

**Division of Science and Engineering  
Department of Environmental Biology**

**Diatom Communities in Lakes and Streams of Varying Salinity  
from South-West Western Australia: Distribution and Predictability**

**Fiona E Taukulis**

**This thesis is presented for the Degree of  
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## **Declaration**

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment 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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## Abstract

The distribution pattern of diatoms from lakes and streams of varying salinity in the south-west of Western Australia was investigated. A total of 95 water bodies were sampled and separated into freshwater (<3 ppt), hyposaline (3-20 ppt), mesosaline (20-50 ppt) and hypersaline ( $\geq 50$  ppt). The south-west and specifically the inland wheatbelt region has been severely influenced by secondary salinisation, due to clearing of native vegetation for agriculture. There has been little research on diatom communities from salt-affected systems, with this data providing the basis for the development of an inference model based on species optima and tolerance limits to salinity.

Physico-chemical variables measured from the study sites were collated and assessed. Salinity ranged from freshwater (0.04) to hypersaline (156.80 ppt), and pH ranged from acidic (2.90) to alkaline (10.51). Dissolved oxygen levels were recorded from 1.11 to 18.67 mgL<sup>-1</sup>, water temperature from 6.30 to 28.10 °C and peripheral vegetation scores from 1 (little or no cover) to 5 (dense cover). Analysis of variance (ANOVA) showed that salinities were significantly higher in standing waters located further inland, compared to flowing waters in high rainfall areas. Hypersaline wetlands displayed significantly lower dissolved oxygen levels, higher water temperatures and reduced peripheral vegetation, compared to freshwaters. The pH of hypersaline sites was also significantly lower, associated with surrounding land use or underlying geology. The data collected provides important baseline information, with implications for aquatic biota.

The community structure of diatoms in relation to varying salinity concentration was explored. An artificial substrate collector (JJ periphytometer) was used to standardise sampling and ensure diatom assemblages were representative of ambient water quality. A total of 217 taxa were identified with the highest diversity observed in freshwater sites (up to 33 species), and limited to less than 15 in hypersaline waters. According to BIOENV, salinity was the key factor influencing diatom community structure. SIMPER analysis found a number of discriminating taxa between salinity ranges, specifically between assemblages from freshwater and hypersaline sites. Taxa such as *Achnantheidium minutissimum* and *Gomphonema parvulum* were

indicative of freshwaters. In comparison, *Amphora coffeaeformis* and *Nitzschia ovalis* were associated with hypersaline water bodies.

Diatom community structure was also examined from 20 hypersaline wetlands in the wheatbelt region with varying pH. Characteristic taxa including *Amphora coffeaeformis*, *Hantzschia* sp. aff. *baltica* and *Nitzschia ovalis* showed a wide tolerance to salinity and pH, or hypersaline acidic conditions. BIOENV analysis found there were no observable differences between diatom assemblages in relation to salinities above 50 ppt and that pH was highly correlated to species composition. The increasing occurrence of acid saline lakes is of concern and is most likely attributable to deep drainage practices and continued use of fertilisers in agricultural areas.

A diatom-based transfer function was developed from the south-west dataset, to document species optima and tolerance limits to salinity. CCA analysis showed that salinity accounted for a significant and independent amount of variation in the diatom data enabling an inference model to be derived. The most successful model was generated using tolerance-downweighted weighted averaging, with a high coefficient of determination and low prediction errors that remained high after jackknifing. The optima of freshwater diatoms were similar to those reported from other regions of the world, although the optima of hypersaline species tended to be higher. Comparatively, the model performed very well, with the potential to be applied in future paleolimnological studies.

In conclusion, this study has shown diatoms to be effective biomonitoring tools, providing the basis for future sampling strategies that assess the biodiversity of salt-affected water bodies in Western Australia. Potential indicator species from different salinity ranges were identified and the sensitivity of diatoms allowed for the development of a statistically robust inference model for the south-west. The reported optima and tolerance limits of important taxa may be further explored to evaluate the success of remediation measures implemented for secondary salinised systems in this region.

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## Chapter 1: General Introduction

### 1.1 Scope and Structure of Thesis

The south-west of Western Australia is considered to be a global hotspot for biodiversity that is currently under threat from secondary salinisation (Halse *et al.* 2003). Specifically, the increasing salinity of lakes and streams in this region is an important environmental issue (Pigram 1986; Alexandra and Eyre 1993; Ghassemi *et al.* 1995), with inland waters playing an integral role in maintaining biodiversity and supporting ecological processes (Williams 1999; National Land and Water Resources Audit 2001). Excessive salinisation results in the collapse of aquatic ecosystems, causing the death of peripheral vegetation, habitat loss, decline in populations of plants and animals and increasing the risk of species extinction. In addition, these water bodies can also become more susceptible to erosion, sedimentation and altered flood regimes. Therefore, monitoring programs consisting of physico-chemical and biological assessments are essential, to determine both current and long-term impacts (Halse *et al.* 2003; Environmental Protection Authority 2006).

