pH groups of wetlands, suggesting that pH was an important contributing factor in the separation of the wetlands. The largest differences in community structure were evident between the acidic waterbodies of Group 1 and the alkaline Group 3 wetlands. Dissimilarities between the dominant taxa of the two groups of wetlands further supported the differences displayed in the overall community structure. The main exception to the otherwise strong separation of the two groups of wetlands was the generally acidic wetland of Blind Roo A. This site, along with other Wagerup wetlands including Blind Roo B was originally created through clay extraction. However, in contrast to the mostly alkaline nature of the other sites, Blind Roo A displays a relatively low pH, probably as a result of large additions of vegetative material (J. John pers. comm.). Despite this, the overall community composition was atypical of acidic wetlands, suggesting that variables other than pH were the over-riding factors in the diatom community structure. Taking the history of the site into consideration it seems likely that unmeasured factors linked to substrate type may be at least partly responsible for influencing the diatom assemblages. Additionally, the Wagerup sites are situated on pastoral land and surrounding land-use practices may have exposed the wetlands to impacts such as nutrient enrichment (Harper 1992). For example *Gomphonema parvulum* , a species able to survive across a range of pH (Schoeman 1973) and known to occur in waters with high nutrient levels (Patrick and Reimer 1975; Silva-Benavides 1996; Gell *et al.* 2002; Soininen 2002) was identified from both Blind

Roo A and Blind Roo B during each sampling period and generally occurred in high numbers.

Despite some limited overlap between the dominant species of the circumneutral and alkaline wetlands (Groups 2 and 3), multivariate analyses demonstrated that the overall community structure of the two groups of wetlands was significantly different during winter and spring. Analyses on the summer data were not carried out because of the low numbers of sites in Group 2 wetlands during that season. Significant differences were also detected between the Group 1 and Group 2 wetlands during winter. However, the aforementioned groups possessed relatively similar community composition in the spring sample. These similarities may have resulted from the fluctuation of sites between the two groups. For example Lakelands (Wi15 and Sr15) was classified as circumneutral (pH 6.73) during winter and acidic during spring (pH 6.04). Accordingly, the diatom community included both acidophilous and circumneutral diatoms with *Brachysira vitrea*, a species known to prefer circumneutral conditions (Battarbee *et al.* 1992) among the dominant taxa. Whereas Kurrajong Village (Wi12 and Sr12) a site classified as acidic during winter and circumneutral during spring, was dominated by species including *Frustulia magaliesmontana* that are known to favour acidic waters (Hall and Smol 1996). The movement of such wetlands between the pH groupings is likely to have contributed to changes in the overall community structure of the groups and led to increased similarities in the community composition. Additionally, three of the four sites identified as circumneutral during spring were all near the lower limit of the circumneutral classification, which may have influenced the type of diatoms present in these sites.

The findings generally suggest that pH is an important influence on diatom distribution, in accordance with other studies including ten Cate *et al.* (1993), Battarbee *et al.* (1997) and Siver *et al.* (2004). The results of the BIO-ENV analysis further support this concept with pH being identified as the variable most closely related to diatom community composition. It should however be noted that the relatively low number of study sites may have affected the reliability of the results. Larger sample sizes generally improve the chances of successfully linking biotic and environmental patterns (Clarke and Warwick 2001). Additionally, the influence of

factors other than pH must be considered. For example the BIO-ENV results showed that the combination of pH and dissolved oxygen displayed a correlation similar to that of pH alone in each season. The effect of dissolved oxygen on species assemblages was highlighted by Schoeman (1973), who listed *Achnanthes*, *Cymbella* and *Fragilaria* as taxa that are generally abundant in highly oxygenated waters. A further consideration is the influence of variables which were not investigated during the present study. Diatom community structure is known to be influenced by a number of factors including the concentration of nutrients such as silica (Cooper 1999) and nitrogen (Saros *et al.* 2003). Calcium concentration is another factor that may help determine community composition (Patrick 1945). Metal concentrations have also been noted as variables which impact upon diatom assemblages (Hirst *et al.* 2002; Gold *et al.* 2003). For example, Anderson *et al.* (1986) identified Al as one of the pH-related factors partly responsible for variation in diatom community structure. Considering the inclusion of acidic mine-void lakes and shallow seasonal wetlands in the current study in conjunction with the known associations between metals such as Al, Fe and pH (Sammut and Lines-Kelly 2000; Sommer and Horwitz 2001), the measurement of metal concentrations would have been particularly relevant.

5.5.3   Seasonal differences in diatom community structure

Patrick (1964) reported that diatom assemblages may vary between seasons in terms of the taxa present and their contribution to overall community structure. Accordingly, there were some differences apparent in some of individual taxa during the current study. However, it is unlikely that these differences resulted in substantial compositional variation between seasons. The acidic sites remaining significantly different from the alkaline sites during each sampling period and both pH groups contained consistently dominant species. For example, taxa such as *Brachysira brebissonii* and *Frustulia magaliesmontana* were dominant taxa in the Group 1 wetlands in each season while *Gomphonema parvulum* and *Staurosira construens* var. *venter* were consistently dominant in the Group 3 wetlands. Differences between the sampling periods were more evident for the intermediate Group 2 wetlands than Group 1 and 3 wetlands but this was probably a reflection of the small number of sites and the comparatively small pH range of Group 2 wetlands. For example, the

lack of significant difference between the Group 1 and Group 2 wetlands during spring was probably related to sites classified as circumneutral in winter being classified as Group 1 wetlands during spring. As discussed in the previous chapter on invertebrate community composition, it is possible that any apparent differences between the sampling periods may have been related to heterogeneity in the data-set than rather than actual seasonal variability. Furthermore, while the results suggested that there was some variation in the dominant taxa between seasons, particularly for the Group 2 wetlands, it is important to note that dominance was assigned by an arbitrary cut-off value of abundance.

While preliminary, these findings tend to suggest that diatoms are influenced by pH regardless of season. It is therefore likely that the group could be successfully used as biological monitors of pH change in a regular monitoring program.

5.6 Conclusion

Of the variables included in the study, pH was the primary determinant of diatom community structure. Multivariate analyses detected differences in diatom assemblages in wetlands on the basis of pH group and geographical region. The largest differences in geographical region were identified between the mostly acidic sites of Collie and the relatively higher pH sites of Wagerup. Differences in the pH groups were most evident between the acidic Group 1 wetlands and the alkaline Group 3 sites, suggesting that many of the diatom taxa inhabiting each of these groups have a specific preference or tolerance to pH. Less defined differences were evident when comparing the Group 2 wetlands with the other groups, possibly as a result of seasonal fluctuations in pH and the subsequent fluidity of the circumneutral wetland group. The inclusion of more circumneutral sites, both natural and human-induced, in future studies would increase understanding of the community structure of circumneutral wetlands and potentially identify more species that favour this pH classification.

The diatoms exhibiting the most specific preferences included *Nitzschia paleaeformis* and *Navicula* aff. *cari*, both of which were common in the acidic wetlands and rarely occurred in circumneutral waters. The species *Brachysira*

brebissonii and *Frustulia magaliesmontana* were also among the dominant taxa in the Group 1 wetlands, occurring abundantly in all seasons. However unlike the former species, both *Brachysira brebissonii* and *Frustulia magaliesmontana* were also regularly identified from circumneutral sites, although generally in lower numbers. Despite this, these taxa are still likely to have potential applications as biological monitors of pH change, indicating pH decline in alkaline waters and possibly detecting pH decline in circumneutral waters through an increase in percentage abundance. *Brachysira vitrea* was dominant in the Group 2 wetlands and may have potential as an indicator of intermediate pH levels.

Most of the dominant species in the alkaline Group 3 wetlands tended to have broader tolerances than the abundant species in the acidic sites. Dominant taxa including *Gomphonema parvulum* and *Staurosira construens* var. *venter* are known to occur over a wide range of pH conditions but do tend to favour neutral and alkaline conditions and may still be useful as indicator species of moderate to high pH. Other abundant Group 3 species such as *Pseudostaurosira brevistriata, Nitzschia palea* and *Cocconeis placentula* are also able to inhabit waters of varying pH but generally prefer higher pH conditions. Additionally, several of the taxa identified as dominant in the Group 3 wetlands have been previously associated with nutrient enriched waters. Further research including the measurement of nutrient concentration would help discern the contributions of pH and nutrient concentration to the distribution patterns of these species and their usefulness as indicators for pH.

Along with the identification of potential indicator species, the current study demonstrated that the overall structure of the diatom communities generally varied between the different wetland pH groups. Furthermore, the results suggest that a relatively strong relationship between diatom community structure and pH is evident in various seasons, suggesting that diatoms would be useful biological monitors of acidification during a regular monitoring program.

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Appendix 5.1: Diatom taxa collected during the summer (Su), winter (Wi) and spring (Sr) 2001 sampling periods.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Achnanthes aff. *linearis* (W Smith) Grunow		Su,Wi,Sr																Su		
Achnanthidium aff. *reidensis* Foged								Su,Wi,Sr												
Achnanthidium binodis Kützing														Su			Wi			
Achnanthidium exiguum (Grunow) Czarnecki	Su	Wi		Wi	Su,Wi			Su		Sr		Wi	Su		Wi	Su,Wi			Sr	Su
Achnanthidium exilis (Kützing) Round & Bukhtiyarova																Su,Wi				
Achnanthidium hungaricum Grunow	Su		Su	Su,Wi	Wi,Sr															
Achnanthidium minutissimum (Kützing) Czarnecki	Wi,Sr	Su,Sr	Sr	Wi,Sr				Su,Wi,Sr		Wi,Sr	Su,Wi,Sr	Su,Wi,Sr		Su,Wi,Sr	Wi	Su,Wi,Sr	Wi		Wi,Sr	Su,Wi,Sr
Achnanthidium oblongella Oestrup			Sr					Su		Wi,Sr					Wi		Su,Sr			
Achnanthidium reidensis Foged	Sr		Su,Wi,Sr	Su,Wi,Sr				Sr					Su							
Achnanthidium sp. 1																Su,Wi,Sr				
Achnanthidium sp. 2			Sr	Su,Wi,Sr				Su,Wi,Sr		Wi,Sr										
Achnanthidium sp. 3					Su															
Amphora fontinalis Hustedt											Su,Sr			Sr						
Amphora ovalis var. *affinis* (Kützing) Van Heurck *ex* DeToni												Wi,Sr								
Amphora ovalis var. *pediculus* (Kützing) Van Heurck *ex* DeToni									Sr	Su										
Amphora subturgida Hustedt	Sr																			
Amphora veneta Kützing	Wi			Wi,Sr	Sr	Sr	Sr				Wi,Sr					Su,Wi		Su		
Amphora ventricosa Gregory																			Wi	
Aulacoseira granulata (Ehrenberg) Simonsen															Su,Wi,Sr				Wi	
Bacillaria paxillifer (O Müller) Hendey																	Wi		Sr	
Brachysira aff. *manfredii* Lange-Bertalot		Sr							Su,Wi,Sr											Su,Wi,Sr
Brachysira aff. *neoexilis* Lange-Bertalot																		Su		
Brachysira brebissonii Ross		Su,Wi,Sr				Su,Wi,Sr	Su,Wi,Sr		Su,Wi,Sr			Su,Wi,Sr			Su,Wi,Sr		Su,Wi,Sr		Su,Wi,Sr	Su,Wi,Sr
Brachysira styriaca (Grunow) Ross *in* Hartley									Wi,Sr						Su,Wi,Sr		Wi		Su,Wi,Sr	
Brachysira vitrea (Grunow) Ross *in* Hartley		Su,Wi,Sr							Su					Su	Su,Wi,Sr				Su,Wi	Sr
Caloneis bacillum (Grunow) Cleve																Wi			Wi	Sr
Cocconeis placentula Ehrenberg	Su,Sr			Su,Sr	Sr					Wi		Wi,Sr			Sr	Su,Sr		Su		
Cocconeis placentula var. *euglypta* (Ehrenberg) Grunow												Sr								
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck												Sr								
Craticula cuspidata (Kützing) Mann *in* Round, Crawford & Mann											Su									
Craticula halophila (Grunow) Mann *in* Round, Crawford & Mann		Wi,Sr		Sr				Su,Wi,Sr		Wi,Sr	Su,Wi			Su,Sr			Su,Wi	Su		Su,Wi
Ctenophora pulchella (Ralfs *ex* Kützing) Williams & Round	Sr			Su						Sr			Su			Sr				
Cyclotella atomus Hustedt								Wi											Su	
Cyclotella meneghiniana Kützing	Su,Wi,Sr		Su,Wi,Sr	Su,Wi	Su,Wi			Su,Wi,Sr		Sr				Sr						
Cyclotella stelligera (Cleve & Grunow) Van Heurck						Wi									Su,Wi,Sr		Sr			Su,Wi,Sr
Cylindrotheca closterium (Ehrenberg) Lewin & Reimann								Su,Wi,Sr												
Cymbella tumida (Brébisson) Van Heurck																	Wi			
Cymbella turgida (Gregory)																				Su,Wi,Sr
Diadesmis confervacea Kützing			Su	Su,Wi,Sr	Su,Wi,Sr									Su			Su			
Encyonema delicatulum (Kützing) Mann																	Wi			
Encyonema gracile Rabenhorst														Su						Wi,Sr
Encyonema minutum (Hilse *ex* Rabenhorst) Mann													Su	Su,Wi,Sr				Su		
Encyonema mesianum (Cholnoky) Mann																Wi				
Encyonema silesiacum (Bleisch *ex* Rabenhorst) Mann								Su							Su				Su	
Encyonopsis microcephala (Grunow) Krammer											Su,Sr			Su,Wi,Sr			Wi			Wi,Sr
Epithemia adnata var. minor (M. Perag. & Hérib.) Patrick														Wi,Sr		Su				

Appendix 5.1 continued: Diatom taxa collected during the summer (Su), winter (Wi) and spring (Sr) 2001 sampling periods.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Epithemia sorex Kützing													Su			Su				
Eunotia bilunaris (Ehrenberg) Mills		Su,Wi,Sr					Wi,Sr										Su	Wi	Sr	Wi,Sr
Eunotia camelus Ehrenberg								Su												Wi
Eunotia exigua (Brébisson ex Kützing) Rabenhorst																	Wi,Sr	Sr		
Eunotia flexuosa Brébisson *ex* Kützing															Su,Wi				Su,Wi,Sr	Wi,Sr
Eunotia formica Ehrenberg																			Su	
Eunotia pectinalis (O. Müller) Rabenhorst	Sr	Su,Wi,Sr	Su,Wi,Sr					Su				Su,Wi,Sr		Wi	Wi	Sr			Wi	Wi,Sr
Eunotia pectinalis var. *minor* (Kützing) Rabenhorst			Wi,Sr		Wi		Su,Wi,Sr		Wi,Sr			Su,Wi,Sr			Su,Wi,Sr		Su,Wi,Sr	Su,Sr	Su,Wi,Sr	Su,Wi,Sr
Eunotia pectinalis var. *recta* A. Mayer *ex* Patrick																				Su,Wi
Fallacia auriculata (Hustedt) D.G. Mann				Su																
Fragilaria capucina Desmaziére														Wi						
Fragilaria punctata John	Su,Wi		Su																	
Fragilaria radians (Kützing) Williams & Round														Sr						
Fragilaria rumpens (Kützing) Carlson	Wi			Su,Wi	Wi									Su		Wi,Sr				
Fragilaria sp. 1														Wi						
Fragilaria vaucheriae (Kützing) J.B. Peterson				Wi												Su				
Fragilariforma virescens (Ralfs) D.M. Williams & Round										Su,Sr			Su		Su				Su	
Frustulia magaliesmontana Cholnoky		Su,Wi,Sr	Sr				Su,Wi,Sr	Su,Wi,Sr				Su,Wi,Sr		Su	Su,Wi		Su,Wi,Sr	Su	Su,Wi	Su,Wi,Sr
Frustulia rhomboides (Ehrenberg) DeToni		Su,Wi,Sr													Su,Wi,Sr		Sr	Su	Sr	Sr
Frustulia rhomboides var. capitata (Mayer) Patrick		Su																		Su
Frustulia rhomboides var. saxonica (Ehrenberg) DeToni		Wi,Sr																	Su	Wi
Frustulia vulgaris (Thwaites) De Toni								Sr												
Gomphonema acuminatum Ehrenberg										Sr				Su,Wi						
Gomphonema acuminatum var. *brebissonii* (Kützing) Cleve															Su				Su,Wi,Sr	
Gomphonema acuminatum var. coronatum (Ehrenberg) Rabenhorst.								Wi												
Gomphonema aff. parvulum Kützing	Su																			
Gomphonema affine Kützing			Sr	Su			Sr			Wi			Sr				Sr			
Gomphonema affine var. *insigne* (Gregory) Andrews				Sr						Sr		Su,Sr								
Gomphonema auritum A. Braun			Su		Su,Sr			Wi												
Gomphonema gracile Ehrenberg										Wi										
Gomphonema lanceolatum Ehrenberg				Wi																
Gomphonema parvulum Kützing	Sr		Su,Wi,Sr	Su,Wi,Sr	Su,Wi,Sr		Wi	Su,Wi,Sr		Wi,Sr	Su	Su,Wi,Sr	Su,Wi,Sr	Sr	Wi,Sr				Wi	Su
Gomphonema truncatum Ehrenberg															Su					
Gomphonema truncatum var. capitatum (Ehrenberg) Patrick in Patrick & Reimer										Wi,Sr			Sr							
Gomphonema undulatum Hustedt				Wi	Wi,Sr															Su
Gyrosigma spencerii (Quekett) Griff. & Henfr.																		Wi		
Hantzschia amphioxys (Ehrenberg) Grunow in Cleve and Grunow			Wi	Wi																
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski				Sr	Wi			Wi						Wi		Su	Wi			
Luticola mutica (Kützing) Mann																	Wi			
Mastogloia elliptica var. dansei (Thwaites) Cleve										Su										
Mastogloia smithii Thwaites														Su						
Navicula aff. *cari* Ehrenberg						Su,Wi			Su,Wi,Sr						Su,Wi,Sr			Su,Wi,Sr		
Navicula aff. *subtilissima* Cleve		Su,Wi,Sr										Wi,Sr			Su,Wi,Sr					
Navicula angusta Grunow																		Su	Wi	
Navicula comoides (Ag.) Peragallo			Sr											Sr						
Navicula cryptocephala Kützing	Su,Wi		Wi	Su,Wi,Sr	Wi,Sr		Wi		Wi			Su		Su,Sr	Su	Su,Wi,Sr			Su,Wi	Su,Wi,Sr

Appendix 5.1 continued: Diatom taxa collected during the summer (Su), winter (Wi) and spring (Sr) 2001 sampling periods.

|	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Navicula notha Wallace																				Su,Wi
Navicula radiosa Kützing	Wi													Su,Wi	Su	Sr			Su,Wi,Sr	Su,Wi,Sr
Navicula rhynchocephala Kützing																				Su,Sr
Navicula rhynchocephala var. *germainii* (Wallace) R.M. Patrick				Su,Sr																
Navicula subrhynchocephala Hustedt													Su							
Navicula symmetrica Patrick	Sr			Sr																
Navicula tripunctata var. *schizonemoides* (Van Heurck) Patrick																	Wi			
Navicula viridula (Kützing) Ehrenberg								Su						Su,Sr						
Nedium affine var. *amphirhynchus* (Ehrenberg) Cleve			Wi					Wi,Sr									Sr			
Neidium iridis (Ehrenberg) Cleve		Su																		
Neidium productum (W. Smith) Grunow																	Su			
Nitzschia aff. *capitellata* Hustedt						Su,Wi														
Nitzschia aff. *linearis* W. Smith															Wi,Sr					
Nitzschia amphibia Grunow	Su,Wi				Su					Wi,Sr			Su,Wi,Sr		Su				Su	
Nitzschia fasiculata Grunow																	Wi			
Nitzschia gracilis Hantzsch			Wi,Sr	Sr	Sr			Su,Wi,Sr			Su		Su							
Nitzschia lorenziana Grunow																	Sr			Sr
Nitzschia obtusa W. Smith															Wi,Sr		Wi		Wi	
Nitzschia palea (Kützing) W. Smith	Su,Wi,Sr	Sr	Wi,Sr	Su,Wi,Sr	Su,Wi			Su,Wi,Sr		Wi	Su,Wi						Wi			Su,Sr
Nitzschia palea var. 1	Sr			Wi,Sr	Wi,Sr											Su,Wi,Sr				
Nitzschia palea var. *tenuirostris* Grunow					Wi							Su				Su,Sr				
Nitzschia paleacea Grunow	Wi,Sr		Wi,Sr		Su					Wi	Su								Wi	
Nitzschia paleaeformis Hustedt		Su,Wi,Sr				Su,Wi,Sr	Su,Wi,Sr		Su,Wi,Sr						Wi		Wi,Sr	Sr		Su,Wi,Sr
Nitzschia romana Grunow			Wi					Su,Sr			Su		Su							
Nitzschia scalpelliformis (Grunow) Grunow															Su,Wi,Sr					
Nitzschia sigma (Kützing) W. Smith.								Su												
Nitzschia sp. 1	Wi	Su,Sr						Sr			Su				Sr		Wi		Wi	
Nitzschia sp. 2						Su,Wi,Sr	Wi,Sr											Wi,Sr		
Nitzschia subinflata Hustedt	Wi																			
Pinnularia biceps Gregory																	Wi			
Pinnularia braunii var. *amphicephala* (A. Mayer) Hustedt			Wi,Sr					Wi												
Pinnularia divergentissima (Grunow) Cleve			Su	Su			Wi,Sr												Su,Sr	
Pinnularia gentilis (Donkin) Cleve								Sr												
Pinnularia gibba Ehrenberg			Su,Wi					Su				Sr			Su				Su,Wi	
Pinnularia gibba var. *parva* (Ehrenberg) Grunow			Sr					Sr												
Pinnularia obscura Krasske			Sr				Wi											Su,Wi,Sr	Wi	
Pinnularia subcapitata Gregory		Su,Wi,Sr		Sr							Su				Wi		Sr		Su,Sr	Wi
Pinnularia viridis (Nitzsch) Ehrenberg			Sr				Sr												Su	
Placoneis elginensis (Gregory) EJ Cox																				Su,Wi,Sr
Planothidium lanceolatum (Brébisson) Round & Bukhtiyarova	Wi				Wi,Sr			Wi		Wi					Su	Wi				
Pseudostaurosira brevistriata (Grunow in Van Heurck) DM Williams & Round	Su,Wi,Sr	Wi	Su		Wi,Sr					Su,Wi,Sr			Su		Su,Sr	Wi	Su,Wi		Su,Wi	Sr
Rhopalodia gibba (Ehrenberg) O.Müller												Su								
Rhopalodia gibberula (Ehrenberg.) O.Müller														Su			Su			Sr
Sellaphora pupula (Kützing) Mereschk.				Su,Wi,Sr	Wi			Su,Wi,Sr									Su			
Sellaphora pupula var. rectangularis (Gregory) Mereschkowsky											Su						Su			Su

Appendix 5.1 continued: Diatom taxa collected during the summer (Su), winter (Wi) and spring (Sr) 2001 sampling periods.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Stauroneis anceps Ehrenberg			Wi					Su											Su	Su,Wi
Stauroneis dubitabilis Hustedt			Su,Wi,Sr												Su				Su,Wi	
Stauroneis kriegeri Patrick																			Wi	
Stauroneis legleri Hustedt							Su,Wi										Su,Sr	Su		
Stauroneis obtusa Lagerstedt		Su,Sr				Sr														
Stauroneis pachycephala Cleve											Su,Wi				Su,Wi		Su,Wi		Su	Su,Wi,Sr
Staurosira construens Ehrenberg																	Wi			
Staurosira construens var. venter (Ehrenberg) Hamilton	Su,Wi,Sr	Wi	Wi	Wi	Su,Wi					Su,Wi,Sr	Su,Wi	Su				Su,Wi,Sr			Su	
Surirella angusta Kützing								Wi,Sr											Su	
Surirella linearis var. *constricta* Grunow																			Sr	
Surirella tenera Gregory		Su,Wi,Sr					Su,Wi,Sr										Su,Wi,Sr	Wi	Wi	
Synedra acus Kützing													Su							
Synedra amphicephala Kützing															Su,Wi,Sr				Su	Su
Synedra ulna (Nitzsch) Ehrenberg.	Wi			Sr				Sr			Su,Sr						Su,Wi			
Tabellaria flocculosa (Roth) Kützing													Su,Wi,Sr				Su,Wi,Sr	Su		
Tabularia tabulata (Agardh) Snoeijs	Wi,Sr									Wi,Sr										
Tryblionella victoriae Grunow										Su,Wi,Sr										

Appendix 5.2: Dominant diatom taxa from the three pH groups. (a) *Achnanthidium minutissimum* valve view (b) *Achnanthidium minutissimum* girdle view (c) *Brachysira brebissonii* (d) *Brachysira styriaca* (e) *Brachysira vitrea* (f) *Cocconeis placentula* (g) *Encyonopsis microcephala* (h) *Eunotia bilunaris* (i) *Eunotia pectinalis* var. *minor*.
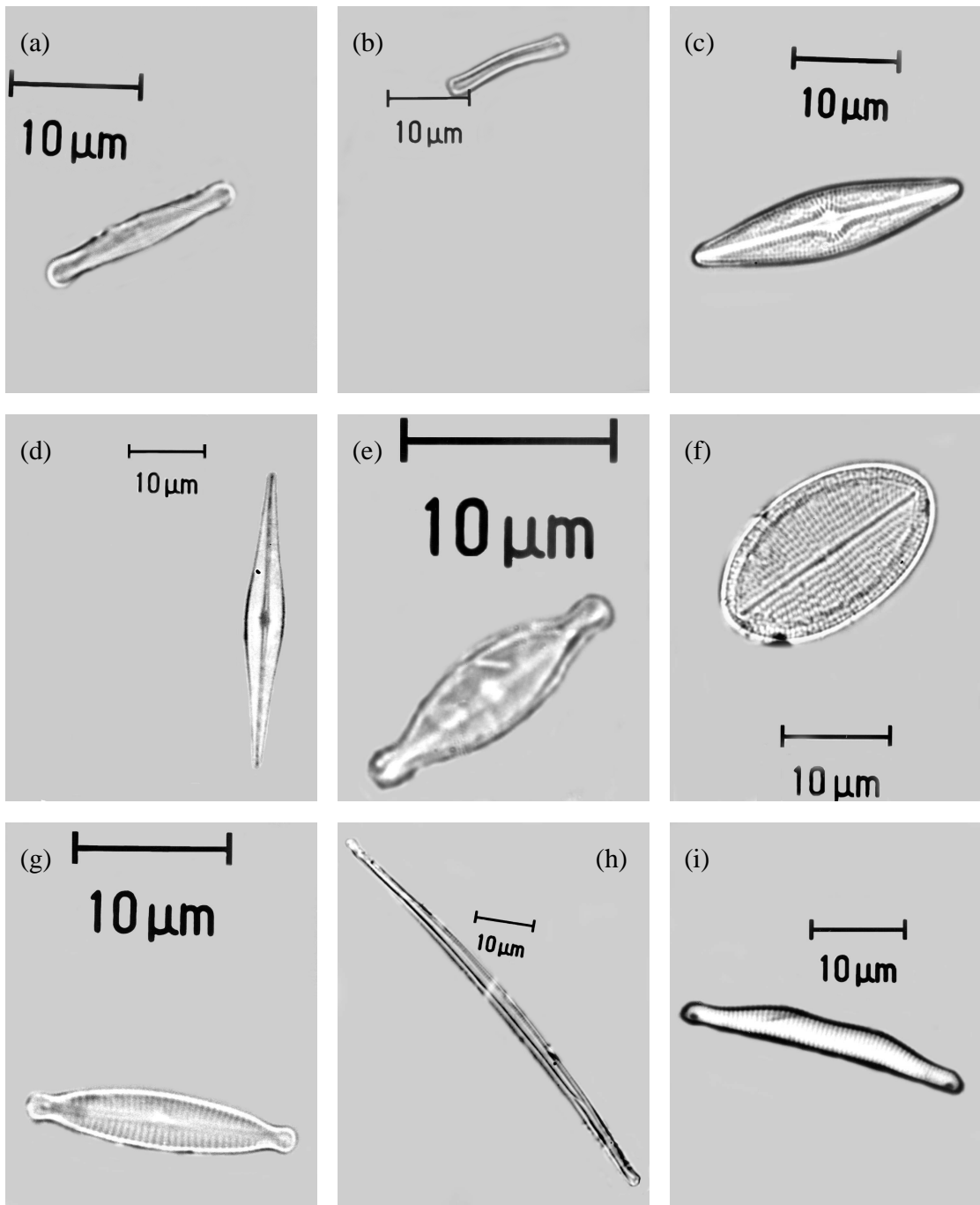
Appendix 5.2 continued: Dominant diatom taxa from the three pH groups. (j) *Frustulia magaliesmontana* (k) *Gomphonema parvulum* (l) *Navicula* aff. *cari* (m) *Navicula cryptocephala* (n) *Nitzschia palea* (o) *Nitzschia palea* var. 1 (p) *Nitzschia paleaeformis* (q) *Pseudostaurosira brevistriata* (r) *Staurosira construens* var. *venter.*
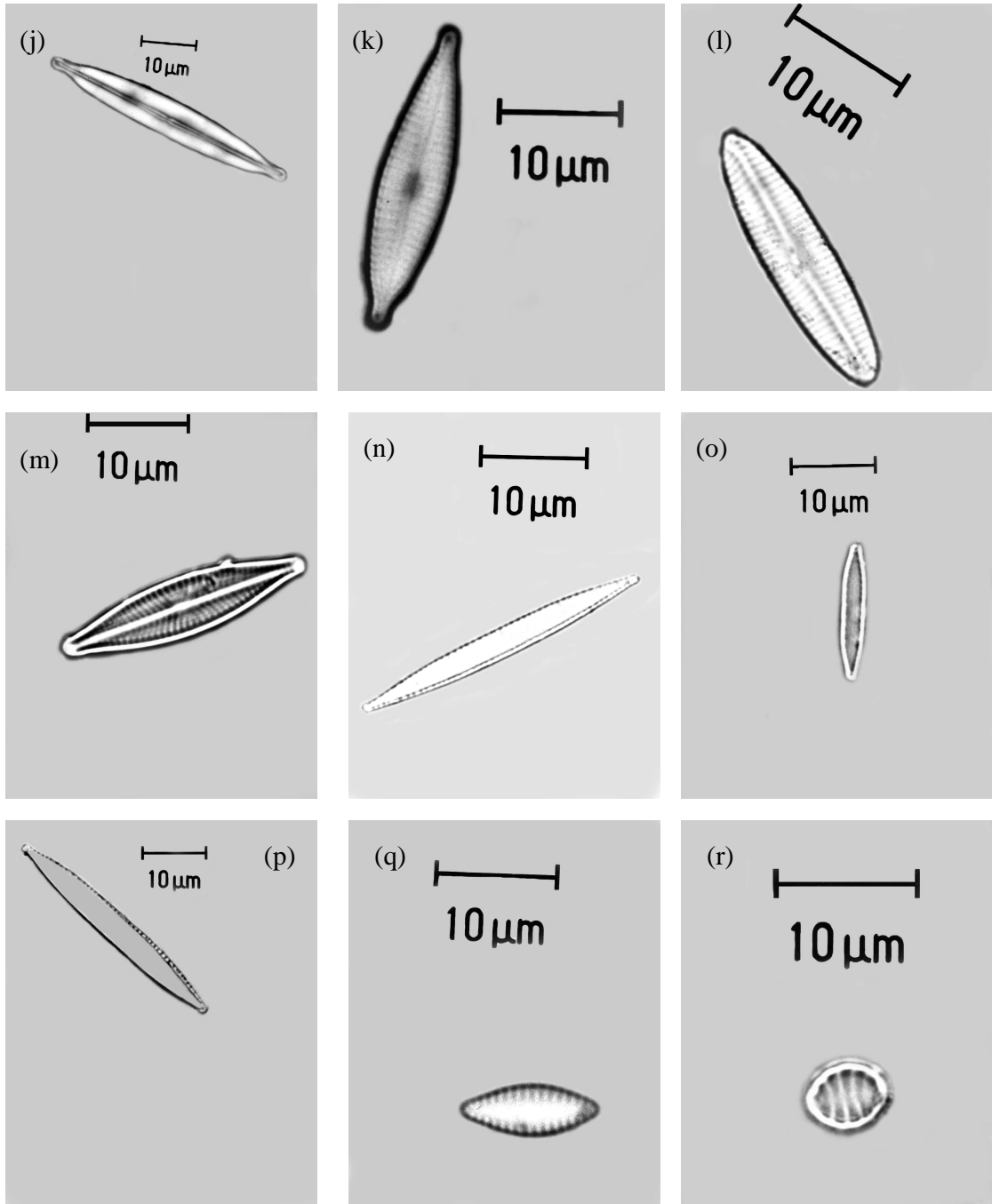
Chapter 6: A comparison of invertebrate and diatom assemblages in wetlands of differing pH in the south-west of Western Australia

6.1 Abstract

The sensitivity of diatom and invertebrate community structure to pH was investigated using data-sets amalgamated from the three seasonal sampling periods. Multi-dimensional scaling ordinations and two way analysis of similarities based on pH groupings of wetlands showed differences in the community composition of diatoms and invertebrates. The largest differences in both the invertebrate and diatom assemblages were detected between the acidic Group 1 wetlands and alkaline Group 3 wetlands. However, the differences displayed by invertebrates were not as strong as those exhibited by diatoms. Differences were also evident between the circumneutral wetlands and the alkaline sites of both biotic groups, with diatoms similarly producing the strongest differences. The community structure of the acidic and circumneutral wetlands did not differ significantly for either biotic group, probably because of the small number of sites and bias towards the lower end of the circumneutral range. There were no significant differences in community composition detected between the seasons for invertebrates or diatoms. The variable of pH produced the optimal correlation for the diatom community patterns and was identified as the single factor most closely related to invertebrate distribution. The strongest correlation for the invertebrate community structure was produced by a two variable combination of pH and temperature. Two-way SIMPER analyses identified a small number of discriminating taxa for both invertebrates and diatoms. However, the invertebrate taxa generally displayed less potential to act as indicators than the diatoms. The results of the study indicate that diatoms are more sensitive to pH than invertebrates and would be the most effective biological monitors of surface water acidification in the south-west of Western Australia. The use of both biotic groups in an integrated monitoring program may be beneficial but this requires further research.

6.2 Introduction

The previous two chapters investigated the influence of pH on the community composition of invertebrates and diatoms in selected wetlands in the south-west of Western Australia respectively. This chapter uses the combined seasonal data-sets of each biotic group to compare the relative sensitivity of the two as biological indicators of pH in the south-west.

The ecology of invertebrates in water bodies of the south-west of Western Australia has been examined in numerous publications (Bunn *et al.* 1986; Davis *et al.* 1991; Balla and Davis 1993; Growns *et al.* 1993; Kay *et al.* 2001). Research into the diatoms inhabiting the region's waters has also been considerable (John 1983; 1993; 1998, 2000; Taukulis and John 2006). However studies investigating both diatoms and invertebrates have been limited, particularly in regard to research on the association between pH and community structure. One of the few studies on Western Australian wetlands incorporating both biotic groups was conducted by Blinn *et al.* (2004) in the Wheatbelt region of the south-west. The study investigated the diatom and microinvertebrate communities of 56 wetlands and while pH was one of the water quality variables measured, the main focus of the research was salinity. Accordingly, the study sites were generally saline and the few displaying low pH values were hypersaline. Thomas and John (2006) investigated the influence of pH on both diatoms and macroinvertebrates but were restricted to five acidic water bodies near Collie. The current study expanded on that research, including the five acidic lakes in a 20 site sampling regime which encompassed a large region of the south-west and included sites over a wide range of pH.

As discussed in previous chapters, there are an increasing number of wetlands in the south-west of Western Australia under threat of acidification (DoE 2005). This has created a need to establish the effectiveness of different organisms as biological indicators of pH. To date, the investigation of biotic communities in wetlands that are currently or potentially at risk of pH decline has generally focused on invertebrates (Clark and Horwitz 2004; McKay and Horwitz 2006). However, the effectiveness of invertebrates as biological indicators of pH as compared with diatoms has not yet been established. The current chapter aimed to address this issue through two main

objectives. Firstly to determine the relative sensitivity of both invertebrates and diatoms to pH and secondly to ascertain which group of organisms would be the most effective biomonitors of pH change in the south-west of Western Australia. A further aim of the study was to identify taxa indicative of different pH levels. The information gained could facilitate the effective monitoring of acidified or threatened systems in Western Australia and may have potential applications in other regions.

6.3 Methods

6.3.1 Field and laboratory procedures

The 20 selected sites (Figure 6.1) were sampled on three occasions. The sampling periods comprised of summer (December – February), winter (June – August) and spring (September – November) of 2001. Environmental variables including pH (pH units), electrical conductivity ($\mu S \ cm^{-1}$), salinity (ppm), temperature (°C), dissolved oxygen (mg L^{-1}) and vegetation score were recorded during each of the three sampling periods. This sampling procedure has been thoroughly described in Chapter 3.

Diatom sampling was conducted during each of the three sampling periods using the artificial substrates, JJ Periphytometers (John 1998). The substrates were recovered after approximately 14 days of immersion, providing sufficient time for the colonization of a climax community (John 1998). The samples retrieved from the periphytometers were prepared following John (1983).

Between 100 -350 frustules were enumerated for each diatom sample and identified using specialized literature (Patrick and Reimer 1966; Foged 1974; Patrick and Reimer 1975; Foged 1978, 1979; John 1983; Hustedt and Jensen 1985; Gasse 1986; Krammer and Lange-Bertalot 1986; Holland and Clarke 1989; Lange-Bertalot and Moser 1994; Ehrlich 1995; John 1998; Snoeijs and Balashova 1998; Camburn and Charles 2000; John 2000; Siver *et al.* 2005). Photomicrographs of the diatoms were taken under oil immersion using a VANOX photomicroscope at 1000x magnification and the permanent slides have been deposited in the International Diatom Herbarium at the Department of Environmental Biology, Curtin University of Technology. A

detailed description of the sampling procedure and laboratory processes was presented in Chapter 5.

Invertebrate sampling was also undertaken at each of the sites during the three sampling periods. A D-framed 250 μm mesh net was swept through the sediment along a 10 m transect, agitating the substrate to dislodge invertebrates. Samples were deposited in labelled containers and preserved using approximately 4% formalin. The collected invertebrate material was sub-sampled in the laboratory using a box sub-sampler based on Marchant (1989). The protocols for invertebrate sorting and enumeration were detailed in Chapter 4.