Diatoms are well-recognised as effective and sensitive biomonitoring organisms (John 2007), although there is limited research available on salt-affected waters from the south-west. Studies by John (1998), Blinn *et al.* (2004), and most recently Taukulis and John (2006), have been restricted, focussing on a particular geographic area or water body type, such as river systems. This thesis investigated the distribution pattern of diatoms, in relation to salinity, from both lakes and streams ranging from freshwater (<3 ppt) to hypersaline ( $\geq 50$  ppt). The research provides comprehensive baseline data on many water bodies that previously have not been examined. Diatom taxa were identified and species optima and tolerance limits to salinity have been reported, the first calibration dataset to be established for this region. These findings prove that diatoms are valuable indicators of salinity that may be used in future aquatic biomonitoring programs.

This thesis has been organised into separate chapters, where salinity throughout the south-west is the common theme, with references and appendices for each chapter. Chapter 1 presents a literature review on key topics including human-induced salinisation of water bodies, the biota of saline lakes and the use of diatoms as biomonitors of aquatic systems. Chapter 2 describes the geographical location of study sites with general comments about the monitoring locations indicative of varying salinities.

Chapter 3 investigates the significance of salinity in relation to water quality and peripheral vegetation and contrasts these results to previous data collected from the south-west. Chapter 4 applies multivariate statistical analysis to separate sites based on diatom assemblages and water quality data. Changes to community structure in response to increasing salinity concentration were also examined. The use of scanning electron microscopy (SEM) was a technique employed to identify species indicative of hypersaline conditions, as outlined in Chapter 5. Specifically, the affects of pH and salinity on diatom composition were explored.

Chapter 6 documents the optima and tolerance limits of dominant taxa to salinity for the development of an inference model. The strength and predictive ability of the transfer function was compared to the performance of similar models developed both in Australia and overseas. A general discussion of the major findings and results of the study is provided in Chapter 7, the final chapter of the thesis. In addition, recommendations have been put forward for the integration of this research into future monitoring strategies for lakes and streams influenced by secondary salinisation in Western Australia.

## **1.2 Global Salinisation**

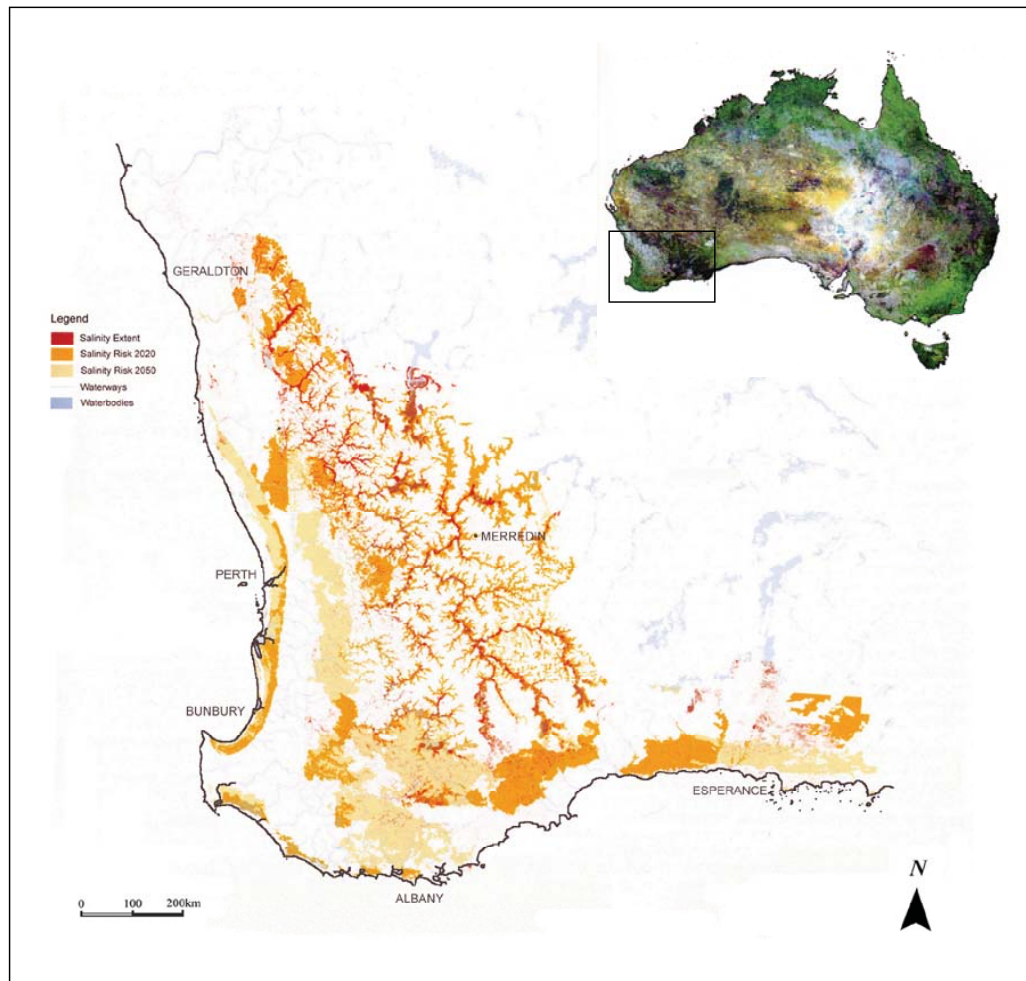
Freshwater is a fundamental resource that is integral to all environmental and societal processes (Gleick 1993). Currently one of the greatest degradation concerns threatening aquatic systems throughout the world is salinity contamination (Meybeck *et al.* 1989; Williams 1999). The term salinisation refers to an increase in the total concentration of dissolved solids in soil or water due to natural processes (primary

salinisation) or human activities (secondary salinisation) (Ghassemi *et al.* 1995). Primary salinisation of lakes and streams can be attributed to processes such as the accumulation of airborne sea-salt, evaporation, and the dissolution of minerals (Hammer 1986; Meybeck *et al.* 1989). In comparison, the cause of secondary salinisation varies depending on the type of land use. In countries such as South Africa, India and China, intensive irrigation and mining activities have led to saline run-off influencing major rivers and tributaries (Salama *et al.* 1999; Fitzpatrick 2002). Agricultural practices have also contributed substantially to salt loads in water bodies from the United States, Argentina and Australia (Chhabra 1996; Williams 2001). In particular, Australia is one of the most well-recognised countries associated with the secondary salinisation, a considerable resource management issue that has been extensively documented (Smith and Finlayson 1988; Alexandra and Eyre 1993; Smith 1998; Pigram 2006).

### **1.3 Salinisation in South-West Western Australia**

Australia is an ancient landmass and primary salinity is prevalent, especially in the arid interior of the continent, where chains of naturally occurring salt lakes are common (Williamson 1990; Ghassemi *et al.* 1995; Chhabra 1996). Secondary salinisation of water bodies has developed since the arrival of Europeans, with the implementation of irrigated and dryland farming methods (Farrington *et al.* 1993). Over 70% of Australia's dryland salinity occurs in the south-west of Western Australia (**Figure 1.1**) and in particular the wheatbelt or inland agricultural zone has been severely impacted (Halse *et al.* 2003; Environmental Protection Authority 2006). Currently, an estimated 1520 km of streams and 72 000 ha of wetland areas are at risk of salinisation in this region, figures that are expected to increase to 2850 km and 80 000 ha by 2050 (National Land and Water Resources Audit 2001).

The severity of the problem is related to the extensive clearing of native vegetation, and its replacement with annual crops and pastures, that use less water (Williamson 1990; Alexandra and Eyre 1993; Ghassemi *et al.* 1995). This alters the hydrological balance of the land and has allowed water that was previously being utilised by deep-rooted plants to enter the groundwater and mobilise salts that are stored in subsoils (Frost *et al.* 2001; Environmental Protection Authority 2006).



**Figure 1.1:** The extent of salt-affected land in south-west Western Australia in 2000 and projected areas at risk of salinisation in 2020 and 2050. Source (Australian State of the Environment Committee 2001; Environmental Protection Authority 2006).



Watertables then rise at a much faster rate than would otherwise occur, bringing seasonally dissolved salts to the surface, producing saline seepage and salt scalds (Stoneman 1976; Hartley and De Vries 1983; Short and McConnell 2001). Subsequent overland and subsurface flow of this saline water into nearby river systems and wetlands has led to increased salinity concentrations (Schofield *et al.* 1988; Davis *et al.* 2003).

The source of salts present in water bodies throughout Western Australia are believed to have originated from the Indian Ocean, having been transported inland via wind and rainfall (Hughes *et al.* 1982; Schofield *et al.* 1988; Davis *et al.* 2003). Over time, these salts have accumulated in the soil profile, reaching an ionic equilibrium that was altered when land clearing regimes began (Hillman 1981). The salinisation of lakes and streams is particularly acute in the wheatbelt region of the state, which is characterised by gently undulating topography and relatively low rainfall (Burvill 1979; Beard 1990). In most catchment areas, the level of threat posed to aquatic systems is largely dependent on these factors, which have the potential to increase salinisation rates (Coram *et al.* 2001; Halse *et al.* 2003).

### *1.3.1 Rainfall and climate*

Western Australia covers an expansive area of 2.52 million square kilometres, over which conditions vary significantly (Gentilli 1979). The south-west region experiences a Mediterranean climate of mild to cool winters with moderate rainfall, and warm to hot dry summers (Burvill 1979; Tinley 1986; Beard 1990; Jaensch and Lane 1992). Rainfall in the far corner of the state is relatively consistent, ranging from 750 mm up to 1500 mm per year. In comparison the wheatbelt has been defined as an area that receives between 300 and 600 mm annually (Bureau of Meteorology *et al.* 1995; Cale *et al.* 2004). Although these figures are considered to be reliable, droughts are known to occur, a factor that can strongly affect the hydrology of standing and flowing waters.

The dry climate experienced in many agricultural areas has compounded salinity problems with high temperatures increasing evaporation rates within catchment zones (Cramer and Hobbs 2002). The low rainfall of inland regions, together with

the internal drainage patterns associated with lakes and rivers mean that salts are retained in the system, with very little flushing occurring (Stephens 1961; Clarke *et al.* 2002). Soil characteristics and topography play an important role in the movement of this salt through subsoils and groundwater, which also affects the ionic equilibrium of water bodies (Pigram 1986).