Identification was carried out to species level where possible using specialised literature (Edward 1964; Watts 1978; Williams 1980; Benzie 1988; Bayly 1992; Lansbury 1995; Shiel 1995; Dean and Suter 1996; Hawking and Smith 1997; Davis and Christidis 1999; Hawking and Theischinger 1999; Suter 1999; St. Clair 2000; Watts 2002; Andersen and Weir 2004; Dean *et al.* 2004). The sub-family Ceratopogoninae was separated into morphotypes and the groups Oligochaeta, Nematoda and Cyclopoida were not identified further. Voucher specimens were placed in a voucher collection located at the Department of Environmental Biology, Curtin University of Technology.
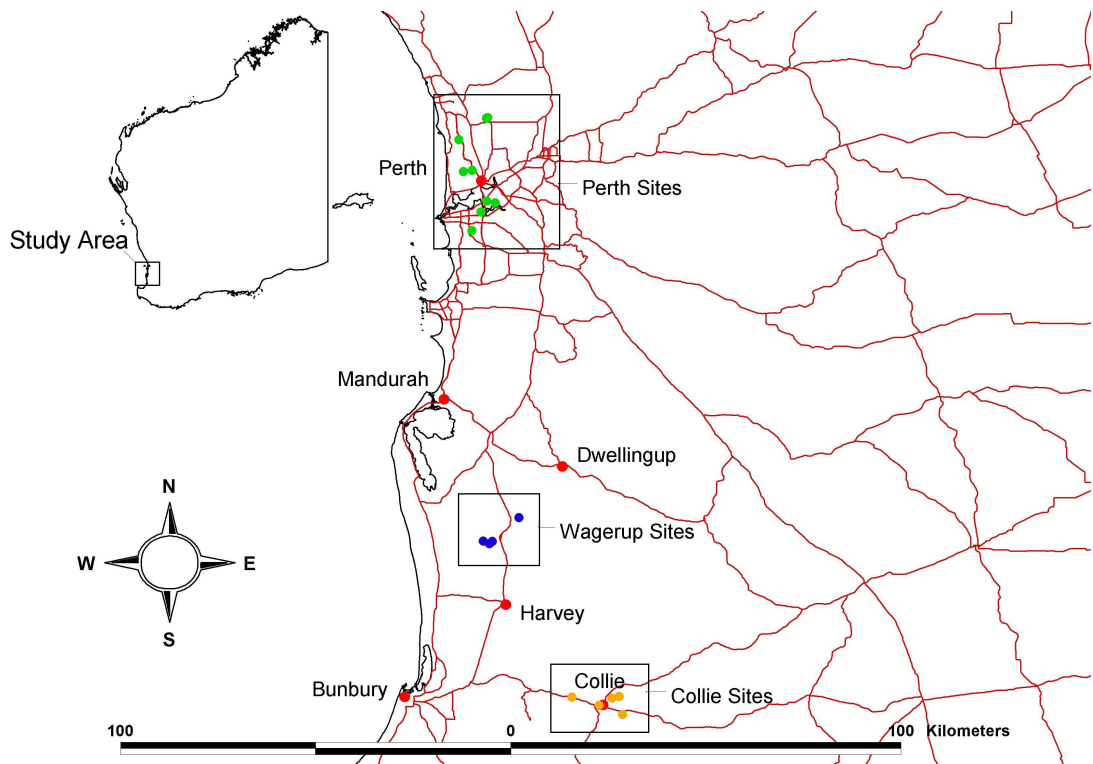
Figure 6.1: Location of the 20 sites in the south-west of Western Australia selected for the seasonal study. Green points indicate the sites located in the Perth Metropolitan Region. Blue points indicate study sites located in the Wagerup area. Yellow points represent sites located in the Collie Basin. Red points represent the nearest neighbouring towns.

6.3.2 Statistical analyses

The community structure of invertebrates and diatoms was investigated using the software package PRIMER 6.0 for Windows Version 6.1.9 (PRIMER-E Ltd 2007). Abundance data from each of the three seasonal samples was amalgamated and both the invertebrate and diatom data-sets were square root transformed. Similarity matrices for the data- sets were constructed using the Bray Curtis similarity measure (Bray and Curtis 1957). Non-metric multi-dimensional scaling (MDS) ordinations (Kruskal and Wish 1978) were employed to identify groups of sites with similar community composition. Differences in community structure among the factors of pH group and season were detected using two-way analyses of similarities (ANOSIM) (Clarke 1993). A maximum of 999 permutations were applied to calculate the probability of the observed values for each analysis. The SIMPER routine (similarity percentages) (Clarke 1993) with a two way crossed layout was employed to identify the taxa which contributed most to the average dissimilarities between the pH groupings across the three seasons. The routine uses the overall percentage contribution of each taxon to the average dissimilarities to determine their importance in discriminating between the two sets of data (Clarke and Gorley 2001). Taxa with relatively high ratios (mean contribution / standard deviation) were identified as consistent contributors to the dissimilarities between the groups and are likely to be useful discriminating taxa (Clarke 1993). The BIO-ENV procedure (Clarke and Ainsworth 1993) used the Spearman rank correlation coefficient (ρ_s) to identify the combinations of environmental variables which produced the matrices most closely correlated with the biotic matrices. Significance was accepted at $p < 0.05$ and $p < 0.01$.

6.4   Results

6.4.1   MDS and ANOSIM

Multi-dimensional scaling (MDS) ordinations based on similarities in invertebrate and diatom community structure respectively displayed some separation but little discrete clustering (Figure 6.2). Differences in biotic patterns were however revealed by overlays of the three pH groupings of wetlands (Figure 6.3). The ordination based on invertebrate data showed a general separation between the acidic Group 1 sites and the alkaline wetlands of Group 3 while the circumneutral Group 2 wetlands occupied an intermediate position (Figure 6.3a). The ordination based on the diatom abundance data displayed a stronger separation between the Group 1 and Group 3 sites. The circumneutral wetlands also formed more defined groupings based on the diatom ordination than those evident in the invertebrate ordination, with some sites located near the acidic Group 1 sites and the remainder clustering near the alkaline Group 3 sites (Figure 6.3b). The circumneutral wetlands such as Kurrajong Village Lake (Site 12) and Tuscan Park (Site 19) that displayed lower pH values (pH < 7) were generally closer to the acidic sites in ordination space. Conversely, circumneutral sites such as Knapping Wetland (Wi11), Lake Moyanup (Wi14) and Exelby Wetland (Wi8) which displayed higher pH values (pH > 7) were more closely associated with alkaline sites. The generally acidic site of Blind Roo A was an exception to the groupings, occurring close to alkaline sites as Blind Roo B.

(a)
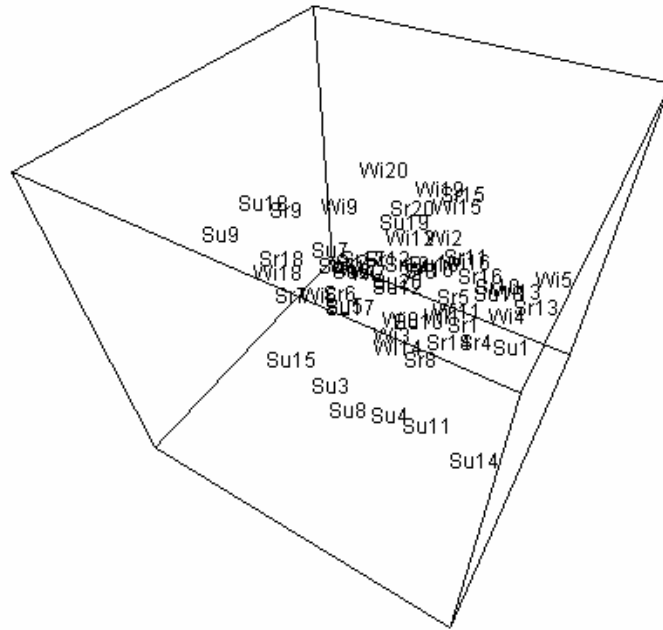
(b)
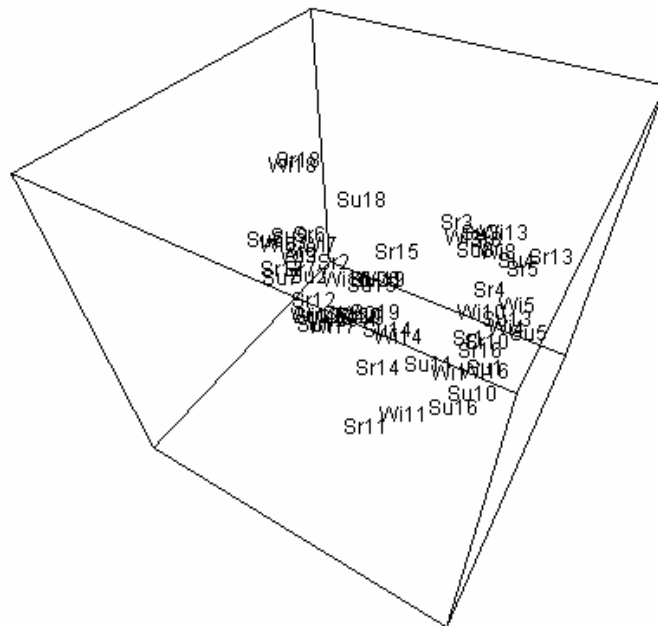
Figure 6.2: Three dimensional multi-dimensional scaling ordination of square root transformed invertebrate and diatom abundance data from all three sampling periods. Site codes are preceded by the seasonal prefix Su to represent the summer sample, Wi to represent the winter sample and Sr to represent the spring sample. (a) Ordination based in invertebrate data-set. Stress = 0.17. (b) Ordination based on diatom data-set. Stress = 0.12.

(a)
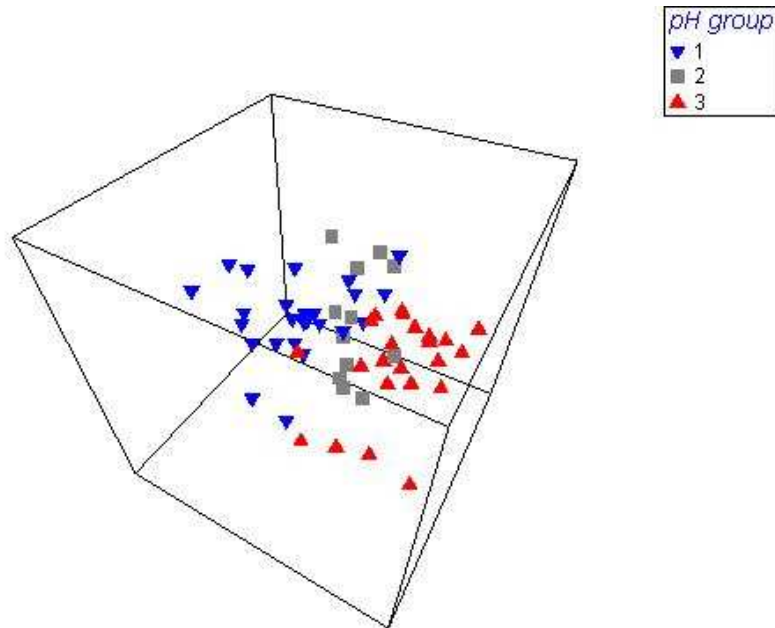
(b)
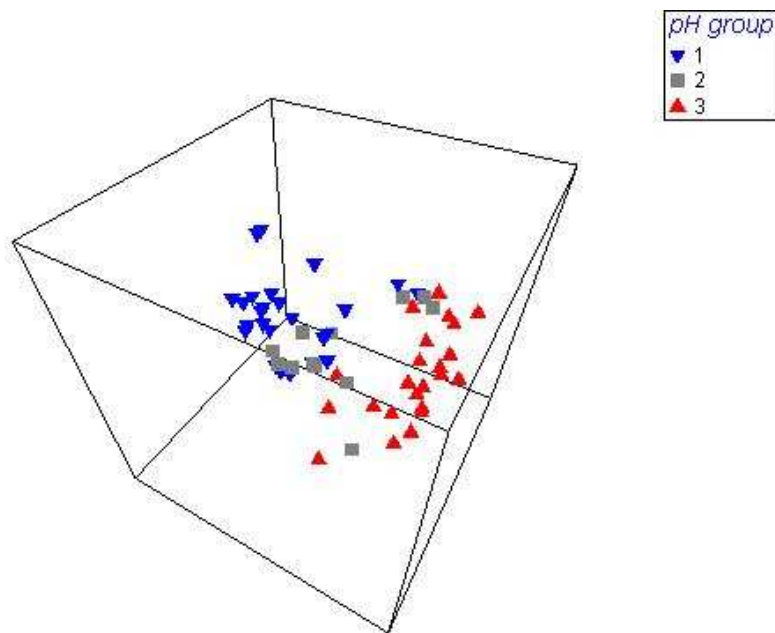
Figure 6.3: Three dimensional multi-dimensional scaling ordination of square root transformed invertebrate and diatom abundance data from all three sampling periods with superimposed symbols representing the three pH groups. (a) Ordination based on invertebrate data. Stress = 0.17. (b) Ordination based on diatom data. Stress = 0.12.

Two way analyses of similarities (ANOSIM) supported the findings of the MDS ordinations, detecting changes in community composition in relation to pH (p < 0.01) for both invertebrates and diatoms (Table 6.1). However the comparatively low global R value for the invertebrate data-set (R = 0.30) indicated that the separation across the pH groups was not substantial. The largest differences in invertebrate community structure were evident between the acidic and alkaline wetlands (p < 0.01) with an R value of 0.44. Differences were also detected between Groups 2 and 3 although to a lesser extent. In contrast, the invertebrate assemblages of the Group 1 and Group 2 wetlands were similar in composition (Table 6.1).

The R values derived from the diatom data were generally higher suggesting that the differences between the groups were more defined than those established from the invertebrate data (Table 6.1). This supports the more distinct clustering evident in the ordination based on diatom data. Similarly to the invertebrate ANOSIM, the acidic and alkaline wetlands displayed the greatest differences in community composition (p < 0.01). However, the R statistic of > 0.70 was relatively high in comparison. The diatom assemblages also varied significantly between the Group 2 and Group 3 wetlands (p < 0.01) but were relatively similar at the Group 1 and Group 2 sites (Table 6.1). The two-way analyses of similarities did not detect any significant seasonal differences in community structure of either invertebrates or diatoms when averaged across each pH group (Table 6.2).

Table 6.1: Results of global and pairwise tests from two-way analyses of similarities (ANOSIM) based on Bray-Curtis similarities in square root transformed invertebrate and diatom abundance data of the three pH groups of wetlands sampled in the south-west of Western Australia across all seasons. Data from all three sampling periods had been included in the analysis. Bold type indicates significant difference ($p < 0.05$).

Biota	pH Groups	R	Probability
Invertebrate	All Groups	0.30	**< 0.01**
	Group 1, Group 2	-0.01	> 0.05
	Group 1, Group 3	0.44	**< 0.01**
	Group 2, Group 3	0.22	**< 0.05**
Diatom	All Groups	0.51	**< 0.01**
	Group 1, Group 2	0.08	> 0.05
	Group 1, Group 3	0.72	**< 0.01**
	Group 2, Group 3	0.38	**< 0.01**

Table 6.2: Results of global and pairwise tests from two-way analyses of similarities (ANOSIM) based on Bray-Curtis similarities in square root transformed invertebrate and diatom abundance data of the three seasons across pH groups of wetlands sampled in the south-west of Western Australia. Data from all three sampling periods had been included in the analysis. Bold type indicates significant difference ($p < 0.05$).

Biota	Season	R	Probability
Invertebrate	All Seasons	0.04	> 0.05
	Summer, Winter	0.04	> 0.05
	Summer, Spring	0.03	> 0.05
	Winter, Spring	0.09	> 0.05
Diatom	All Seasons	-0.07	> 0.05
	Summer, Winter	-0.12	> 0.05
	Summer, Spring	-0.02	> 0.05
	Winter, Spring	-0.09	> 0.05

6.4.2 BIO-ENV analyses

The BIO-ENV analyses determined that the strongest correlation between the matrices for the environmental variables and faunal distribution across all wetlands was produced by the two variable combination of pH and temperature ($\rho_s = 0.53$), followed by the three variable combination of pH, electrical conductivity and temperature ($\rho_s = 0.52$) (Table 6.3). The single variable with the strongest correlation to the invertebrate community structure was pH ($\rho_s = 0.48$). Diatom community composition was most closely correlated to the variable of pH alone ($\rho_s = 0.61$) with the two variable combination of pH and electrical conductivity producing the next highest correlation ($\rho_s = 0.49$) (Table 6.3).

Table 6.3: A summary of BIO-ENV results giving the combinations of environmental variables with the highest rank correlations between the abiotic and square root transformed invertebrate and diatom similarity matrices for all three sampling periods as measured by Spearman rank correlation (ρ_s). A correlation cut-off of $\rho_s < 0.40$ was applied. The optimal correlations are presented in bold type. Veg = vegetation score, EC = electrical conductivity, Temp = temperature, DO = dissolved oxygen.

Biota	n	Variables	ρ_s
Invertebrate	1	pH	0.48
	2	**pH, Temp**	**0.53**
	2	pH, EC	0.44
	3	pH, EC, Temp	0.52
	3	Veg, pH, Temp	0.46
	4	Veg, pH, EC, Temp	0.48
	4	pH, EC, Temp, DO	0.42
	5	Veg, pH, EC, Temp, DO	0.40
Diatom	1	**pH**	**0.61**
	2	pH, EC	0.49
	2	pH, DO	0.48
	2	Veg, pH	0.42
	2	pH, Temp	0.40
	3	pH, EC, DO	0.43

6.4.3   SIMPER analyses

SIMPER analyses were used to identify the invertebrate and diatom taxa which predominantly accounted for differences in the community structure of the pH groups of wetlands (Table 6.4 and 6.5). The largest differences in invertebrate assemblages were recorded between the Group 1 and Group 3 wetlands (mean dissimilarity of 91.09 %). These differences were largely attributable to crustaceans such as the cladoceran *Macrothrix indistincta* which occurred solely in the Group 1 wetlands and the ostracod *Sarscypridopsis aculeata* which was abundant in the Group 3 wetlands (Table 6.4). The other dominant crustaceans Cyclopoida and *Calamoecia tasmanica subattenuata* were both recorded in higher numbers from the Group 1 sites. Nematodes, oligochaetes and the dipteran larvae *Tanytarsus fuscithorax/semibarbitarsus*, Ceratopogoninae 7 and Ceratopogoninae 2 were also more common in the Group 1 wetlands. Other dominant taxa included *Tasmanocoenis tillyardi,* juvenile Corixidae, *Polypedilum nubifer* and *Micronecta robusta,* all of which occurred more abundantly in the Group 3 sites. *Sarscypridopsis aculeata* contributed relatively consistently to the differences in the invertebrate assemblages of Groups 1 and 3 (consistency ratio ≥ 1) and could potentially be a useful discriminating species (Table 6.4).

The Group 2 and Group 3 wetlands displayed a mean dissimilarity of 81.58%. The taxa contributing most to differences in invertebrate community structure were largely attributable to *Sarscypridopsis aculeata*, followed by oligochaetes, nematodes and *Daphnia carinata* (Table 6.4). The ostracod was most commonly recorded from the Group 3 wetlands while oligochaetes, *Daphnia carinata* and nematodes generally inhabited the circumneutral waters of Group 2 wetlands. The crustaceans *Calamoecia tasmanica subattenuata*, *Calamoecia attenuata*, cyclopoid copepods, *Chydorus* sp., *Alona quadrangularis* and *Boeckella triarticulata* also occurred most frequently in Group 2 wetlands. *Tanytarsus fuscithorax/semibarbitarsus* was also more common in the circumneutral sites. *Micronecta robusta*, juvenile corixids and *Candonocypris novaezelandiae* were among the taxa more commonly recorded from the Group 3 wetlands. Taxa that occurred consistently and may be useful discriminators included *Sarscypridopsis aculeata*, oligochaetes, nematodes and cyclopoid copepods (ratio ≥ 1) (Table 6.4).

According to ANOSIM, the invertebrate assemblages of the Group 1 and Group 2 wetlands were not significantly different and therefore SIMPER results for these groups have been omitted.

Table 6.4: Taxa contributing to differences between pH groups based on two way similarity percentage (SIMPER) analysis of square root transformed invertebrate data with pH group and season as factors. Taxa are listed in decreasing order of their contribution to the average dissimilarity between the groups. A taxa cut-off was applied at a cumulative percent dissimilarity of 50%. Consistency ratios indicate the usefulness of a taxon in discriminating between groups - larger values suggest a higher level of discrimination. The percent and cumulative percent contribution to variation between the groups are also presented.

Taxa	Average Abundance		Consistency Ratio	Percent	Cumulative Percent
	Group 1	Group 3	Mean Dissimilarity = 91.09 %		
Sarscypridopsis aculeata	0.34	3.56	1.05	7.40	7.40
Macrothrix indistincta	2.32	0.00	0.58	5.23	12.63
Oligochaeta	1.63	1.58	0.90	4.88	17.51
Nematoda	1.80	1.13	0.80	4.82	22.34
Tanytarsus fuscithorax /semibarbitarsus	1.93	0.10	0.96	4.05	26.38
Ceratopogoninae 7	1.41	0.00	0.45	3.71	30.09
Tasmanocoenis tillyardi	0.16	1.41	0.73	3.12	33.22
Cyclopoida	1.26	1.03	0.98	3.08	36.30
Corixidae (juvenile)	0.15	1.40	0.67	2.79	39.09
Calamoecia tasmanica subattenuata	1.33	0.04	0.60	2.66	41.76
Polypedilum nubifer	0.08	1.14	0.57	2.64	44.39
Ceratopogoninae 2	1.12	0.00	0.58	2.39	46.79
Micronecta robusta	0.00	1.13	0.69	2.34	49.13
	Group 2	Group 3	Mean Dissimilarity = 81.58 %		
Sarscypridopsis aculeata	1.13	3.56	1.40	8.48	8.48
Oligochaeta	2.22	1.58	1.04	4.90	13.37
Nematoda	2.05	1.13	1.41	4.74	18.11
Daphnia carinata	1.52	1.10	0.67	4.26	22.37
Cyclopoida	1.73	1.03	1.35	3.88	26.25
Calamoecia tasmanica subattenuata	1.56	0.04	0.54	3.14	29.39
Micronecta robusta	0.33	1.13	0.94	3.12	32.51
Corixidae (juvenile)	0.64	1.40	0.78	2.88	35.39
Calamoecia attenuata	1.18	0.09	0.71	2.72	38.11
Chydorus sp.	0.99	0.14	0.36	2.57	40.68
Candonocypris novaezelandiae	0.10	0.97	0.71	2.44	43.12
Alona quadrangularis	1.05	0.00	0.53	2.19	45.31
Tanytarsus fuscithorax/semibarbitarsus	0.87	0.10	0.81	2.19	47.50
Boeckella triarticulata	0.78	0.28	0.49	2.17	49.67

Analyses based on diatom abundance data identified the highest mean dissimilarity between Group 1 and Group 3 wetlands (average dissimilarity = 95.07 %). Species contributing most to differences included *Brachysira brebissonii*, *Nitzschia paleaeformis* and *Frustulia magaliesmontana* which were abundant in the acidic sites and *Gomphonema parvulum* and *Staurosira construens* var. *venter* which generally occurred in the alkaline sites. *Achnanthidium minutissimum*, *Pseudostaurosira brevistriata*, *Navicula cryptocephala* and *Nitzschia palea* were also among the taxa that commonly inhabited the alkaline Group 3 wetlands. In contrast the species *Navicula* aff. *cari* and *Eunotia pectinalis* var. *minor* occurred more frequently in acidic waters. *Brachysira brebissonii, Gomphonema parvulum, Frustulia magaliesmontana* and *Eunotia pectinalis* var. *minor* were the taxa which contributed most consistently to differences between the two wetland pH groups (consistency ratio ≥ 1) (Table 6.5).

Taxa contributing most to the dissimilarity in the diatom assemblages of the Group 2 and Group 3 wetlands included *Achnanthidium minutissimum, Gomphonema parvulum, Frustulia magaliesmontana*, *Staurosira construens* var. *venter* and *Navicula cryptocephala*. The species *Achnanthidium minutissimum* and *Frustulia magaliesmontana* were both relatively abundant in the Group 2 wetlands and occurred less frequently in the Group 3 wetlands. *Staurosira construens* var. *venter, Gomphonema parvulum* and *Navicula cryptocephala* were more abundant in alkaline waters. *Cocconeis placentula* and *Amphora ovalis var. affinis* were restricted to the Group 3 wetlands while *Eunotia pectinalis* var. *minor*, *Brachysira brebissonii* and *Brachysira styriaca* occurred at Group 2 sites but not in Group 3 wetlands. Species identified as possible discriminators (consistency ratio ≥ 1) included *Gomphonema parvulum, Navicula cryptocephala* and *Nitzschia palea* and *Eunotia pectinalis* var. *minor. Eunotia pectinalis*, a species recorded frequently in the circumneutral wetlands and rarely in the alkaline wetlands, were also shown to have consistently contributed to dissimilarities between the wetland groups (Table 6.5).

Table 6.5: Taxa contributing to differences between pH groups based on two way similarity percentage (SIMPER) analysis of square root transformed diatom data with pH group and season as factors. Taxa are listed in decreasing order of their contribution to the average dissimilarity between the groups. A taxa cut-off was applied at a cumulative percent dissimilarity of 50%. Consistency ratios indicate the usefulness of a taxon in discriminating between groups - larger values suggest a higher level of discrimination. The percent and cumulative percent contribution to variation between the groups are also presented.

Taxa	Average Abundance		Consistency Ratio	Percent	Cumulative Percent
	Group 1	Group 3	Mean Dissimilarity = 95.07 %		
Brachysira brebissonii	3.00	0.00	1.09	5.93	5.93
Nitzschia paleaeformis	2.81	0.00	0.86	5.63	11.55
Gomphonema parvulum	0.53	2.40	1.18	4.40	15.95
Staurosira construens var. *venter*	0.14	2.35	0.84	4.40	20.35
Frustulia magaliesmontana	2.36	0.08	1.10	4.23	24.58
Achnanthidium minutissimum	0.48	1.93	0.83	3.77	28.35
Pseudostaurosira brevistriata	0.43	1.65	0.62	3.68	32.04
Navicula cryptocephala	0.26	1.67	0.93	3.03	35.06
Navicula aff. *cari*	1.43	0.00	0.57	3.03	38.09
Nitzschia palea	0.38	1.33	0.96	2.62	40.71
Cocconeis placentula	0.11	1.26	0.58	2.55	43.26
Eunotia pectinalis var. *minor*	1.31	0.00	1.00	2.32	45.58
Encyonopsis microcephala	0.12	0.83	0.42	1.77	47.35
Surirella tenera	0.84	0.00	0.60	1.56	48.92
	Group 2	Group 3	Mean Dissimilarity = 88.91%		
Achnanthidium minutissimum	2.67	1.93	0.92	5.56	5.56
Gomphonema parvulum	1.32	2.40	1.33	4.60	10.16
Frustulia magaliesmontana	2.04	0.08	0.78	4.31	14.47
Staurosira construens var. *venter*	0.23	2.35	0.87	4.24	18.72
Navicula cryptocephala	0.63	1.67	1.19	3.44	22.15
Eunotia pectinalis var. *minor*	1.79	0.00	1.13	3.39	25.55
Pseudostaurosira brevistriata	0.34	1.65	0.65	2.93	28.48
Nitzschia palea	0.96	1.33	1.03	2.77	31.24
Eunotia pectinalis	1.45	0.08	1.23	2.72	33.96
Cocconeis placentula	0.00	1.26	0.56	2.61	36.57
Brachysira brebissonii	1.24	0.00	0.95	2.55	39.12
Amphora ovalis var. *affinis*	0.00	0.47	0.31	2.08	41.20
Nitzschia palea var. 1	0.00	0.83	0.78	2.04	43.24
Amphora veneta	0.30	0.62	0.73	1.98	45.22
Encyonopsis microcephala	0.50	0.83	0.48	1.90	47.12
Brachysira styriaca	1.03	0.00	0.51	1.90	49.02

6.5   Discussion

6.5.1   Distribution patterns of invertebrates and diatoms

Multivariate analyses demonstrated that both invertebrate and diatom community structure was influenced by pH. The largest variations in the biota were detected between the acidic Group 1 and alkaline Group 3 wetlands, suggesting that at least some of the taxa within the two groups displayed a narrow pH tolerance. Differences were also detected between Group 3 and the circumneutral Group 2 wetlands, although to a lesser extent. The less defined differences between these groups were probably related to the comparatively small pH range. This is likely to have resulted in a greater overlap of taxa than was evident between the acidic wetlands of Group 1 and the alkaline Group 3 sites.

In contrast to the other comparisons, the biotic patterns of the Group 1 and Group 2 wetlands did not significantly differ. The similarities in the community structure were probably attributable to a number of factors. Firstly, as previously noted, smaller pH ranges increase the number of taxa able to exist across the pH groups. Additionally, the relatively small number of circumneutral sites included in the study and the tendency of the circumneutral sites to be at the lower end of the circumneutral pH range may both have compounded the similarities. The seasonal fluctuation of some sites between the acidic and circumneutral groupings is also likely to have contributed to the compositional affinities between the two groups.

The influence of pH on biotic patterns has been documented in studies on both invertebrates (Kimmel *et al.* 1985; Feldman and Connor 1992) and diatoms (Charles 1985; Battarbee *et al.* 1997; Ledger and Hildrew 2001; Siver *et al.* 2004). The current study similarly identified pH as an important factor in the distribution of the respective biotic groups. However, while both invertebrates and diatoms displayed significantly different assemblages for the two of the three wetland pH group comparisons, the results indicated that differences between the invertebrate communities were not substantial. Conversely, analyses of the diatom abundance data showed strong differences between the Group 1 and Group 3 wetland communities and higher values than the invertebrates for the remaining comparisons.

These findings suggest that while both groups were influenced by pH, diatoms displayed a greater sensitivity to the variable.

Results of the BIO-ENV tests reiterated the findings of the other multivariate analyses, revealing that pH was the single factor most closely correlated to invertebrate and diatom community composition ($\rho = 0.48$ and $\rho = 0.61$ respectively). The highest correlation between diatom community structure and environmental patterns was achieved through the single factor of pH, while the two factor combination of pH and temperature produced the strongest correlation for invertebrates. Additionally, the correlation between diatom distribution and pH was higher than that of the invertebrate data-set, further emphasizing the greater sensitivity of diatoms to pH. These findings support earlier research on a sub-set of the current sites which similarly suggested that diatoms were more sensitive to pH than invertebrates (Thomas and John 2006).

However, as evidenced by both MDS and BIO-ENV results, the influence of variables other than pH on the two biotic groups cannot be discounted. For example, the MDS on diatom abundance data positioned the generally acidic site of Blind Roo A (Site 3) close to the highly alkaline site of Blind Roo B (Site 4). Both wetlands were created through clay extraction and it is likely that unmeasured water chemistry variables related to substrate type may have been the over-riding factors in the diatom community structure. BIO-ENV results similarly highlighted the effect of variables other than pH, displaying the influence of factors such as temperature on invertebrate community composition. Additionally, when considering the influence of variables on community structure it is important to note that the relationship between biotic patterns and the measured environmental variables is not a definitive causal association. The variables included in the study may instead have been highly correlated with other potential causal parameters that were not measured (Clarke and Warwick 2001).

Factors which were not measured during the current study and are known to influence both invertebrate and diatom community structure include metal concentrations (Hirst *et al.* 2002), water colour (Growns *et al.* 1993; Wunsam *et al.* 2002) and nutrient enrichment (Cheal *et al.* 1993; Kelly 1998). Diatom assemblages

may also be impacted by factors such as grazing (Burton *et al.* 1994) while competition (Nielson *et al.* 1999) and predation can alter invertebrate community structure (Crowder and Cooper 1982; Bendell and McNicol 1987; Knapp *et al.* 2001).

6.5.2 Invertebrate and diatom taxa

Two way SIMPER results supported the findings of the MDS and ANOSIM analyses, determining that the largest differences in both invertebrate and diatom community structure were evident between the acidic Group 1 and alkaline Group 3 sites. One of the major contributors to dissimilarities in the invertebrate assemblages of two groups was the cladoceran *Macrothrix indistincta*. The species occurred exclusively in acidic waters during this study and has previously been collected from two gnammas in the state, one of which was acidic (Bayly 1997). The dipteran larvae *Tanytarsus fuscithorax/semibarbitarsus* and Ceratopogoninae 2 and 7 were also relatively abundant in the Group 1 wetlands. Sommer and Horwitz (2001) recorded an increased abundance of Ceratopogonidae larvae in an acidifying lake on the Swan Coastal Plain and *Tanytarsus fuscithorax/semibarbitarsus* has also been associated with low pH waters (Randall and Cox 2003). However, while these taxa appear to favour low pH, they did not consistently contribute to the dissimilarities between the pH groups and therefore may not be useful discriminating taxa. Nonetheless, their presence in low pH conditions suggests that further research may be warranted.

In contrast, the ostracod *Sarscypridopsis aculeata* was common in the alkaline wetlands and was the only species identified as having consistently contributed to differences between the groups (consistency ratio $\geq$ 1). However, the reasonably broad pH tolerance of the taxon noted during other studies (Davis and Rolls 1987; Growns *et al.* 1993) suggests that pH is not the sole influence on distribution. For example, the species is known to be tolerant of eutrophic conditions (Gifre *et al.* 2002) and may be partly influenced by variables such as nutrient concentration.

The mayfly *Tasmanocoenis tillyardi* and juvenile corixids also contributed to differences between the groups, with higher numbers in the alkaline wetlands. Mayflies are generally sensitive to low pH (Harvey 1989) and records of this species

suggest that it prefers waters exceeding pH 6 (Storey *et al.* 1993). Conversely, juvenile corixids have been identified from waters over a range of pH (Growns *et al.* 1993) and are probably unsuitable indicators of alkaline conditions during these early life stages. The other common Group 3 taxa *Polypedilum nubifer* and *Micronecta robusta* have both been previously recorded in high numbers from alkaline sites such as Lake Monger (Edward 1964; Davis and Rolls 1987) but are not restricted to high pH waters. For example *Micronecta robusta* was collected from five sites with pH < 6.50 during Bayly's (1997) study of gnammas on granite outcrops.

Nematodes, oligochaetes and cyclopoid copepods occurred in both pH groups but were slightly more abundant in the acidic sites. The greater presence of these taxa in the acidic wetlands requires further investigation. It is possible that increased taxonomic resolution may reveal species within these groups with narrow pH tolerances and potential to act as indicators of pH change.

Sarscypridopsis aculeata was among the species differentiating between the Group 2 and Group 3 wetlands but as previously discussed, factors other than pH may have been partly responsible for the distribution of this species. Oligochaetes, nematodes and cyclopoid copepods were more common in the Group 2 wetlands than Group 3. Their relatively high consistency ratios suggest that they are likely to be useful discriminators for the circumneutral Group 2 wetlands. *Daphnia carinata* was also more common in the Group 2 sites but having been previously recorded from both circumneutral and alkaline waters (Mitchell and Williams 1982; Lund and Davis 2000), it is probable that the higher abundance may be related to factors other than pH.

In contrast, the lower pH of the Group 2 wetlands was likely to have been a primary factor in the higher abundance of *Tanytarsus fuscithorax/semibarbitarsus, Calamoecia tasmanica subattenuata* and *Calamoecia attenuata.* The calanoid copepod *Calamoecia attenuata*, which is endemic to Western Australia (Maly and Bayly 1991) has been recorded over a range of pH (Growns *et al.* 1993) but appears to favour circumneutral wetlands. For example, the species has previously been recorded from circumneutral wetlands (pH 6.68 - 7.27) in the south of the state (Storey *et al.* 1993) and was most commonly recorded from waters of intermediate

pH during the current study. Similarly, *Calamoecia tasmanica subattenuata* has been recorded from wetlands over a range of pH (Growns *et al.* 1993) but is reported to prefer humic waters (Bayly 1992). Other species contributing to dissimilarities between the circumneutral and alkaline sites included *Alona quadrangularis*, a cladoceran that generally appears to favour circumneutral conditions (Rundle and Ramsay 1997) and the calanoid copepod *Boeckella triarticulata.*

Juvenile corixids, *Micronecta robusta* and the ostracod *Candonocypris novaezelandiae* were also involved in distinguishing the between the two groups, with higher abundances in the Group 3 sites. However, the environmental preferences of the juvenile corixids remain difficult to discern and *Micronecta robusta* and *Candonocypris novaezelandiae* are likely to be at least partly influenced by factors other than pH.

The SIMPER analysis of diatom abundance data displayed comparatively higher mean dissimilarities than those generated from the invertebrate data set. Species contributing most to differences between the Group 1 and Group 3 sites included *Nitzschia paleaeformis* and *Brachysira brebissonii*, both of which are known to inhabit low pH waters (Stokes and Yung 1986; Denys and van Straaten 1992; Kapfer 1998; Gaiser and Johansen 2000). Other abundant Group 1 taxa included *Frustulia magaliesmontana* and *Navicula* aff. *cari,* which have previously been identified from acidic mine-lakes in south-western Australia (John 1993) Additionally, *Brachysira brebissonii, Frustulia magaliesmontana* and the acidophilous *Eunotia pectinalis* var. *minor* (Foged 1978) were among the species that contributed consistently to dissimilarities between the Group 1 and Group 3 sites (consistency ratio $\geq$ 1) and were thereby identified as good discriminators.

Species such as *Nitzschia paleaeformis* that were restricted to acidic sites but did not consistently contribute to the dissimilarities between groups provide valuable information about the wetlands they inhabit and would be worth investigating further. However, taxa such as *Brachysira brebissonii* and *Frustulia magaliesmontana* are more likely to be present in a greater number of acidic sites and as such are potentially more useful for biomonitoring pH over wide areas.

The SIMPER analysis identified *Gomphonema parvulum* as a potentially useful discriminator for the Group 3 sites. As evidenced by the current study, the species is able to inhabit waters over a broad range of pH but tends to favour neutral and alkaline conditions (Schoeman 1973). *Gomphonema parvulum* has also been associated with nutrient enriched waters (Butcher 1947; Patrick and Reimer 1975) and this may have been a factor in its distribution.

Staurosira construens var. *venter* was also abundant in the Group 3 wetlands. Similarly to *Gomphonema parvulum*, this taxon has been recorded over a range of pH but tends to prefer moderate conditions (Gasse 1986). *Pseudostaurosira brevistriata* (formerly *Fragilaria brevistriata*), a species previously recorded from both circumneutral and alkaline waters (Gasse 1986; Siver *et al.* 2005) also contributed to differences between the alkaline wetlands and the other pH groups. The higher abundance of these species in alkaline waters compared to acidic sites is probably attributable to their pH preferences. However, it is likely that unmeasured factors such as nutrient concentration may have influenced the distribution of these species among the circumneutral and alkaline sites. Subsequently, the inclusion of nutrient concentration as an environmental variable would be a useful addition to future research.

Navicula cryptocephala also contributed to dissimilarities between Group 3 wetlands and the remaining pH groups. Additionally, the species was identified as a potentially useful discriminator between the circumneutral and alkaline sites (consistency ratio $\geq$ 1.0). This supports previous research which suggests that the species is widely distributed in lakes and rivers (Patrick and Reimer 1966) and has a pH optimum of around 8 (Cholnoky 1968). *Achnanthidium minutissimum* was more common in the Group 3 wetlands than in the acidic sites but concomitantly was more abundant in the Group 2 sites than in Group 3. This is in agreement with Foged (1979) who mostly recorded the species in waters with a pH of 6.5-7.5 and Gasse (1986) who identified the species from weakly acidic to weakly alkaline waters.

The acidophilous species of *Brachysira brebissonii*, *Frustulia magaliesmontana*, *Eunotia pectinalis* and *Eunotia pectinalis* var. *minor* (Foged 1979) were also among

the species that separated the circumneutral sites from alkaline waters. Additionally, the *Eunotia* spp. consistently contributed to dissimilarities between the groups and were identified as potentially useful discriminators for Group 2. Conversely, *Cocconeis placentula* and *Nitzschia palea* were more common in the Group 3 wetlands. This is supported by Cholnoky (1968) which described the taxa as having pH optima of around 8.00 and 8.40 respectively. Furthermore *Nitzschia palea* was one of the species that consistently contributed to dissimilarities between the two groups.