### *1.3.2 Soils and topography*

Soils representative of saline areas in the wheatbelt of Western Australia are referred to as saline loams and usually begin at the margin of salt pans or depressions. Saline soils are followed by red loams on slopes which can be heavy at first with high proportions of clay that eventually become more sandy. On higher ground a widespread arrangement of sandy soils are known to occur overlaying laterite or clay to varying depths (Burvill 1979; Pigram 1986; Beard 1990). The south-west of the state is generally hillier, with river systems forming a large number of complex valleys. The soils of the elevated regions are dominated by ironstone gravels, in contrast to the fertile, low-lying floodplains that surround rivers and streams (Burvill 1979).

The formation of water bodies throughout Western Australia is linked to topography. The landscape has been shaped by the process of erosion, which has occurred over thousands of years leaving behind well-defined drainage lines from ancient river systems. Some of these systems, located in the outer wheatbelt regions have now developed into chains of salt lakes that remain relatively dry and rapidly absorb any run-off (Pilgrim 1979; Bettenay 1984). However, in years of unusually heavy rainfall, flushing will occur producing an organised flow, connecting the lakes through smaller tributaries. The only rivers currently active during these high rainfall periods are situated along a narrow band on the western margin of the wheatbelt. Along the west and south coast of Western Australia where rainfall is more reliable, lakes are common and can remain inundated year round. Rivers in this region also flow steadily in winter and in some areas flow consistently even in drier months (Pilgrim 1979; Beard 1990).

Changes in topography and soil types of specific catchment areas influences the movement of saline water that drains into nearby lakes and streams (Cramer and Hobbs 2002). The flow of groundwater can direct stored salts towards the soil surface or alternatively into nearby water bodies, affecting peripheral vegetation (Hartley and De Vries 1983). One of the most important implications of increased salt loads is the potential reduction in diversity of vegetation, which provides essential habitats for terrestrial and aquatic organisms (Marcar *et al.* 1995).

### 1.3.3 Riparian vegetation

Distinctive vegetative zones surround watercourses and lakes throughout Western Australia. Vegetation typical of freshwater lakes and streams further south in high rainfall areas is highly variable and dependent on soil type as well as inundation period (Waters and Rivers Commission and Department of Conservation and Land Management 1997b). Along the banks of rivers and their tributaries *Eucalyptus rudis* (flooded gum) is generally dominant interspersed with *Melaleuca* species including *M. preissiana* and *M. raphiophylla* (Beard 1990; Waters and Rivers Commission and Department of Conservation and Land Management 1997b). Closer to the waters edge rushes and sedges such as *Lepidosperma tetraquetrum* are common, occurring on seasonally moist or wet sandy loams along watercourses and winter depressions (Beard 1990). A number of species from the *Baumea* genus (for example *B. articulata*) are also widespread along the margins of freshwater lakes and creeklines in the southern regions (Waters and Rivers Commission and Department of Conservation and Land Management 1997b).

In wheatbelt areas, peripheral vegetation is determined predominantly by the salinity of the associated water body. Along the western margin of the wheatbelt region where the Avon River and its tributaries are situated, soils are well drained and support large stands of trees including *Eucalyptus loxophleba* (York gum), *Eucalyptus rudis* (flooded gum) and *Casuarina obesa* (swamp sheoak) which line stream banks (Beard 1990; Bamford 1995). Understorey vegetation is usually composed of *Melaleuca* species such as *M. raphiophylla* or *M. cuticularis* as well as common rush and sedge species from the *Juncus* and *Baumea* genera (Waters and

Rivers Commission and Department of Conservation and Land Management 1997a). Salt depressions occur elsewhere, supporting a unique complex of plants able to tolerate extreme temperatures and high salt levels. These include samphires such as *Halosarcia* (*H. halecnomoides* and *H. indica*) and *Sarcocornia* (*S. blackiana* and *S. quinqueflora*) species, whose shrub-like habit can completely dominate low lying regions (Datsun 2002). *Melaleuca* complexes occur on sandy rises behind samphire communities, with the density of vegetation reflecting the level of disturbance from surrounding land use (Beard 1990).

Currently 18 million hectares of native vegetation has been cleared for agricultural purposes throughout Western Australia (Berry 1997). Few stands of native plant species surrounding lakes and streams affected by salinisation remain. Those that are left are under threat from increasing salinity as well as waterlogging associated with rising groundwater levels (Marcar *et al.* 1995; Cramer and Hobbs 2002; McFarlane and Williamson 2002). The continued loss of riparian vegetation has the ability to further concentrate salts within aquatic systems (Lymbery *et al.* 2003).

#### *1.3.4 Salinisation of flowing and standing waters*

In Western Australia, the rate of secondary salinisation in flowing waters (rivers and streams) compared to standing waters (lakes and wetlands) varies greatly (**Figure 1.2**). Rivers and streams located in high rainfall areas to the south of the state are predicted to experience only moderate increases in salinity, as salts are regularly flushed from the system (Schofield *et al.* 1988). In low rainfall zones however, such as those located in the eastern wheatbelt region, a 50-fold increase in stream salinities is estimated in some catchments (Sadler and Williams 1981; Steering Committee for Research on Land Use and Water Supply 1989).