6.5.3 Comparative usefulness of the discriminating taxa

According to the SIMPER results, four invertebrate taxa consistently contributed to the dissimilarity between pH groups (consistency ratio > 1) and could be considered good discriminators. Three of the four taxa, cyclopoid copepods, nematodes and oligochaetes occurred across all pH groups but were more abundant in the Group 1 and Group 2 wetlands than in the alkaline Group 3 sites. The final taxon identified as a good discriminator was *Sarscypridopsis aculeata.* This ostracod also occurred across all pH groups but displayed the highest abundance in the Group 3 wetlands. However, while the species is often collected from alkaline sites (Gifre *et al.* 2002), studies have also noted its frequent presence in eutrophic waters (Cheal *et al.* 1993; Gifre *et al.* 2002). Subsequently, further research may be required to determine the over-riding factor influencing the distribution of *Sarscypridopsis aculeata.*

In contrast to the invertebrates, seven diatom taxa consistently contributed to differences in the diatom assemblages of the pH groups. Moreover, at least four of the species display potential to act as indicator species for pH. *Brachysira brebissonii* and *Frustulia magaliesmontana* both of which are known to be acidophilous species (Foged 1979) were identified as good discriminators of the acidic sites in relation to alkaline Group 3 wetlands. The taxa were also collected from Group 2 sites although with lower average abundances than recorded for the Group 1 wetlands. This pattern of occurrence suggests that *Brachysira brebissonii* and *Frustulia magaliesmontana* could potentially be used as indicator species in one of two ways. Firstly, their presence could be used to identify pH decline in alkaline waters and secondly shifts in their abundance may be a useful indicator of pH changes in circumneutral waters.

The species *Eunotia pectinalis* and *Eunotia pectinalis* var. *minor* were identified as good discriminators between Group 2 and the remaining wetland pH groups. Once again, the species were not restricted to a single pH group, instead occurring at both acidic Group 1 and circumneutral Group 2 sites. Similarly to *Brachysira brebissonii* and *Frustulia magaliesmontana,* they either did not occur at Group 3 sites or were present in negligible numbers. Subsequently both *Eunotia pectinalis* and *Eunotia pectinalis* var. *minor* are likely to be valuable indicator species for pH decline in programs monitoring currently neutral to alkaline wetlands.

The species *Gomphonema parvulum*, *Navicula cryptocephala* and *Nitzschia palea* were identified as good discriminators for the alkaline Group 3 wetlands. However, their usefulness as indicators of alkaline conditions may require further investigation. For example while *Gomphonema parvulum* is mostly common in circumneutral and alkaline waters (Foged 1974) it is also capable of tolerating large pH fluctuations (Schoeman 1973). Additionally, the species has been associated with nutrient enriched waters by authors including Schoeman (1973) and Patrick and Reimer (1975) and may be responding to the nutrient concentrations rather than pH. The presence of large numbers of *Nitzschia palea* is also considered to be an indicator of eutrophic conditions, while *Navicula cryptocephala* is also likely to be tolerant of nutrient enrichment (Schoeman 1973).

These findings suggest that the diatoms are probably more useful for discriminating between wetland pH groups. However, it is also important to take into consideration the level of taxonomic resolution used. Invertebrates are a diverse group, comprising many species from several phyla (Abel 1989) whereas diatoms belong to a single division (Bacillariophyceae) (John 2007). Consequently some of invertebrate taxa including Nematoda and Oligochaeta were only identified to coarse taxonomic levels, while the diatoms were identified to genus or species. Organisms may have variable environmental requirements which cannot be discerned through coarse level identification. In contrast, lower level identification is likely to provide more specific information on ecological preferences and increase the probability of identifying effective indicator taxa. Therefore, while SIMPER identified invertebrates such as Oligochaeta and Cyclopoida as discriminating taxa, their usefulness is probably limited at the current level of identification.

6.5.4 Comparability of the biotic groups

It is possible that the aforementioned taxonomic disparities have resulted in an unbalanced comparison between the two biotic groups, creating a bias towards diatoms. The extent to which differing levels of identification influence the comparative sensitivity of the invertebrates and diatoms to water quality variables such as pH is an issue worthy of examination in future research.

Despite this, comparison of the two groups using the current levels of identification is relatively justifiable when considering the objectives of the present study. The research aimed to facilitate the use of effective biological monitoring tools for pH change, through the identification of the most sensitive biotic group. Therefore, diatoms and invertebrates were compared using levels of taxonomic resolution considered feasible for routine monitoring programs in the south-west of Western Australia. Previous studies in the south-west using similar levels of identification include Growns *et al*. (1993) which identified nematodes and oligochaetes to phylum and class respectively and most other groups to genus or species and John (1998) who identified diatom taxa to species level during research on rivers in the south-west. Moreover, studies from outside of Western Australia which have compared both invertebrates and diatoms have similarly left some invertebrate groups at coarse taxonomic levels while identifying diatoms to species (Hirst *et al.* 2002; Newall *et al.* 2006).

6.5.5 The application of invertebrates and diatoms as biological monitors

Based on the results of the current study, diatoms were more sensitive to pH than invertebrates in the selected wetlands of the south-west of Western Australia. However, although diatoms are likely to be the most effective indicators in relation to water quality changes such as pH, the usefulness of invertebrates as indicators should not be discounted. For example, a study using diatoms and macroinvertebrates to classify sites in a Victorian river system suggested that while diatoms were more indicative of water quality changes, macroinvertebrates were also responsive to habitat (Newall *et al.* 2006). Research on Finnish rivers similarly noted

that diatom community structure was closely related to chemical variables while invertebrates were more regulated by physical factors such as channel width (Soininen and Könönen 2004). Following concurrent studies on the effects of urbanization on Victorian streams (Sonneman *et al.* 2001; Walsh *et al.* 2001), it was suggested that diatoms were potentially more useful as indicators of nutrient enrichment, whereas macroinvertebrates may have applications as indicators of catchment disturbance (Sonneman *et al.* 2001). Chessman *et al.* (1999) also noted that diatoms were probably more responsive to water quality factors such as pH and salinity, whereas invertebrates were possibly influenced by variables such as altitude and catchment area.

Physical variables were not extensively explored during the current study and the sensitivity of invertebrates to factors other than water quality at the selected study sites cannot be assessed. The inclusion of such factors would be a valuable addition to future research on the usefulness of invertebrates and diatoms as biological monitoring tools for Western Australian wetlands. Nonetheless, it seems probable that using both groups in an integrated biological monitoring program would provide a more comprehensive assessment of the integrity of sampling sites. The potential for using both groups as biomonitors has also been noted in reports released as part of the National River Health Program (Metzeling 2001; Townsend 2001). The reports, from Victoria and the Northern Territory respectively, suggested that the inclusion of diatoms as biological monitors might complement the AusRivAs macroinvertebrate assessments already being used.

6.5.6   Seasonal variation of invertebrates and diatoms

Based on the results of the two-way ANOSIM with season and pH group as factors, neither invertebrates nor diatoms exhibited significant seasonal differences in community structure. Linke *et al.* (1999) noted that many studies currently deal with the issue of potential seasonal variation by restricting sampling to a particular season. This technique can be problematic though, because it may obscure important variation in community structure (Linke *et al.* 1999). Nonetheless, where seasonal differences are not found to be substantial, as was evident with the current study, restricting sampling to a specific season is probably justifiable, albeit dependent on

the objectives of the study. If early detection of the impacts of pH decline is the major objective of a monitoring program, it remains advisable to sample as frequently as budgetary and staffing constraints allow.

6.6 Conclusion

The results of the multivariate analyses suggested that the community structure of both invertebrates and diatoms was influenced by pH, with the largest differences in the two biotic groups evident between the acidic and alkaline sites. Diatoms displayed a stronger relationship with pH than invertebrates in the selected wetlands and were accordingly classified as the group most sensitive to pH.

Based on these findings, diatoms may be recommended for use in biological monitoring programs for surface water acidification, a serious problem affecting many wetlands in the south-west region due to a decline in rainfall. However, while the current study primarily advocates the use of diatoms for the biological monitoring of pH decline, it seems likely that the inclusion of both invertebrates and diatoms may provide additional information on the overall integrity of threatened and acidified systems. The benefits gained from using both groups in biological monitoring programs for acidification is an aspect that requires further research.

Many invertebrate and diatom taxa contributed to differences between the community structure of the three pH groups but only those which consistently contributing to dissimilarities were identified as good discriminators and hence potential indicator taxa. The invertebrates identified as good discriminators included cyclopoid copepods, nematodes and oligochaetes, all of which were more common in acidic and circumneutral wetlands than in alkaline sites. However, the occurrence of these taxa across all pH groups and their respectively coarse levels of identification decrease their ability to act as effective indicators. The ostracod *Sarscypridopsis aculeata* was the only other invertebrate identified as a potential indicator species but once again lacked potency because of a broad pH tolerance and a possible affinity with nutrient enriched waters. In contrast, several of the diatoms including *Brachysira brebissonii, Frustulia magaliesmontana* and *Eunotia pectinalis* var. *minor* have the potential to be used as effective indicators of pH decline.

The current study has established that diatoms are likely to be more effective biological monitors of pH decline than invertebrates in wetlands in the south-west of Western Australia. It is hoped that the findings of the study facilitate the monitoring of threatened water bodies in the region by encouraging the inclusion of diatoms in future biological assessments.

6.7 References

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Chapter 7: A diatom-based pH inference model for wetlands in the south-west of Western Australia

7.1 Abstract

Multivariate analyses were used to explore the relationship between diatoms and environmental variables in 40 wetlands in the south-west of Western Australia. A total of 144 diatom species were recorded from the study, with 113 taxa displaying a maximum abundance of $\geq$ 1%. Canonical correspondence analysis based on the 113 species data-set demonstrated that diatom distribution was most closely correlated with the variables of pH and electrical conductivity. Variance partitioning indicated that pH explained the greatest amount of variation in the diatom data and was suitable for the development of diatom-based transfer functions. The diatom-based pH models were produced using weighted averaging (WA) and tolerance-downweighted weighted averaging (Tol-WA). WA with classical deshrinking generated the best transfer function with a high apparent r^2 of 0.96 and a jackknifed r^2 of 0.85. However, the model displayed a relatively high prediction error (RMSEP = 0.66), which is likely to be improved by amalgamation with other data-sets. The model has provided increased knowledge on the relationship between diatoms and pH in wetlands of the south-west of Western Australia. It may now be applied in the reconstruction of pH, used in modern monitoring programs or incorporated into a larger training set for these purposes.

7.2 Introduction

The previous chapter of the thesis was a comparative study of the sensitivity of invertebrates and diatoms to pH. The demonstrated relationship between diatom community structure and pH prompted further investigation into the influence of this variable on diatom assemblages. Therefore, the current section of the thesis aims to develop a diatom-based transfer function for pH using water bodies from the south-west of Western Australia.

Acidification of surface waters has been documented in countries including Australia (Sommer and Horwitz 2001), Canada (McNicol *et al.* 1995), Sweden (Renberg *et al.* 1990), Finland (Meriläinen and Huttunen 1990) and the United States (Driscoll *et al.* 2001; Fitzhugh *et al.* 2001). In Western Australia, acidification is commonly associated with the oxidation of acid sulphate soils (EPA 2006) whereas acidification in many other regions of the world has mostly been attributed to acidic deposition (Økland and Økland 1986; Schindler 1988).

The emergence of surface water acidification as an important environmental issue has led to the increased use of diatoms as indicators of pH (Battarbee *et al.* 1999). One method commonly employed to assess acidification or pH decrease is the development of diatom-based inference models for palaeolimnological reconstructions. For example, a pH transfer function for Finnish Lapland was derived using diatom data from 45 lakes (Korhola *et al.* 1999). Training sets collated from 54 and 42 water bodies respectively have been used to develop diatom-based pH models for Canada (Hall and Smol 1996; Enache and Prairie 2002). In contrast, a pH inference model for the north-eastern United States (Dixit *et al.* 1999) was derived from a data-set of 237 lakes. Other diatom-based transfer functions for pH include models by Reid (2005) and Kilroy *et al.* (2006)from different areas of New Zealand.

Australian inference models for pH include transfer functions based on Tasmanian highland lakes (Vyverman *et al.* 1996) and south-eastern Australian water bodies (Tibby *et al.* 2003; Philibert *et al.* 2006). In Western Australia, inference models for pH have been restricted to a single model from the Perth Metropolitan Region of the Swan Coastal Plain (McHugh 2004). However, a major limitation of this transfer

function was the poor representation of sites in the lower pH range. The training set developed for the current study incorporates a greater number of acidic sites and includes wetlands from a larger area of the south-west of Western Australia. A further difference is the use of artificial substrates (JJ Periphytometers) for the collection of diatoms, ensuring uniformity of sampling as well as measurement of the ambient pH experienced by the living diatom community at the time of collection (John 1998).

The aims of the current study were to explore the relationship between diatom distribution and environmental variables in the study sites and derive a diatom-based pH transfer function for the region. The development of a pH inference model for the south-west of Western Australia has potential applications for palaeolimnological studies and modern monitoring programs.

7.3 Methods

7.3.1 Study sites

A total of 40 water bodies from the south-west of Western Australia were initially selected for the development of a pH model (Figure 7.1). The sites were each represented by a single sampling event which occurred between 2000-2004, generally during spring. A more detailed description of the study sites used in the model has been provided in Chapter 2 of this thesis.

7.3.2 Field and laboratory methods

Environmental variables were measured at each of the sites following the procedure outlined in Chapter 3. The full environmental data-set is presented in Appendix 7.1. Periphytic diatoms were collected from each site using the artificial substrate collector – the JJ Periphytometer. Artificial substrate collectors were submerged at the sites for a period of approximately 14 days and provided a uniform surface for colonisation (John 1998). Diatom slide preparation followed the methods outlined in John (1983). A comprehensive description of the methods used for diatom collection, preparation and enumeration was provided in Chapter 5 of this thesis.

7.3.3 Statistical analyses

A total of 144 species were initially included in the study. A reduced data set of 113 species was generated through the removal of species which did not display a maximum relative abundance of $\geq$ 1% (ter Braak and Juggins 1993). Diatom abundance data was square-root transformed prior to ordination but was left untransformed for the development of the transfer functions. The environmental variable of electrical conductivity was $\log_{10}$ transformed to reduced skewness.

Detrended correspondence analysis (DCA) (Hill and Gauch Jr. 1980) with detrending by segments was used to investigate patterns in diatom community structure of the 40 sites and assess the most suitable model for predicting species response to environmental variables. The length of the gradients generated by the DCA exceeded 4.0 standard deviation units, suggesting that a unimodal method of ordination such as canonical correspondence analysis (CCA) would be most appropriate (Lepš and Šmilauer 2003). Subsequently CCA was used to explore the relationship between diatom community structure and environmental variables (ter Braak 1986). The significance of the ordination axes (p <0.05) were tested using unrestricted Monte Carlo permutation tests with 999 permutations. A list of site codes and species abbreviations presented in the CCA ordinations and the corresponding site and species names are presented in Appendices 7.1 and 7.2. CCAs constrained to single environmental variables and Monte Carlo permutation tests (999) were employed to identify variables which contributed significantly to the variance in the diatom data (p <0.05). The amount of variance explained by these parameters was established by performing a CCA using significant environmental variables only. Partitioning of variance was carried out through a series of partial CCAs to determine the unique contribution of each significant variable and the interaction between these variables (Borcard *et al.* 1992). Analyses were performed using the program CANOCO Version 4.53 (ter Braak and Smilauer 2004).

Transfer functions for pH were developed using the statistical program C^2 Data Analysis Version 1.4 Beta (Juggins 2003). The methods used to derive the transfer functions included weighted averaging (WA) and tolerance-downweighted weighted averaging (TOL-WA) regression and calibration with both classical and inverse

deshrinking (Birks *et al.* 1990). Averages are taken twice during the WA technique, shrinking the range of the estimated environmental variable. The use of a deshrinking regression allows the amount of shrinkage to be estimated (ter Braak and Juggins 1993). The WA regression coefficients generated for each species represent the abundance weighted means and abundance weighted standard deviations and gives an estimate of the pH optima and tolerances of the individual taxa (Birks *et al.* 1990).

The predictive ability of the transfer functions were evaluated in terms of the correlation coefficient (r^2) between the observed to inferred pH, the root mean square of the error (RMSE) (observed – inferred) and the maximum bias. The strength of the relationship between observed and inferred values is represented by r^2 and prediction errors are indicated by the RMSE. The maximum bias represents the tendency of the model to over or under-estimate along an environmental gradient (Gasse *et al.* 1995). Values were derived from both transfer functions on the full data–set (apparent values) and from the jackknife 'leave-one out' cross validation method. Sites which displayed a difference between the observed and jackknife-inferred pH values of greater than 25% of the total pH range were classified as outliers and removed from the data-set (Gasse *et al.* 1995).
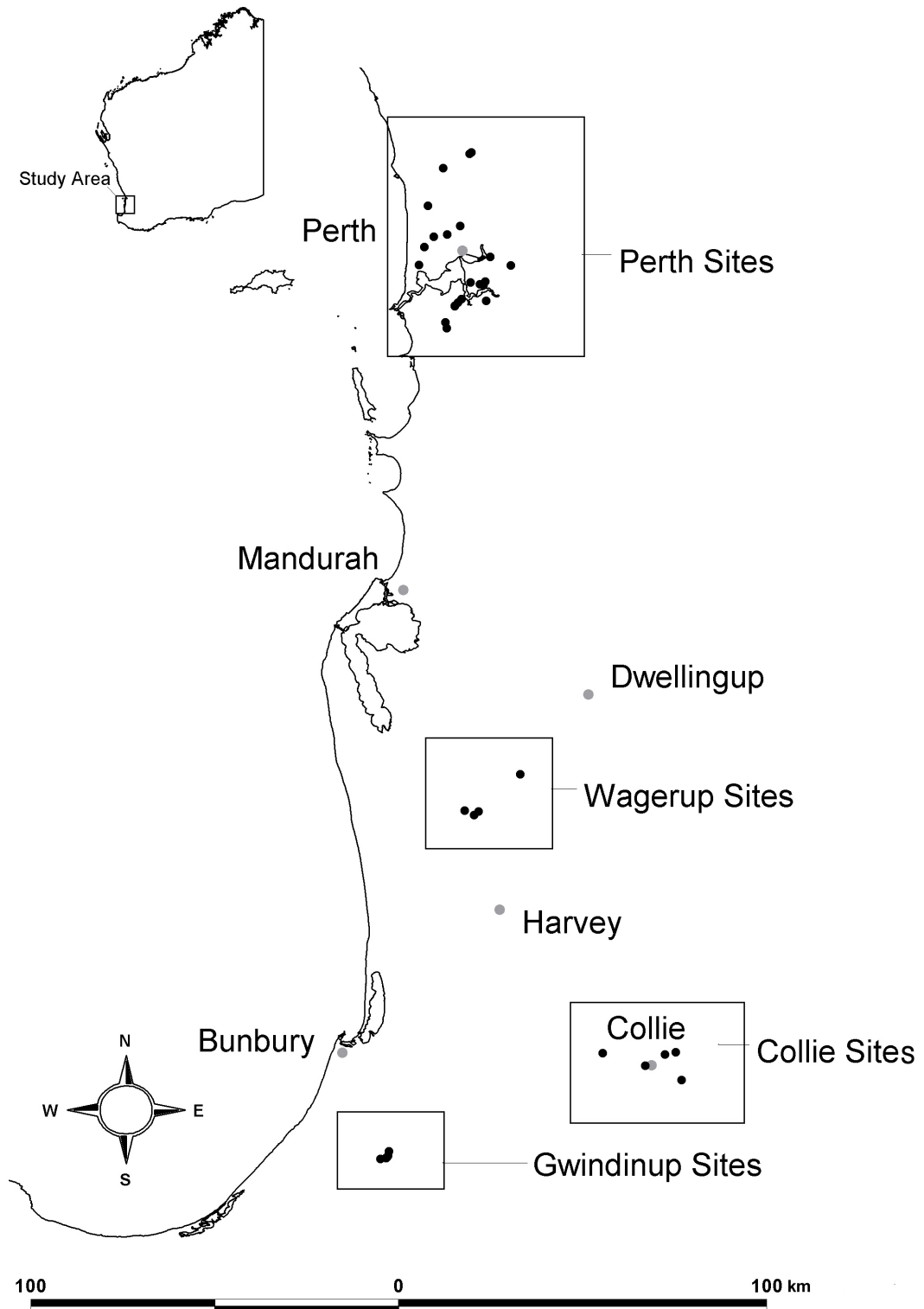
Figure 7.1: Location of the 40 sites in the south-west of Western Australia selected for use in the diatom-based transfer function for pH. Black points indicate the study sites located in the Perth Metropolitan Region. Grey points represent the neighbouring towns.

7.4 Results

7.4.1 Environmental variables

The 40 wetlands included in the study ranged in pH from 3.12 at M37 (Stockton Tailings Pond) to 9.30 at M21 (Knapping Wetland). Electrical conductivity varied from 174.30 μS cm^{-1} at M23 (Lake Brockman) to 3220.00 μS cm^{-1} at M24 (Lake Claremont), with most sites displaying values under 1000.00 μS cm^{-1}. Dissolved oxygen ranged from 1.75 to 18.10 mg L^{-1}.The lowest temperature recorded was 16.30 ºC while the maximum recorded was 25.60 ºC. Fringing vegetation ranged from sparse to relatively dense with vegetation scores from 1-5 (Appendix 7.1).

7.4.2 Ordinations

A total of 144 species were identified from the 40 wetlands, with 113 species displaying a maximum abundance of < 1%. Detrended correspondence analysis performed on the 113 species data-set showed that the first two canonical axes had eigenvalues of 0.75 and 0.53 respectively and cumulatively accounted for 13.80% of the variance in the diatom data (Table 7.1). The low percentage variance explained is common to data-sets with many zero values and high numbers of taxa (Dixit *et al.* 1999; Davies *et al.* 2002). Gradients of 6.14 for axis 1 and 4.67 for axis 2 were recorded from the DCA, supporting the use of CCA for further analyses (Table 7.1).

Table 7.1: A summary of DCA results for the data-set of 40 sites and 113 taxa sampled in the south-west of Western Australia.

	Axis 1	Axis 2	Axis 3	Axis 4	Total Inertia
Eigenvalues	0.75	0.53	0.34	0.25	
Lengths of gradient	6.14	4.67	3.35	3.15	
Cumulative % variance of species data	8.10	13.80	17.40	20.20	
Sum of all eigenvalues					9.28

Species-environment correlations from CCA with the five environmental variables were high with $r = 0.95$ for axis 1 and $r = 0.91$ for axis 2. The first two axes explained 11.30% of the cumulative variation in the species data ($\lambda_1 = 0.63$, $\lambda_2 = 0.42$) (Table 7.2). Although the percentage variation accounted for by axes 1 and 2 was low, they were both significant ($p < 0.01$) and displayed eigenvalues similar to

those generated by the DCA ($\lambda_1 = 0.75$ and $\lambda_2 = 0.53$). This suggests that the five environmental variables accounted for the majority of variation in the species data.

Table 7.2: A summary of CCA results for the data-set of 40 sites and 113 taxa sampled in the south-west of Western Australia using five environmental variables – pH (pH units), electrical conductivity (μS cm^{-1}), temperature (°C), dissolved oxygen (mg L^{-1}) and vegetation score.

	Axis 1	Axis 2	Axis 3	Axis 4	Total Inertia
Eigenvalues	0.63	0.42	0.25	0.23	9.28
Species-environment correlations	0.95	0.91	0.83	0.88	
Cumulative % variance of the species data	6.80	11.30	14.00	16.50	
Cumulative % variance of the species-environment relation	37.30	62.00	76.70	90.20	
Sum of canonical eigenvalues					1.70

The variables of pH and electrical conductivity were the environmental parameters that primarily explained the variation in the diatom data (Figure 7.2). The variable of pH was closely associated with axis 1, displaying an inter-set correlation of 0.93. This is demonstrated by the gradient from acidic to alkaline sites along that axis. Electrical conductivity accounted for the greatest variability along the second axis ($r = 0.79$) and sites possessing the highest values for this parameter were positioned in the upper two quadrants of the biplots (Figure 7.2a). A group of outlier sites including sites M6 (Blue Waters), M9 (Ewington 2), M11 (Gnangara Lake) and M37 (Stockton Tailings Pond) located in the upper left quadrant indicate low pH and relatively high electrical conductivity. The single outlier site of M24 (Lake Claremont) in the upper right quadrant recorded the highest electrical conductivity of the study (3220.00 μS cm^{-1}) (Figure 7.2a). A list of sites codes and corresponding site names is presented in Appendix 7.1. The influence of the two main variables is also displayed by the positioning of the species on the species-environment CCA biplot (Figure 7.2b). Taxa such as *Brachysira brebissonii, Eunotia pectinalis* var. *minor* and *Nitzschia paleaeformis* were situated in the upper left quadrant, displaying a relationship with waters of low pH and relatively high conductivity in comparison with other sites. In contrast, species such as *Frustulia rhomboides, Eunotia pectinalis* and *Eunotia bilunaris* were in the lower left quadrant and were associated with low pH and low electrical conductivity. The species *Nitzschia frustulum* was related to relatively high conductivity and alkaline conditions (Figure 7.2b).
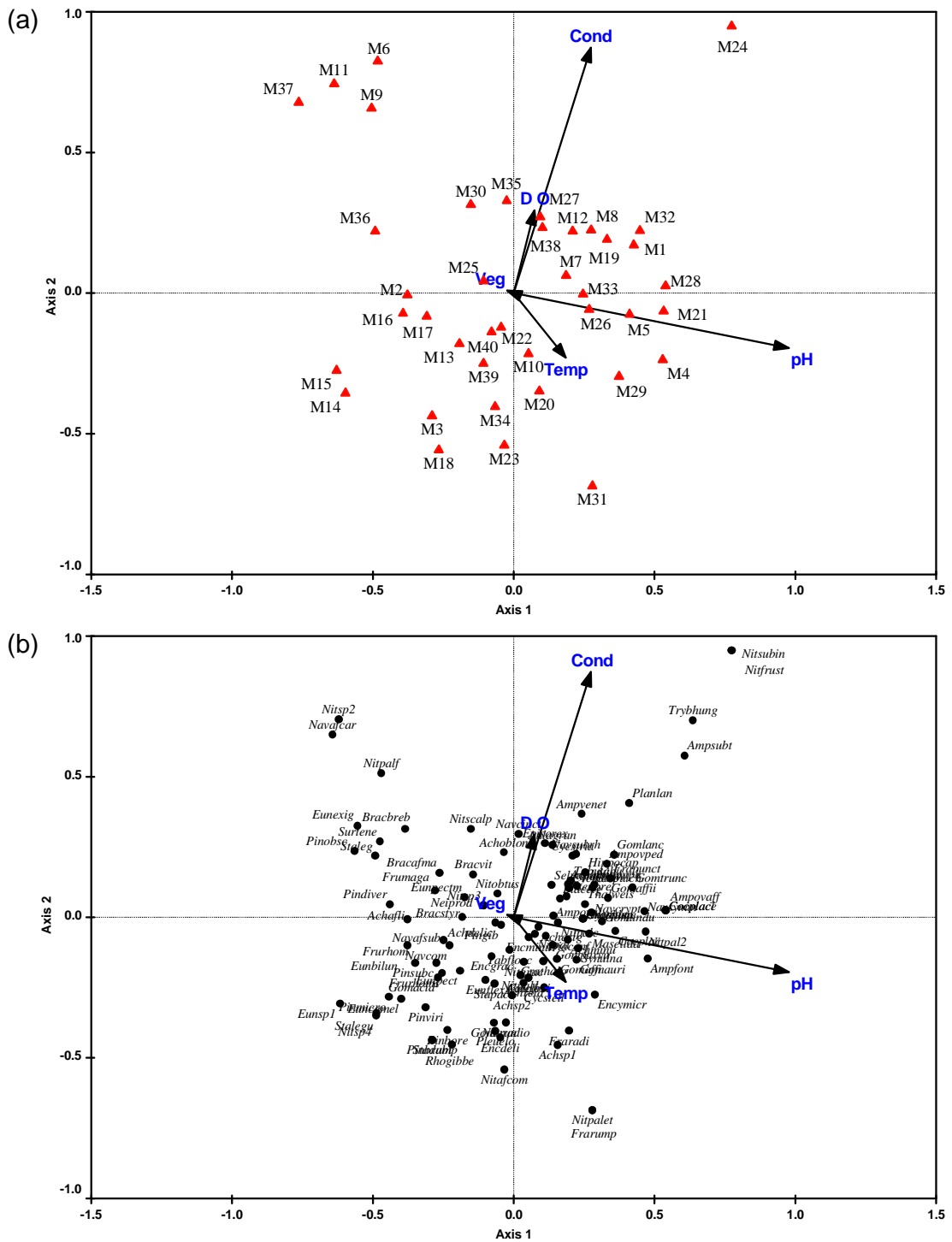
Figure 7.2: CCA ordination biplots based on the data-set of 40 sites, five environmental variables and 113 species (maximum abundance of $\geq$ 1%). Environmental variables are displayed as vectors and the vector length indicates the relative importance of the variable in contributing to the variance in species data. Cond = electrical conductivity; DO = dissolved oxygen; Temp = temperature; Veg = vegetation score. (a) Site environment biplot. (b) Species environment biplot.

CCAs of the data constrained to single environmental variables found that pH and electrical conductivity were the only significant variables (p < 0.01). A further CCA using the two variables captured 10.94% of the variance in the diatom data over the first two axes (λ_1 = 0.63, λ_2 = 0.42), a similar value to the 11.30 % of variation explained by axes 1 and 2 when including the five environmental variables. The variable of pH made a unique contribution of 6.60% to the variance in the diatom data while electrical conductivity contributed 4.25%. The interaction between the two variables was low (0.09%), indicating that they each accounted for an independent section of the variance in diatom data (Figure 7.3). The low interaction coupled with the higher unique contribution of pH suggests that the variable is suitable for the development of a transfer function.
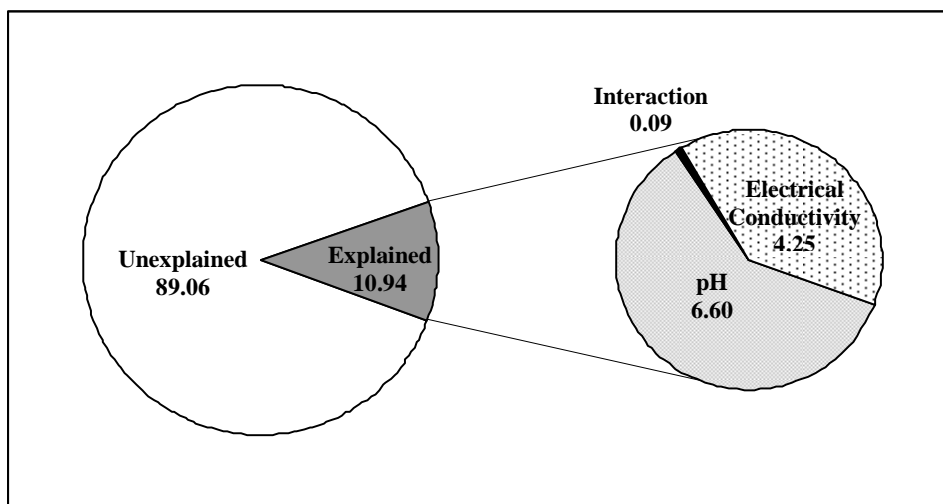
Figure 7.3: Results of partitioning of variance in the diatom data using the significant variables of pH and $\log_{10}$-transformed electrical conductivity. The diagram displays the percentage of variance in the data which is explained or unexplained and the unique contribution and interaction of the environmental variables.

7.4.3 Diatom-based transfer function for pH

Weighted averaging pH transfer functions were developed using the 40 site data-set. There was a strong correlation between the observed and inferred pH values of the 40 sites with an apparent r^2 > 0.90 for both WA and tolerance- downweighted WA models. The highest correlation coefficient (r^2) and lowest RMSE were derived using tolerance-downweighted WA, whereas the simple WA models performed better

under cross validation by jackknifing. Each of the models displayed lower r^2 and higher RMSEs and maximum bias values following jackknifing (Table 7.3).

The sites M3, M4, M22 and M25 displayed residuals of greater than 25% of the total pH range and were identified as outliers according to the methods of Gasse *et al.* (1995). Removal of these water bodies generated a reduced data-set of 36 sites and 109 species. Each of the models constructed from the reduced data-set displayed relatively high correlation coefficients ($r^2 > 0.95$) and increased predictive power. Tolerance-downweighted WA models generally performed better than simple WA models in terms of apparent values, generating higher correlation coefficients (r^2) and lower RMSEs. Under jackknifing, the highest r^2 values and lowest RMSE and maximum bias were produced by the simple WA model using classical deshrinking (Table 7.3). The relatively strong linear relationship between the observed and diatom-inferred pH generated from the WA model is illustrated in Figure 7.4a-b. The figure also shows the slightly uneven spread of sites along the pH gradient, with the highest number contained within the upper pH range. Figure 7.4c shows a trend between the residuals (jackknifed) and observed pH values (Figure 7.4c) where the model tends to over-estimate values at the low end of the pH range and under-estimate at the high end of the pH scale.

Table 7.3: A summary of the performance of simple weighted averaging (WA), tolerance-downweighted WA (Tol-WA) models for pH using inverse and classical deshrinking and cross-validation by jackknifing. Table includes statistics on the initial data-set of 40 samples and the reduced data-set of 36 samples from wetlands in the south-west of Western Australia.

Deshrinking Type	Model	40 samples			36 samples		
		r^2	RMSE	Max. Bias	r^2	RMSE	Max. Bias
Inverse	WA	0.91	0.50	0.38	0.96	0.36	0.26
	Tol-WA	0.95	0.35	0.19	0.97	0.28	0.23
	Jack. WA	0.75	0.83	1.18	0.85	0.68	1.10
	Jack. Tol-WA	0.72	0.89	1.97	0.82	0.76	1.74
Classical	WA	0.91	0.52	0.39	0.96	0.36	0.19
	Tol-WA	0.95	0.36	0.30	0.97	0.28	0.25
	Jack. WA	0.75	0.82	1.05	0.85	0.66	1.03
	Jack. Tol-WA	0.72	0.88	1.89	0.82	0.75	1.68

7.4.4 pH optima and tolerances

The 109 taxa included in the final model and the simple weighted average pH optima and tolerance for each taxon have been presented in Appendix 7.2. The number of sites in which each taxon occurred, the maximum percentage abundance, and the number of effective occurrences (N_2) for each species, values derived from Hill's N_2 diversity index (Hill 1973), have also been included. The estimated optima of species with low N_2 values are likely to be less reliable as a result of the fewer occurrences (Gasse *et al.* 1995).

The pH optima of species included in the final transfer function ranged from 3.35 to 9.19. Estimated tolerances were similarly variable with the lowest being 0.07 pH units and the highest being 2.65. The pH optima and tolerance ranges of the most abundant species (those which occurred with a maximum abundance of $\geq$ 1% and were present in greater than five of the 36 sites) have been presented in Figure 7.5. Each of these common taxa (Figure 7.5) displayed Hill's N_2 values > 2 with most having N_2 values of > 3. Species such as *Brachysira brebissonii, Nitzschia paleaeformis, Pinnularia divergentissima*, *Pinnularia microstauron* and *Frustulia magaliesmontana* displayed low optima of < pH 5.00 while the estimated optima of taxa such as *Cocconeis placentula, Achnanthium minutissimum, Navicula cryptocephala, Synedra ulna* and *Nitzschia amphibia* exceeded pH 7.50 (Figure 7.5). The species with optima below pH 5 display tolerance ranges within the acidic pH range (pH < 6.50) and could be considered potential indicator species for acidic conditions.

(a)

(b)

(c)
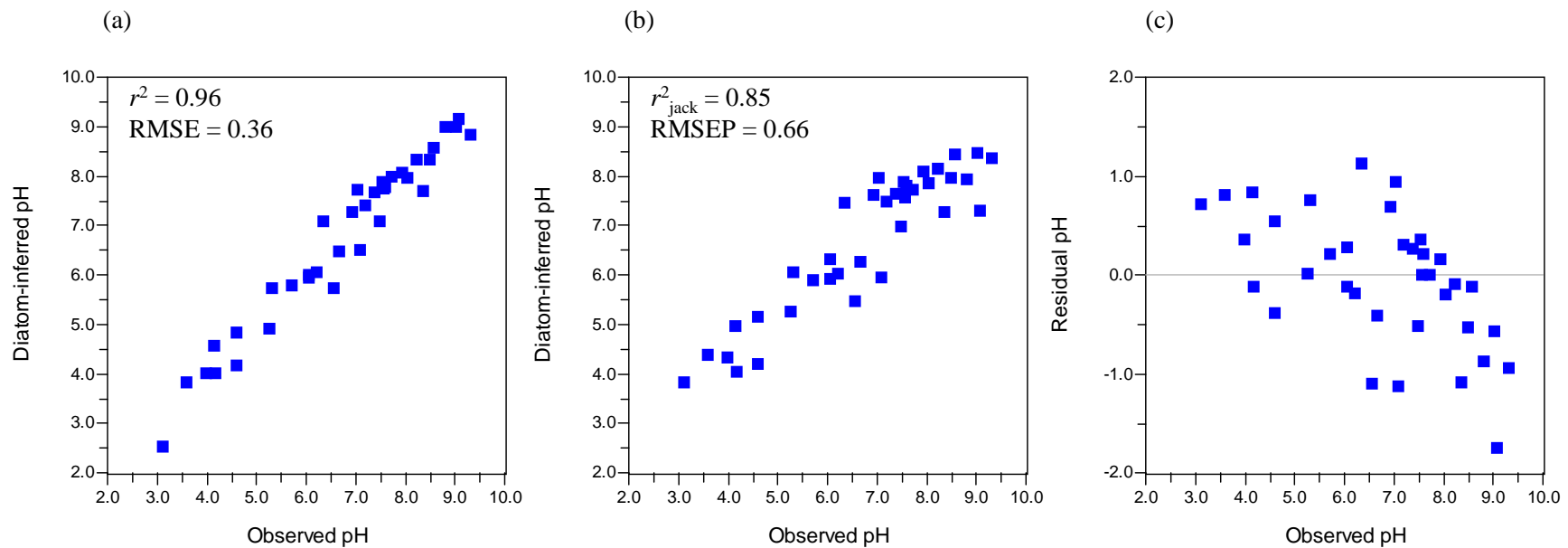
Figure 7.4: Plots of the relationships between (a) observed pH and diatom-inferred pH (b) observed pH and diatom-inferred pH under jackknifing and (c) observed pH and residual pH under jackknifing. Plots were constructed from the results of the simple WA model with classical deshrinking using the data-set of 36 sites and 109 species.
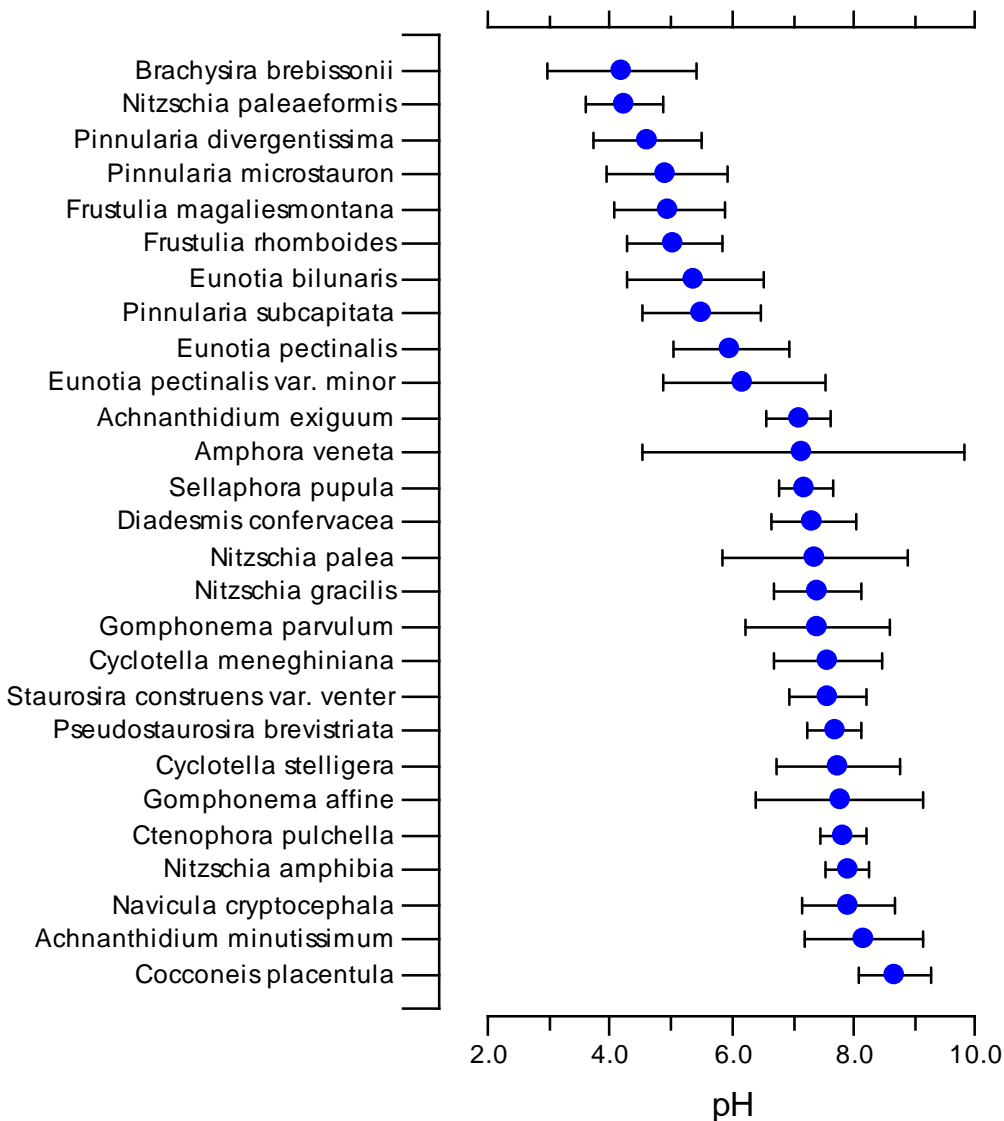
Figure 7.5: The estimated pH optima (abundance weighted means) and tolerances (abundance weighted standard deviations) for diatom species that occurred with a maximum abundance of $\geq$ 1% and were present in > five sites in the simple WA model with classical deshrinking based on the data-set of 36 sites and 109 species.