In lakes and wetlands, the concentration of salts may have a more severe effect due to evaporative processes that occur during dry, summer months (Boulton and Brock 1999). In the south-west of Western Australia, freshwater lakes are becoming saline and naturally occurring salt lakes situated further inland are also showing a steady rise in salinity (Williams 1999; 2001). It is expected that salts will continue to leach

into these systems far in the future causing irreversible changes before a new hydrological equilibrium is reached (Sadler and Williams 1981; Hartley and De Vries 1983). The analysis of the ionic concentration of these water bodies is an important tool that can be used to monitor secondary salinisation.

### *1.3.5 Ionic concentration of water bodies*

The nature of the constituent ions in aquatic systems in Western Australia has a distinct pattern that can be influenced by environmental factors (Swaine and Schneider 1971). The oceanic origin of salts mean that the ionic spectrum generally follows that of seawater, with characteristic displays of cations and anions (Hughes *et al.* 1982; McComb and Lake 1990). Previous studies on lakes and streams in the wheatbelt region showed the dominance of cations: Na>Mg>Ca>K and anion proportions of: Cl>SO<sub>4</sub>>HCO<sub>3</sub>. Sodium values accounted for 75-95 % of the cation equivalence, with chloride contributing to 87-97 % of anion equivalence (Geddes *et al.* 1981). The dominance of sodium and chloride is typical of wetlands and rivers in the south-west, with concentrations of these ions increasing with elevated salinity levels (Hughes *et al.* 1982; Williams 1983).

The most exact way to determine the salinity of a given water body is to sum the measured quantities of the individual ions, a time consuming and tedious method (Schofield *et al.* 1988; Williams 1998a). However, other surrogate measures of salinity can be employed, with the most accurate being those expressed on the basis of mass of total ions per mass of solution (gkg<sup>-1</sup>, ppt), or as the mass of total ions per volume of solution (gL<sup>-1</sup>). Differences between these units are negligible in freshwaters or at low salinities, and are essentially interchangeable. At higher concentrations though, this is not possible due to the density of concentrated salts in solution. Generally it is preferable to present salinity in ppt or gkg<sup>-1</sup>, however gL<sup>-1</sup> tends to be more widely used, having been incorporated into various classification systems throughout the world (Williams 1998a; Boulton and Brock 1999).



**Figure 1.2:** Salinisation of flowing and standing waters from inland areas of southwest Western Australia. Flowing waters: (a) Avon River, (b) Mackie River, (c) Moore River and (d) Mortlock River. Standing waters: (e) Kevills Lake, (f) Lake Wallambin, (g) Nomans Lake and (h) Yenyening Lakes.

A number of classification regimes that divide inland saline waters into distinct categories can be found in the literature (cited in Hammer 1986), including those by Kolbe (1927), Beadle (1943), Williams (1964), Fan (1981) and Hammer (1983). Freshwaters are accepted as those with a salinity of less than  $3 \text{ gL}^{-1}$ , an arbitrary value put forward by Williams in 1964. Hammer (1986) separates saline waters into hyposaline ( $3\text{-}20 \text{ gL}^{-1}$ ), mesosaline ( $20\text{-}50 \text{ gL}^{-1}$ ) and hypersaline (greater than or equal to  $50 \text{ gL}^{-1}$ ), with a suggestion that an extreme hypersalinity category may also be justified. In terms of the response of aquatic biota, it is obvious that not all species will fit precisely into the boundaries of any classification system, and that some degree of overlap will always occur (Hammer 1986).

### 1.3.6 Biodiversity and salinity

The concept of biodiversity in lakes and streams is related to salinity as well as other environmental factors including human interference (Nelson *et al.* 2003). In freshwaters ( $<3$  ppt) all major groups of biota are generally represented, however species diversity can be affected by variables such as pH and nutrient levels (Williams 1998b). In saline waters reduced species richness is typically observed with less taxonomic complexity in response to increasing salinity concentrations (Borowitzka 1981; McComb and Lake 1990; Brendonck and Williams 2000).

The hostile conditions presented by hypersaline lakes ( $\geq 50$  ppt) mean that only a small number of organisms can survive, with specific morphological adaptations for growth and reproduction (Larsen 1980). A range of microorganisms such as bacteria (*Halobacterium*) and algae (*Dunaliella*) inhabit saline environments, and certain larger macrophytes (*Ruppia*) can also tolerate increasing salt loads (Borowitzka 1981; Brock 1981; Geddes *et al.* 1981). A number of invertebrates from the Crustacean (*Parartemia*) and Gastropod (*Coxiella*) groups are common, however larger aquatic fauna (for example fish) are generally absent from concentrations in excess of 100 ppt (Geddes *et al.* 1981; Williams 1981). Although many inland salt lakes are associated with reduced biodiversity, they contain many important species that have specific functions, providing intrinsic ecological value (Williams 1998a).

The conservation of lakes and streams throughout Western Australia has become a key resource management issue (Bresler 1981; Loh and Stokes 1981). Both freshwater and saline environments have a unique array of biodiversity, much of which remains undescribed in the scientific literature (Halse 1998). The Western Australian Government has launched a number of documented strategies to address concerns relating to salinity and the conservation of aquatic flora and fauna (Government of Western Australia 1996; State Salinity Council 1998; 2000). These reports detail the use of biological monitoring organisms as part of an integrated approach to the salinisation problem.