7.5 Discussion

7.5.1 The relationship between diatom assemblages and environmental variables

Canonical correspondence analysis detected a strong relationship between diatom community structure and pH with electrical conductivity the only other measured variable to display a relatively strong correlation with the structure of the assemblages. Sites such as the Collie water bodies of M6 (Blue Waters) and M9 (Ewington 2) have developed in abandoned mine voids which intercept the water table (Commander *et al.* 1994). As a result, these sites displayed low pH and higher electrical conductivity in comparison with many of the other sites, conditions typically linked to acid mine drainage (Kelly 1988). The shallow Perth wetland of Gnangara Lake displayed similar pH and electrical conductivity to the aforementioned water bodies. These conditions are attributable to the oxidation of acid sulphate soils exacerbated by increasing groundwater abstraction (McHugh 2004) and the direct exposure of sulphidic sediment as a result of dredging for diatomaceous earth (John and Kupfer 1994). *Navicula* aff. *cari, Nitzschia paleaeformis* and *Eunotia exigua,* species which have been previously identified from low pH waters associated with mining (John 1993; Kapfer 1998; Verb and Vis 2000) were commonly associated with the low pH and higher electrical conductivity sites in the study. Taxa such as these could potentially be useful indicators of human-induced acidification.

Species such as *Eunotia bilunaris* were more commonly recorded from sites with low pH and low electrical conductivity such as the Gwindinup water bodies. These sites were highly coloured (J. John pers. comm.) and it is likely that their low pH is mostly related to the presence of organic acidity rather than anthropogenic disturbance. This has resulted in a different suite of acidophilous diatom species than those recorded from sites such as Gnangara. In contrast, *Nitzschia frustulum* was one of the species most closely related to high pH and relatively high electrical conductivity in comparison to the other sites. The taxon occurred in very high numbers in Lake Claremont (M24), a site which displayed a pH of approximately 9.0 and the highest electrical conductivity recorded during the study. The taxon has also

been commonly recorded from alkaline and high conductivity lakes in East Africa (Hecky and Kilham 1973) and Mexico (Davies *et al.* 2002).

7.5.2 Transfer function performance

Transfer functions using weighted averaging performed better than tolerance-downweighted WA under cross validation by jackknifing and were selected for use in the current study. WA models with classical deshrinking displayed slightly greater predictive ability than those models using inverse deshrinking. Classical regression deshrinks more than inverse regression and is therefore more reliable at the low and high ends of the pH range (Birks *et al.* 1990). The predictive ability of the final transfer function, as indicated by the correlation coefficients, was strong (apparent r^2 of 0.96 and r^2_{jack} of 0.85). The model was less robust however when taking the RMSEP and maximum bias into consideration. The r^2 values of the current model were similar or often higher than those generated by diatom-based pH models in other regions. For example, a Canadian pH transfer function developed by Hall and Smol (1996) produced a similar apparent r^2 of 0.88 while a lower apparent r^2 of 0.80 was derived from a transfer function based on data from 55 lakes in Minnesota (Ramstack *et al.* 2003). Another inference model, derived from an eastern Australian data-set, generated an r^2_{jack} of 0.77 (and a RMSEP of 0.35pH units) (Tibby *et al.* 2003). The r^2_{jack} of 0.91 based on diatom data from 20 subalpine pools in New Zealand (Kilroy *et al.* 2006) was similar to the current study while another New Zealand study gave a lower r^2_{jack} of 0.72 (Reid 2005).

In contrast, the RMSEP and maximum bias under cross validation were relatively high in comparison to many of the other pH models. For example, transfer functions developed by Enache and Prairie (2002) and Kilroy *et al.* (2006) had RMSEPs of 0.25 and 0.18 respectively. The higher RMSEP of the current transfer function indicated reduced predictive power. The predictive errors were highlighted by the pattern evident in the residuals, showing the tendency of the model to over-estimate at the lower end of the pH range and under-estimate at the higher end. An earlier Western Australian pH transfer function (McHugh 2004) based on wetlands in the Perth Metropolitan Region had similar findings, with a high RMSEP and jackknifed maximum bias. However, the r^2_{jack} value of the current model was comparatively higher ($r^2_{jack} = 0.85$) than that recorded by McHugh (2004) (r^2_{jack} value < 0.6).

Poor performance under jackknifing has previously been related to heterogeneity within the data-set, a relatively low number of sites and fewer samples from the low end of the environmental gradient (Reed 1998; McHugh 2004). The current training set contained a greater number of acidic sites than the previous Western Australian pH transfer function (McHugh 2004). However a slightly uneven spread of sites along the pH gradient, a relatively low number of sampling sites and heterogeneity of sites remain as factors affecting the present study.

It is probable that an increased number of sites would improve the predictive ability of the model, as demonstrated by Wilson *et al.* (1996) during the development of a salinity transfer function for western North America. An increase in the number of sites along the pH gradient would improve the reliability of the pH ranges for each taxon and provide more accurate estimates of the optima, especially for rare species (Battarbee *et al.* 1999). Furthermore, the addition of sites from areas geographically intermediate between the Collie, Wagerup and Perth sites may be beneficial in improving the robustness of the model by providing a more comprehensive spread of sites across the geographical range and potentially increasing the pH range sampled.

7.5.3 Estimated pH optima and tolerances of the diatom taxa

Many of the species identified as common in the current study displayed relatively small pH tolerances ranges. For example, frequently occurring species with pH optima between 5.00 and 6.50 generally displayed tolerance ranges within acidic or circumneutral levels and could be potentially useful as indicators of pH decrease in neutral to alkaline waters. Additionally, many of the abundant species displayed N_2 values > 3, suggesting that the estimated optima for these species were likely to be more reliable than the optima of less abundant taxa.

Some of the estimated pH optima for species known to inhabit acidic and circumneutral wetlands were much lower in the current pH model than in the transfer function developed for the Swan Coastal Plain (McHugh 2004). The low number of sites at the acidic end of the pH gradient in that study may have resulted in the over-estimation of optima for species including *Eunotia pectinalis* (optima = 8.27) and

Eunotia pectinalis var. *minor* (optima = 8.03). The current pH model included a higher number of low pH sites and estimated optima of species in the acidic to circumneutral range were often comparable to those derived from other regions. For example, the present study displayed an estimated optima of 7.17 for *Tabellaria flocculosa,* in keeping with the optima of 7.30 was generated from a Minnesota data-set (Ramstack *et al.* 2003). The estimated optima for *Eunotia pectinalis* was 6.00, comparing well to the optima of 6.01 presented by Camburn and Charles (2000) and the current optima of 5.40 for *Eunotia bilunaris* was similar to that given by a Tasmanian study by Vyverman *et al.* (1996) (pH = 5.59). The optima of 5.07 produced for *Frustulia rhomboides* was within the range of 4.59 to 6.20 discerned from various studies (Dixit *et al.* 1999; Camburn and Charles 2000; Enache and Prairie 2002; Kilroy *et al.* 2006).

It is however important to take prediction errors and effective number of occurrences (N^2) into account when considering the optima from the lower pH range. The low N^2 value of some species indicates that their optima may not be as reliable as those generated for more commonly occurring species. Nonetheless, some of the more common species including *Eunotia pectinalis, Eunotia bilunaris, Frustulia rhomboides, Frustulia magaliesmontana, Pinnularia divergentissima, Nitzschia paleaeformis* and *Brachysira brebissonii* may be potentially useful as indicators of pH decline.

The estimated optima of many species in the higher pH range were also comparable to other studies. Examples include *Cocconeis placentula* (pH optima = 8.67) which was given an optima of 8.5 by Ramstack *et al.* (2003), *Navicula cryptocephala* (pH optima = 7.9) which had an optima of 8.1 according to Dixit *et al.* (1999) and Ramstack *et al.* (2003) and *Pseudostaurosira brevistriata* for which the current study and Dixit *et al.* (1999) both generated an optima of 7.70. The highest N_2 values were generally recorded for sites with pH optima > 7.00 and this indicates that lower predictive error is probable for reconstructions in the higher pH range.

Discrepancies between the values calculated from the current model and other transfer functions may be related to factors such taxonomic consistency and the size of the data-set (Gell 1997). As noted by Wilson *et al.* (1996) larger data-sets improve

the estimates of the species optima and tolerances. Bias in site selection across the environmental variable's range is also likely to contribute to differences between estimated optima (Davies *et al.* 2002). A further issue to consider is the role of other environmental variables in the distribution of diatoms and the impact this may potentially have on the results of a transfer function based on a single environmental variable. For example while the largest amount of variance in the diatom data for this study was related to pH, electrical conductivity was also shown to make a significant contribution.

7.5.4 Use of artificial substrates

Many training sets are compiled from diatoms in surface sediment samples (Wilson *et al.* 1996; Davies *et al.* 2002; Bloom *et al.* 2003). The major advantage of using surface sediments is that the values generated by the transfer function are appropriate to core samples (Cooper *et al.* 1999). However, there can be problems associated with the use of this technique for diatom collection. Firstly, there is generally uncertainty about the age of the surface layers of sediment. Secondly, the surface sediments may be contaminated by taxa from older reworked sediment (Battarbee *et al.* 1999).

 In contrast, the use of artificial substrates ensures that the diatoms collected were growing at the time of sampling and therefore reflect the ambient environmental conditions (John 1998). A disadvantage of their use is that unlike surface sediment which can be collected in a single trip, artificial substrates require both deposition and collection. However, because surface sediments display diatom communities integrated over time, it is recommended that environmental variables should be measured seasonally (Battarbee *et al.* 1999) thus increasing the number of sampling trips associated with surface sediment assemblages. Subsequently, the use of artificial substrates or surface sediment for collection of diatoms should be governed by the aims of the study. If the intent of the study is to use the data for both monitoring and reconstructive purposes it is possible that artificial substrates may provide the most suitable means of collection.

7.5.5   Applications of the transfer function

Diatom methods are already being incorporated into the management of acidified waters in other regions of the world (Battarbee *et al.* 1999). Similarly, the diatom-based pH model produced by the current study has the potential to be integrated into management programs for threatened and acidified waters in Western Australia or amalgamated with a larger training set for this purpose. The use of the artificial substrates rather than the collecting diatoms from the surface sediment implies that the information gained from the model would be of particular relevance to modern monitoring programs. Furthermore, the inclusion of acidic wetlands impacted by factors such as acid-mine drainage and the oxidation of acid sulphate soils mean that the model generated has the potential to be used as a tool to evaluate restoration efforts in waters impacted by these types of acidification. Future research including a greater number of sites impacted by the aforementioned factors would be likely to further increase the reliability of the model and help identify indicator species for these types of acidification.

The model may also have applications for reconstructing past pH. The benefits of reconstruction in terms of management include the provision of long-term data on the natural variability of a water body (Enache and Prairie 2002). This information can then be used to differentiate between waters that are naturally acidic and those that have recently become acidified (Battarbee *et al.* 1999). However, it is important to note that while the model is relatively strong, the high RMSEP suggests that reconstruction should be interpreted with caution, particularly at the lower end of the pH range.

7.6   Conclusion

The diatom-based pH transfer function produced by this study has improved upon the previous Western Australian model by including wetlands from a larger range of pH, incorporating sites affected by various acidification processes and sampling over a wider geographical area. Furthermore, the current transfer function had increased statistical power. However, while still useful, the model is less robust than some of the pH transfer functions from other regions. Increasing the data in the training set through further sampling or amalgamation with an existing data-set would be likely

to strengthen the predictive ability of the model. This would also improve the transfer function's potential to be used as a tool in palaeolimnological studies or programs monitoring the restoration of acidified waters.

7.7 References

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Appendix 7.1: List of sites, codes and measurements of environmental variables. pH (pH units), electrical conductivity (μS cm^{-1}), temperature (°C), dissolved oxygen (mg L^{-1}) and vegetation score (1-5).

Site	Code	pH	Conductivity	Temperature	Dissolved Oxygen	Vegetation Score
Bibra Lake	M1	8.23	1386.00	16.90	4.17	4.00
Black Diamond Lake	M2	5.26	447.00	19.00	8.27	3.00
Blind Roo A	M3	5.86	249.00	17.70	5.72	3.00
Blind Roo B	M4	9.29	395.00	21.90	10.18	2.00
Blue Gum Lake	M5	8.57	780.00	20.00	6.60	4.00
Blue Waters	M6	3.98	1485.00	17.60	9.11	1.00
Booragoon Lake	M7	7.57	1111.00	21.60	5.60	5.00
Dog Swamp	M8	7.58	700.00	16.30	8.77	1.00
Ewington II Lake	M9	4.16	1380.00	18.80	8.69	3.00
Exelby Wetlands	M10	7.19	423.00	22.30	7.15	2.00
Gnangara Lake	M11	3.59	1689.00	23.90	8.28	2.00
G.O. Edwards Park	M12	7.37	748.00	16.30	8.77	2.00
Gwindinup 1	M13	6.20	280.00	21.60	9.91	1.00
Gwindinup 2	M14	4.60	280.00	21.10	5.57	3.00
Gwindinup 3	M15	4.14	396.00	17.40	1.75	3.00
Gwindinup 4	M16	5.30	382.00	19.70	8.90	3.00
Gwindinup 5	M17	5.70	330.00	21.20	10.30	2.00
Gwindinup 6	M18	6.04	204.00	19.60	5.35	2.00
Herdsman Lake	M19	8.04	1124.00	22.30	7.42	3.00
Jack Finney Lake	M20	7.48	323.00	22.00	7.60	2.00
Knapping Wetlands	M21	9.30	603.00	24.20	11.03	3.00
Kurrajong Village Lake	M22	6.73	392.00	20.20	8.81	2.00
Lake Brockman	M23	7.08	174.30	20.60	8.30	1.00
Lake Claremont	M24	9.06	3220.00	17.00	10.10	1.00
Lake Gillon	M25	6.24	522.00	18.40	7.86	2.00
Lake Goollelal	M26	7.93	989.00	22.80	4.26	4.00
Lake Gwelup	M27	7.53	502.00	22.60	18.10	4.00
Lake Monger	M28	9.03	785.00	22.30	8.35	2.00
Lake Moyanup	M29	8.81	355.00	22.90	10.70	3.00
Lakelands	M30	6.04	1125.00	22.50	8.27	4.00
Neil McDougall Park	M31	8.50	179.50	19.90	7.57	2.00
North Lake	M32	8.37	1698.00	20.50	4.38	4.00
Perry Lakes	M33	7.71	1059.00	25.10	3.57	2.00
Piney Lakes	M34	6.94	478.00	25.60	4.38	4.00
Sheldrake Park	M35	6.34	669.00	17.30	10.10	1.00
Stockton Lake	M36	4.59	582.00	17.70	9.02	3.00
Stockton Tailings Pond	M37	3.12	1332.00	18.00	8.83	4.00
Tomato Lake	M38	7.02	884.00	21.40	8.23	2.00
Tuscan Park	M39	6.56	351.00	21.80	7.36	2.00
Wallsend Lake	M40	6.66	447.00	19.00	8.27	4.00

Appendix 7.2: List of the 113 taxa present with a maximum abundance of $\geq$ 1% in at least one site. Included are the taxonomic authority and taxon code for each of the 113 species. Further information including the total number of sites in which the taxon occurred (N), the Hill's N_2 Diversity Index, the maximum percentage abundance (Max %) and the pH optima and tolerance are presented for the 109 species included in the final WA transfer function.

Taxon	Code	N	Max %	N_2	Optimum	Tolerance
Achnanthes aff. *linearis* (W Smith) Grunow	Achafli	1	6.13	1.00	5.26	0.84
Achnanthidium aff. *reidensis* Foged	Achafrei	1	18.00	1.00	7.19	0.84
Achnanthidium delicatulum Kützing	Achdelic	4	3.00	2.85	6.30	1.00
Achnanthidium exiguum (Grunow) Czarn.	Achexig	12	78.00	2.06	7.10	0.54
Achnanthidium hungaricum Grunow	Achhung	3	3.33	2.19	7.46	1.48
Achnanthidium minutissimum (Kützing) Czarnecki	Achminu	18	64.67	6.83	8.19	0.96
Achnanthidium oblongella Oestrup	Achoblon	4	36.67	1.07	6.36	1.01
Achnanthidium reidensis Foged	Achreid	4	2.67	2.72	7.25	0.38
Achnanthidium sp. 1	Achsp1	1	13.67	1.00	8.50	0.84
Achnanthidium sp. 2	Achsp2	2	1.00	1.92	7.53	0.60
Amphora fontinalis Hustedt	Ampfont	2	23.33	1.55	9.19	0.35
Amphora ovalis Kützing	Ampoval	5	22.00	1.96	7.49	0.63
Amphora ovalis var. affinis (Kützing) Van Heurck *ex* DeToni	Ampovaff	1	1.33	1.00	9.03	0.84
Amphora ovalis var. pediculus (Kützing) Van Heurck *ex* DeToni	Ampovped	1	1.33	1.00	8.04	0.84
Amphora subturgida Hustedt	Ampsubt	2	2.33	1.99	8.68	0.59
Amphora veneta Kützing	Ampvenet	6	10.67	4.11	7.17	2.65
Anomoeoneis sphaerophora (Ehrenberg) Pfitzer	Anosphae	1	1.33	1.00	7.71	0.84
Aulacoseira granulata (Ehrenberg) Simonsen	Aulagran	3	6.67	2.82	7.02	1.19
Brachysira aff. *manfredii* Lange-Bertalot	Bracafma	4	7.00	2.21	5.70	1.90
Brachysira brebissonii Ross	Bracbreb	9	55.00	2.85	4.21	1.23
Brachysira styriaca (Grunow) Ross *in* Hartley	Bracstyr	4	54.33	3.21	6.26	1.16
Brachysira vitrea (Grunow) Ross *in* Hartley	Bracvit	3	22.33	1.27	6.10	0.46
Cocconeis placentula Ehrenberg	Cocplac	10	60.00	2.76	8.67	0.59
Cocconeis placentula var. euglypta (Ehrenberg) Grunow	Cocplace	1	6.67	1.00	9.03	0.84
Cocconeis placentula var. lineata (Ehrenberg) Van Heurck	Cocplacl	1	2.33	1.00	9.03	0.84
Craticula cuspidata (Kützing) Mann *in* Round, Crawford & Mann	Cratcusp	1	1.00	1.00	7.71	0.84
Craticula halophila (Grunow) Mann *in* Round, Crawford & Mann	Crathalo	4	6.45	3.09	6.99	1.77
Ctenophora pulchella (Ralfs *ex* Kützing) Williams & Round	Ctepulch	6	4.33	3.15	7.83	0.38
Cyclotella meneghiniana Kützing	Cycmene	13	5.33	8.20	7.59	0.89
Cyclotella stelligera (Cleve & Grunow) Van Heurck	Cycstell	6	9.67	2.97	7.77	1.02
Cyclotella striata (Kützing) Grunow	Cycstria	1	2.33	1.00	7.37	0.84
Cylindrotheca closterium (Ehrenberg) Lewin & Reimann	Cylclost	1	20.67	1.00	7.19	0.84
Diadesmis confervacea Kützing	Diaconf	9	56.00	2.18	7.34	0.70
Encyonema delicatulum (Kützing) Mann	Encdeli	1	22.67	1.00	7.08	0.84
Encyonema gracile Rabenhorst	Encgrac	1	2.00	1.00	6.66	0.84
Encyonema minutum (Hilse *ex* Rabenhorst) Mann	Encminu	1	0.33	1.00	8.81	0.84
Encyonopsis microcephala (Grunow) Krammer	Encymicr	4	70.33	1.21	8.70	1.19
Epithemia sorex Kützing	Episorex	2	24.59	1.05	7.53	0.11
Eunotia bilunaris (Ehrenberg) Mills	Eunbilun	11	41.67	4.86	5.40	1.12
Eunotia camelus Ehrenberg	Euncamel	5	10.00	3.06	5.13	1.09
Eunotia exigua (Brébisson ex Kützing) Rabenhorst	Eunexig	2	3.67	1.18	4.47	1.04
Eunotia flexuosa Brébisson *ex* Kützing	Eunflex	2	18.67	1.21	6.57	0.07
Eunotia pectinalis (O. Müller) Rabenhorst	Eunpect	13	83.00	4.75	6.00	0.94
Eunotia pectinalis var. *minor* (Kützing) Rabenhorst	Eunpectm	10	22.00	3.42	6.21	1.33
Eunotia sp. 1	Eunsp1	2	24.00	1.71	4.28	0.33
Fragilaria punctata John	Frapunct	3	5.00	1.99	8.11	0.49
Fragilaria radians (Kützing) Williams & Round	Fraradi	2	1.67	1.88	8.16	1.22
Fragilaria rumpens (Kützing) Carlson	Frarump	1	10.33	1.00	8.50	0.84
Frustulia magaliesmontana Cholnoky	Frumaga	6	36.67	3.27	4.98	0.90
Frustulia rhomboides (Ehrenberg) DeToni	Frurhom	8	10.00	2.79	5.07	0.79
Frustulia rhomboides var. *saxonica* (Ehrenberg) DeToni	Frurhoms	2	3.22	1.57	5.69	1.29
Gomphonema acidoclimatiforme	Gomacid	4	13.00	2.47	5.99	0.42
Gomphonema affine Kützing	Gomaffi	7	12.00	3.26	7.78	1.38
Gomphonema affine var. *insigne* (Gregory) Andrews	Gomaffii	4	10.33	2.39	8.04	0.82
Gomphonema auritum A. Braun	Gomauri	3	2.67	2.67	7.95	0.86
Gomphonema lanceolatum Ehrenberg	Gomlanc	2	1.67	1.98	7.93	0.56
Gomphonema parvulum Kützing	Gomparvu	19	27.00	9.02	7.43	1.19
Gomphonema truncatum Ehrenberg	Gomtrun	2	3.00	1.60	6.51	1.34
Gomphonema truncatum var. *capitatum* (Ehrenberg) Patrick in Patrick & Reimer	Gomtruncc	3	7.67	1.66	8.76	0.74
Gomphonema undulatum Hustedt	Gomundu	3	3.33	1.92	8.33	0.51
Gyrosigma kutzingii (Grunow) Cleve	Gyrokut	1	1.33	1.00	7.71	0.84

Appendix 7.2 continued: List of the 113 taxa present with a maximum abundance of $\geq$ 1% in at least one site. Included are the taxonomic authority and taxon code for each of the 113 species. Further information including the total number of sites in which the taxon occurred (N), the Hill's N_2 Diversity Index, the maximum percentage abundance (Max %) and the pH optima and tolerance are presented for the 109 species included in the final WA transfer function.

Taxon	Code	N	Max %	N_2	Optimum	Tolerance
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	Hippocap	4	4.67	1.87	7.33	0.78
Mastogloia elliptica var. *dansei* (Thwaites) Cleve	Masellda	1	1.00	1.00	7.93	0.84
Navicula aff. *cari* Ehrenberg	Navafcar	3	65.33	1.68	3.35	0.93
Navicula aff. *subtilissima* Cleve	Navafsub	3	13.87	1.19	5.40	1.14
Navicula cincta (Ehrenberg) Ralfs	Navcinct	2	6.67	1.47	6.48	0.48
Navicula comoides (Ag.) Peragallo	Navcom	1	0.67	1.00	6.04	0.84
Navicula cryptocephala Kützing	Navcrypt	14	14.67	7.39	7.92	0.75
Navicula radiosa Kützing	Navradio	4	5.00	2.40	6.93	0.48
Navicula subrhynchocephala Hustedt	Navsubrh	3	1.00	2.58	7.36	0.56
Navicula symmetrica Patrick	Navsymm	1	1.00	1.00	8.23	0.84
Neidium productum (W. Smith) Grunow	Neiprod			NA		
Nitzschia aff. *commutata* Grunow	Nitafcom	1	1.00	1.00	7.08	0.84
Nitzschia amphibia Grunow	Nitamph	8	9.00	3.28	7.91	0.35
Nitzschia frustulum (Kützing) Grunow	Nitfrust	1	68.33	1.00	9.06	0.84
Nitzschia gracilis Hantzsch	Nitgrac	6	4.67	3.20	7.42	0.74
Nitzschia obtusa W. Smith	Nitobtus	5	5.33	1.82	6.30	0.67
Nitzschia palea (Kützing) W. Smith	Nitpal	14	37.00	5.05	7.38	1.52
Nitzschia palea var. 1	Nitpal1	1	1.33	1.00	8.50	0.84
Nitzschia palea var. 2	Nitpal2	2	10.67	1.42	8.29	0.24
Nitzschia palea var. *tenuirostris* Grunow	Nitpalet	1	2.00	1.00	8.50	0.84
Nitzschia paleacea Grunow	Nitpalc	2	15.00	1.09	8.18	0.86
Nitzschia paleaeformis Hustedt	Nitpalf	7	76.33	3.22	4.24	0.64
Nitzschia scalpelliformis (Grunow) Grunow	Nitscalp	1	1.00	1.00	6.04	0.84
Nitzschia sp. 2	Nitsp2	3	6.00	2.23	3.51	0.65
Nitzschia sp. 3	Nitsp3			NA		
Nitzschia sp. 4	Nitsp4	4	7.00	2.48	4.86	0.84
Nitzschia subinflata Hustedt	Nitsubin	1	1.33	1.00	9.06	0.84
Pinnularia borealis Ehrenberg	Pinbore	2	2.00	1.80	6.09	0.11
Pinnularia braunii var. amphicephala (A. Mayer) Hustedt	Pinbramp			NA		
Pinnularia divergentissima (Grunow) Cleve	Pindiver	7	11.67	3.98	4.62	0.88
Pinnularia gibba Ehrenberg	Pingib	1	0.33	1.00	7.71	0.84
Pinnularia microstauron (Ehrenberg) Cleve	Pinmicro	6	24.00	3.64	4.93	1.01
Pinnularia obscura Krasske	Pinobsc	3	27.33	1.22	3.40	1.96
Pinnularia subcapitata Gregory	Pinsubc	9	4.00	6.93	5.51	0.97
Pinnularia viridis (Nitzsch) Ehrenberg	Pinviri	2	3.00	1.22	5.85	1.33
Placoneis elginensis (Gregory) EJ Cox	Placelg	4	10.67	1.32	7.57	0.81
Planothidium lanceolatum (Brébisson) Round & Bukhtiyarova	Planlan	4	1.67	3.13	8.16	1.12
Pleurosigma elongatum W. Smith	Pleuelo	1	1.67	1.00	6.94	0.84
Pseudostaurosira brevistriata (Grunow in Van Heurch) DM Williams & Round	Pseubre	8	46.18	4.39	7.71	0.45
Rhopalodia gibberula (Ehrenberg.) O.Müller	Rhogibbe	2	3.00	1.22	6.10	0.44
Sellaphora pupula (Kützing) Mereschk.	Selpupul	7	30.67	3.84	7.21	0.44
Stauroneis dubitabilis Hustedt	Stadubi			NA		
Stauroneis legleri Hustedt	Staleg	1	3.33	1.00	4.59	0.84
Stauroneis legumen	Stalegu	4	9.00	2.51	4.77	0.86
Stauroneis pachycephala Cleve	Stapach	2	3.33	1.20	6.70	0.30
Staurosira construens var. venter (Ehrenberg) Hamilton	Stasconv	11	66.33	7.17	7.59	0.62
Surirella tenera Gregory	Surtene	3	21.67	1.37	4.59	0.41
Synedra ulna (Nitzsch) Ehrenberg	Synulna	5	19.67	3.07	8.04	0.78
Tabellaria flocculosa (Roth) Kützing	Tabflocc	4	0.67	3.77	7.17	1.33
Tabularia tabulata (Agardh) Snoeijs	Tabutabu	3	12.33	1.72	7.57	0.40
Thalassiosira weissflogii (Grunow) Fryxell and Hasle	Thalweis	2	4.00	1.17	7.70	0.09
Tryblionella hungarica (Grunow) DG Mann	Trybhung	2	2.67	1.25	8.91	0.95

Chapter 8: Conclusion

8.1 Synthesis

A number of wetlands in the south-west of Western Australia have been impacted by acidification, while others are considered susceptible (Environmental Protection Authority 2006), justifying the development of suitable monitoring tools for evaluating the impacts of acidification. This thesis focused on the use of invertebrates and diatoms in a biological monitoring role primarily aimed at assessing their potential as indicators of pH decline. The objectives of the study were addressed through the examination of the community structure and environmental variables of 20 wetlands. Previous studies have identified season as a factor influencing invertebrate community structure (Bunn *et al.* 1986; Balla and Davis 1995) and sampling was therefore undertaken in three periods. In addition, data from a further 20 sites were amalgamated with the data from the spring sampling of the 20 seasonal sites to create a training set for the development of a diatom-based pH inference model.

The investigation of the environmental variables measured in conjunction with invertebrate and diatom sampling (Chapter 3) revealed a wide range of values for most parameters. This was probably attributable to the seasonal sampling regime (Schmidt and Rosich 1993; Kinnear and Garnett 1999) and the heterogeneity of the study sites in terms of size, geology, hydrology and surrounding land-use. The variable of electrical conductivity displayed a consistent trend with comparatively higher mean and maximum values in the acidic Group 1 wetlands. These values were probably mostly attributable to the inclusion of sites associated with mining (Kelly 1988) and shallow seasonal sites impacted by the oxidation of acid sulphate soils (McHugh 2004). The presence of organic acids may also have contributed to the higher conductance of Group 1 wetlands (Boulton and Brock 1999). The results suggest that factors such as electrical conductivity are likely to be elevated in acidifying water bodies and highlight the potential influence of factors other than pH on community structure.

The seasonal sampling regime revealed some variation in the environmental parameters of individual sites as well as within pH groups. Despite this, temperature, a variable known to be influenced by season (ANZECC and ARMCANZ 2000) was the only parameter to display a strong pattern of seasonal variation across the pH groups. The environmental parameters explaining the largest amounts of variation in the multivariate analyses differed between seasons.

Examination of invertebrate community structure over the three sampling periods (Chapter 4) determined that pH influenced invertebrate distribution, a finding consistent with previous studies (Simpson *et al.* 1985; Courtney and Clements 1998; Woodcock *et al.* 2005). The acidic Group 1 and alkaline Group 3 wetlands displayed significantly different assemblages and differences albeit smaller, were also detected between the circumneutral and alkaline group. In contrast, the community compositions of the acidic and circumneutral groups were not significantly different, implying that a reasonable number of taxa from these sites occurred across the two pH ranges. It has been suggested that macroinvertebrate families in Western Australia are probably tolerant of low pH (Kay *et al.* 2000) and this may partly explain the number of invertebrates able to inhabit both acidic and circumneutral water bodies.

Taxa which exhibit narrow and specific tolerances are generally considered ideal indicators (Abel 1989). This was supported by the current study which identified *Macrothrix indistincta* and *Alona quadrangularis* as potential indicators of acidic and circumneutral waters respectively, based on their restricted pH tolerances. However, even species with wider tolerances may have applications as biological indicators of acidification. For example, *Calamoecia tasmanica subattenuata* was commonly recorded from acidic and circumneutral wetlands in the current study and could potentially act as an indicator of pH decline in neutral to alkaline waters.

The results of the study did however suggest that pH was not the sole factor governing invertebrate distribution. While pH was found to be the most closely correlated single variable, the strongest correlations between biotic patterns and environmental variables were generally achieved through a combination of parameters. It is difficult to speculate on the importance of these variables however,

as the low number of sites in comparison to the number of variables may have influenced the reliability of the results (Stevens 1992). The seasonal nature of the sampling regime did not appear to have a substantial impact on the sensitivity of invertebrates to pH and therefore the group could be used to monitor the ecological impacts of pH in various seasons.

The variable of pH was also found to influence diatom community structure (Chapter 5), corresponding with previous studies including John (1993), Kwandrans (1993) and Battarbee *et al.,* (1997). Significant differences in community composition were evident between each of the three pH groups for two of the three sampling periods. The acidic and alkaline water bodies displayed the most divergent assemblages while differences were less obvious for the circumneutral group. The study identified a number of species with potential to act as indicators of pH change. *Nitzschia paleaeformis, Brachysira brebissonii* and *Frustulia magaliesmontana* were mostly abundant in the Group 1 wetlands and could be considered potential indicators of acidification. The common species in the Group 3 wetlands were generally less specific in their pH preferences than the common species in the acidic wetlands. Nonetheless, species including *Gomphonema parvulum, Staurosira construens* var. *venter* and *Nitzschia palea* may have applications as indicators of moderate to alkaline pH levels.

There were some seasonal differences apparent in the diatom data (Chapter 5) but multivariate analysis (Chapter 6) demonstrated that these differences were not significant. Furthermore, the variable of pH was found to be an important influence in the community structure of diatoms in all seasons.

Comparisons of the amalgamated seasonal data-sets of invertebrates and diatoms (Chapter 6) indicated that diatoms were more responsive to pH than invertebrates. These results are in accordance with the findings of previous studies which have suggested that diatoms are more sensitive to water quality than invertebrates (Chessman *et al.* 1999; Newall *et al.* 2006). Physical variables were not focused upon in the current study and the inclusion of these variables in future research would help clarify the factors governing invertebrate distribution. It does however seem likely that an integrated monitoring program including both invertebrates and

diatoms would provide a more comprehensive overview of the integrity of the aquatic systems.

Diatom-based transfer functions for pH have been developed for various areas of the world including the Swan Coastal Plain in south-western Australia (Gasse *et al.* 1995; Vyverman *et al.* 1996; Dixit *et al.* 1999; Enache and Prairie 2002; McHugh 2004; Kilroy *et al.* 2006). The association between diatoms and pH demonstrated in the current study (Chapters 5 and 6) suggested that a pH inference model could be developed for a larger area of the south-west. Multivariate based exploration of the relationship between the environmental variables and diatom community structure of the 40 model sites confirmed this with pH accounting for a large proportion of the variation in the data. The estimated pH optima and tolerances generated by the model enabled the identification of potential indicator species. For example, *Brachysira brebissonii*, *Nitzschia paleaeformis* and *Frustulia magaliesmontana* displayed estimated optima of < 5.00 and tolerances within the acidic pH classification, suggesting they may have potential applications as indicators of low pH.

The current transfer function could potentially be used in pH reconstructions or programs monitoring the ecological impacts of acidification in the south-west of Western Australia. Use of the current model or a model derived from an integrated data-set in either capacity would aid in the monitoring and management of impacted or threatened wetlands in the region.

8.2 Conclusions and recommendations for future research

This research has provided valuable knowledge on the response of invertebrate and diatom community structure to pH in water bodies in the south-west of Western Australia. The insight gained from the study can now be translated to monitoring programs aiming to assess the ecological impacts of acidification. In addition, the study has provided a basis for further research, leading to an even greater understanding of invertebrates and diatoms as tools for the biological monitoring of pH in the south-west region. Accordingly, a number of recommendations have been outlined below.

The seasonal sampling regime of the current study prevented the inclusion of a large number of sites. While trends were evident between the biotic groups and pH, the relatively small sample sizes probably lead to reduced statistical power and an increased chance of Type II errors (Stevens 1992). Future studies in this area would be likely to benefit from a larger data-set and increased statistical robustness. Moreover, a larger data-set might facilitate the investigation of additional pH classifications, namely extremely acidic and extremely alkaline. Future research should also include a larger number of circumneutral sites to provide greater understanding of the biotic communities inhabiting this pH type. It is important to note that the circumneutral classification is less stable than the acidic and alkaline classification, probably as a result of the smaller pH range. Future studies encompassing different regions of the south-west would also benefit from the inclusion of wetlands with an even spread of pH.

Factors such as nutrient concentrations and colour are known to influence biotic community structure (Cheal *et al.* 1993; Soininen 2002; Wunsam *et al.* 2002; Tibby 2004; Chambers *et al.* 2006). The inclusion of variables such as these in future monitoring programs would help clarify the extent to which pH influences community structure. This would in turn assist with identifying potential indicator species for pH. Additionally, while pH is the principal factor linked with acidification (Battarbee *et al.* 1999) it is recommended that the measurement of other related variables including sulphate concentration, alkalinity, Al concentration and the total of all anions and cations (Thomas *et al.* 1992; Sommer and Horwitz 2001) be included in future research.

The current study included water bodies acidified through a number of different mechanisms. Passy (2006) has suggested that the origin of acidity (e.g. organic versus inorganic) may be of similar importance to the pH decline in terms of community composition. An expanded data-set focusing on sites acidified by sources including humic acids, acid mine drainage and the oxidation of acid sulphate soils would allow further research into the relevance of this topic for waters of the south-west of Western Australia.

A study on aquatic fauna by Trayler *et al.,* (1996) noted that a lack of historical or baseline data restricts the ability to assess the impacts of anthropogenic disturbances. In the case of diatoms, the use of the pH inference model generated by the current study provides the capacity to compile valuable historical information on sites potentially at risk of acidification. With an appropriate training set, models for other parameters could be generated and used for a similar purpose.

Aside from the applications in palaeolimnological studies, the diatom-based pH inference model could also be integrated into monitoring programs for surface waters. It is however recommended that the data be amalgamated into a larger training set with a more even spread of sites along the pH gradient to increase the predictive capabilities of the model and provide more accurate estimations of the pH optima and tolerances of diatom taxa.

The key recommendation to emerge from this study is the value of using biological monitoring as part of an integrated program for assessing the impacts of pH change. Of the two biotic groups included in this study, diatoms were identified as the most responsive to pH and would be the principle organisms recommended for use in this capacity. Declining rainfall associated with climate change (CSIRO 2001) is likely to lead to the increased exposure of sulphidic soils and the acidification of more wetlands. As a result, diatom monitoring will become more relevant. It is important to note however that invertebrates may still provide valuable information in regard to pH change, as shown by this research. Given the potentially serious impacts of acidification on wetlands in the south-west of Western Australia, particularly in terms of biodiversity, it is likely that a comprehensive monitoring program including both diatoms and invertebrates would be desirable. An integrated monitoring program such as this would provide an early indication of the effects of pH decline, reflect any changes in the overall integrity of the systems and would assist in conservation and restoration efforts.

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