#### **1.4 Biological Monitoring of Aquatic Systems**

Biological monitoring refers to the use of biota to detect changes in the water quality of lakes and streams (Cairns and Pratt 1993; Davis and Christidis 1999). As aquatic organisms integrate the effects of their surroundings they can reflect the total state of the environment (Gower 1980). Biomonitoring is often used, as chemical analyses alone tend to lack the responsiveness necessary to assess the overall health of a water body (John 2003). Measurements obtained even at frequent intervals can miss significant variations that may be detected by observing the diversity of aquatic organisms (Abel 1989; Loeb 1994). However, the incorporation of biological and physico-chemical information is essential to produce a complete view of system dynamics (Friedrich *et al.* 1992; Meybeck *et al.* 1992). Regular collection of data using both biomonitors and water quality variables provides the basis for the development of effective rehabilitation programs (Gower 1980).

Indicator species that can be used for these purposes range from microorganisms such as bacteria and algae to larger groups of biota including macroinvertebrates and fish (Ghetti and Ravera 1994). The choice of biomonitor should be based on the individual requirements of the sampling regime as well as the overall aim of the study (Norris and Norris 1995). For example, the use of bacteria could be employed to assess the potability of water supplies for human consumption, where as macroinvertebrate communities may be sampled to determine the impact of mine effluent on nearby waterways (Dills and Rogers 1974; Gower 1980). Each group of

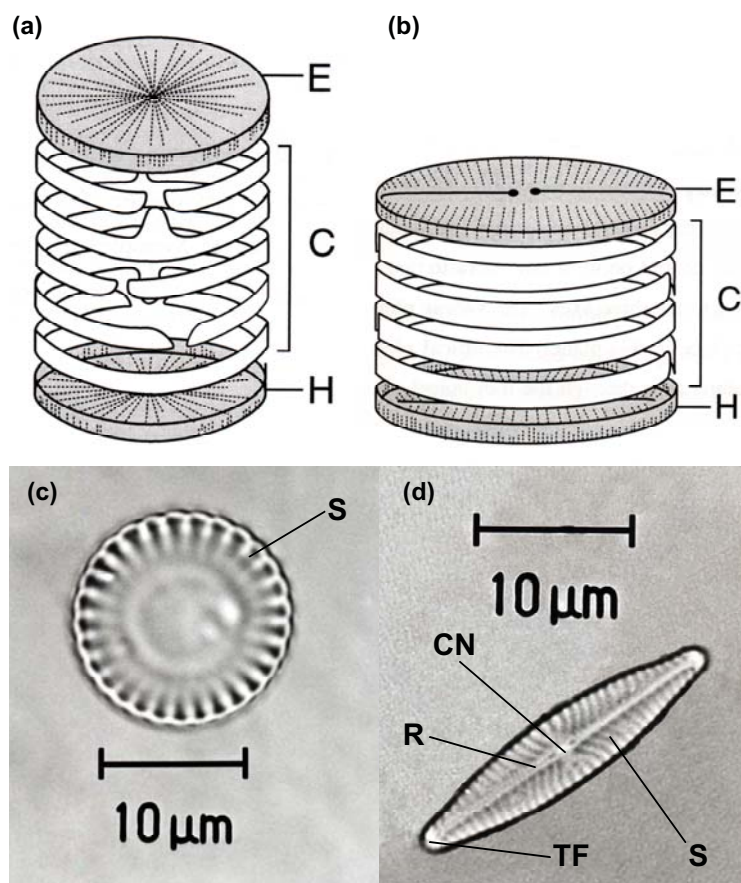


organisms has their own specific merits for detecting changes in water quality conditions, however one of the most successful and commonly used, is the unicellular microalgae diatoms (John 2000).

#### *1.4.1 Diatoms as biological monitors*

Diatoms belong to the Division Bacillariophyta and have a cosmopolitan distribution in almost all aquatic environments (Cox 1991; Round 1991; Schofield and Davies 1996; Stevenson and Pan 1999). Currently there are over 900 validly published genera (Fourtanier and Kociolek 1999), that may represent more than 100 000 species (Round *et al.* 1990). Their most prominent morphological feature is a silicious cell wall, referred to as a frustule (**Figure 1.3**). It consists of two overlapping halves called a hypotheca and epitheca (upper and lower valves), connected by a cingulum made of thin girdle bands (John 2000). Fine markings called striae are a noticeable feature on the cell wall, forming distinct patterns that are considered unique to each species (Battarbee *et al.* 2001; John 2003). A comprehensive discussion on diatom biology and systematics has been presented in Round (1990).

As important primary producers in most aquatic systems, diatoms are close to the site of nutrient and energy transfer, showing changes in water quality before higher trophic levels (Patrick 1970; 1994). They have also shown to be more specific in their preference and tolerance of environmental conditions when compared to other biota (John 1998). Their sensitivity is such, that even slight variations in the physical and chemical environment of a water body will be reflected by a shift in species dominance (Rott 1991; Round 1991; Stevenson and Pan 1999).



**Figure 1.3:** Diatom frustule structure. (a) Centric and (b) pennate diatoms, where E-epitheca, C-cingulum with girdle bands and H-hypotheca. Source (John 2000). Light microscopy view ( $\times 1000$ ) of diatoms. (c) Centric and (d) pennate diatoms, where CN-central nodule (thickened with silica), R-raphe (longitudinal groove), S-striae (pore) and TF-terminal fissure (external ending of raphe).

Diatoms also have the advantage that samples can be permanently prepared, enabling archiving for future reference and comparative purposes over extended periods of time (Cox 1991; Stevenson and Pan 1999; John 2003). This attribute means that the morphology of individual species has been extensively studied and is well-documented (Whitton 1991). Taxonomic research is continuing to evolve and recent significant changes have eased identification problems with the intention of promoting the use of this microalgae to a wider audience (John 2000).

Early monitoring studies by Patrick in the 1950s established the effectiveness of diatoms in assessing the overall health of aquatic systems and provided stimulus for similar research around the world (John 1998). Recent papers have documented the response of diatom assemblages to changes in water quality such as acidification (Vinebrooke and Graham 1997; Hinderer *et al.* 1998), eutrophication (Sonneman *et al.* 2001; Soininen 2002) and salinisation (Clavero *et al.* 2000; Blinn and Bailey 2001). The tolerance limits of different species can be used to provide an important diagnostic tool for the past and present assessment of water bodies affected by problems such as increasing salt loads.

#### *1.4.2 Diatoms and salinity*

Extensive research has been carried out to investigate the response of diatom community structure to varying salinities, which led to the development of the halobion preference system by Kolbe (1927) (cited in Hammer 1986), which was later modified by Hustedt (1953) (cited in Fritz *et al.* 1999). This system classifies diatoms according to their ecological tolerances to changing salinity concentration. For example, species indicative of saline conditions can be referred to as polyhalobous species, whereas those that can tolerate a wide range of salinities are known as oligohalobous (Foged 1978). It is important to note that this regime was generated primarily for marine environments, and should therefore be applied only to water bodies that show the characteristic ionic composition of seawater (Fritz *et al.* 1999).

The use of diatoms as indicators of salinity in flowing waters (including streams, estuaries and rivers) has been well-documented (Main and McIntire 1974; Ehrlich and Ortal 1979; Daemen *et al.* 1982; Snoeijs 1999). Saline species have been shown to dominate estuarine systems when salt intrusions occur, usually during summer months (Sullivan 1982; John 1988). Studies carried out by Blinn and Bailey (2001) established that increasing salt loads in streams led to a decrease in the diversity of diatom assemblages. Changes in salinity concentration have also been responsible for determining community structure in rivers and tributaries (van der Molen 1998; Potapova and Charles 2003).

Research into the tolerance limits of diatom species identified from inland saline lakes and wetlands has been widely published (Sullivan and Moncreiff 1988; Ehrlich 1995; Gabellone *et al.* 2001). Daemen *et al.* (1982) and Blinn (1993) found species diversity was relatively high in the lower salinity ranges. Clavero *et al.* (2000) and Gell (2002) demonstrated similar results showing that taxa numbers became limited as salt loads increased due to evaporative processes. Studies by Compère (1994) documented the affect of extreme salinity levels on assemblages in lakes and wetlands, with only a few diatoms able to survive the hostile conditions.

The use of fossil diatom records to reconstruct lake history and salinity trends is also a common field of research (Fontes *et al.* 1985; Gell 1998; Sherrod 1999; Taffs 2001; Gasse 2002; Zalat 2003). Changes in the salinity concentration of a water body over time are associated with shifts in dominant species (Wasell 1992). For example, Radle *et al.* (1989) discovered a transition from fresh to saline taxa when reconstructing lake sedimentary records. In comparison, analysis of sediments by Flower *et al.* (2001) showed that over time diatom assemblages indicated a decrease in the salinity of wetlands. The sensitivity of diatoms was also used to determine past variations in the ionic concentration of inland lakes, reflected by a change in the species composition (Fritz *et al.* 1991).

Extensive studies on past and present conditions within lakes and streams have shown that salinity level is a key factor in establishing the distribution pattern of

diatom community structure (Snoeijs 1999; Stevenson and Pan 1999). It is also important to recognise that increasing salt loads can affect other environmental variables such as nutrients and metal availability, which can influence species composition (Fritz *et al.* 1999). When using diatoms to monitor water quality variables such as salinity, it is important that standardised methods be incorporated into the sampling regime (Lowe and Gale 1980; John and Hellenen 1998).

#### *1.4.3 Artificial substrates and diatom sampling*

Diatoms are generally the most abundant microalgae found living on substrates in aquatic systems (Lowe and Gale 1980). Monitoring these communities can be carried out by scraping the surface of natural substrates such as rocks and macrophytes. Alternatively, artificial substrates including glass slides or clay tiles can be introduced to the water body allowing for colonisation and collection of diatom species (Cairns 1982; Aloï 1990). This method of sampling is considered to be a valuable approach when the objectives of the study require precise assessments over a range of different habitats (Stevenson and Pan 1999; Barbiero 2000).

Artificial substrates have a number of advantages including that they provide a uniform sampling surface, are readily available and provide standardisation between sites (Aloï 1990; Barbiero 2000; John 2003). This is particularly useful in water bodies that are subject to fluctuating salinities, a problem exacerbated by evaporative processes and unpredictable rainfall. They are also inexpensive, easy to handle and minimise disruption to the surrounding environment (Lamberti and Resh 1985; Biggs 1988). Most importantly, the use of artificial substrates within an aquatic system should provide an accurate representation of the naturally occurring diatom community (Aloï 1990).

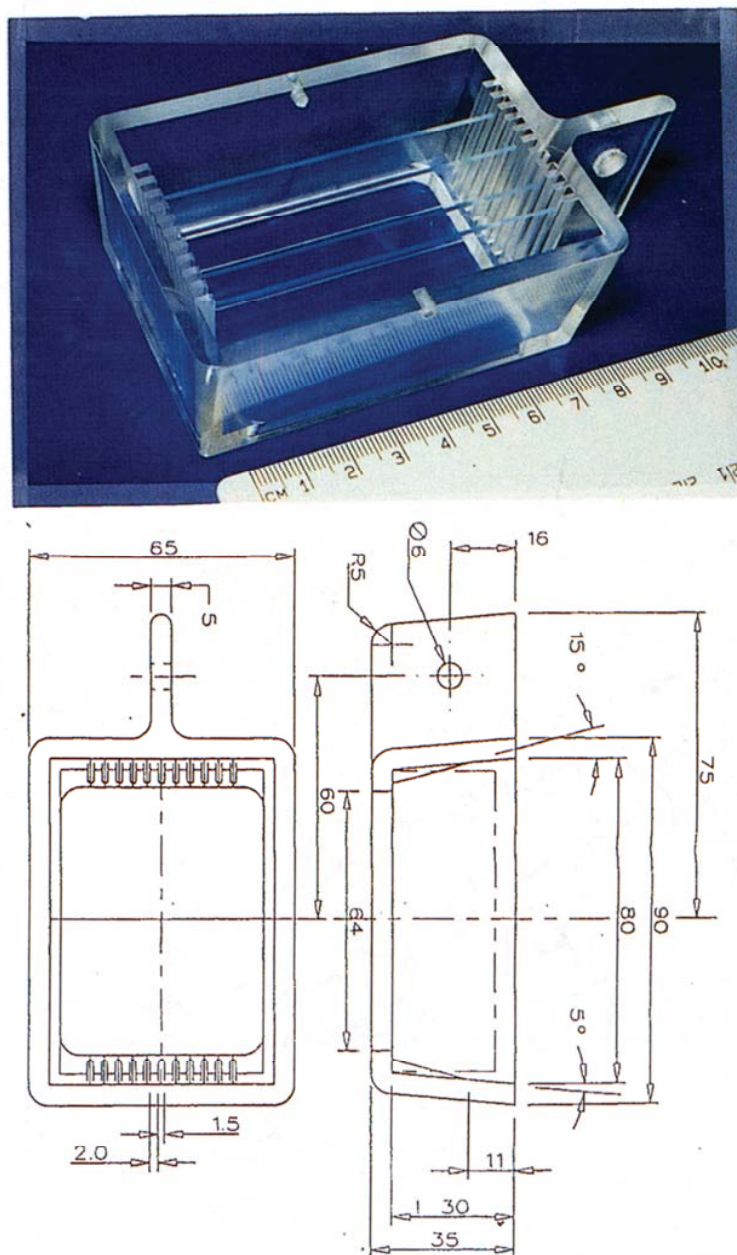
Artificial substrate collectors have successfully linked diatoms to fluctuations in a number of water quality variables. Chessman (1985) investigated their colonisation on glass slides to assess the impact of nutrient inputs from surrounding land use. Acs and Kiss (1993) monitored species found on artificial substrates to determine temporal changes in flowing waters. John (1998) related diatom assemblages to pH

and salinity using a collection device known as the JJ periphytometer (**Figure 1.4**). These studies have demonstrated the value of artificial sampling methods when the priority of the research project is to detect changes in environmental conditions such as salinity (Stevenson and Pan 1999). Quantitative results obtained in this manner can be subjected to a range of statistical analyses to aid the conservation and management of lakes and streams.

### **1.5 Diatom-Based Inference Models**

The sensitivity of diatoms to a number of factors including salinity, pH, heavy metals and nutrients has been used to develop inference models that can predict environmental conditions in aquatic habitats (Stevenson and Pan 1999). These models, also known as transfer functions are based on species composition in relation to the variable of interest, from representative wetlands within a geographic region (Philibert *et al.* 2006). Generally it is preferable to compile large numbers of study sites in order to increase the predictive ability of a transfer function along a select gradient (Battarbee *et al.* 2001). A diverse community structure and consistent taxonomic identification can also improve model performance (Stevenson and Pan 1999).

Before developing a predictive model, the target variable must first be demonstrated to account for the highest amount of variance, in relation to the diatom data. Multivariate techniques such as canonical correspondence analysis (CCA) are ideal for these purposes (Battarbee *et al.* 2001). The most common method for generating a transfer function is weighted averaging (WA), a technique which incorporates the relative abundance of taxa, the optimal conditions of each taxon and their specific tolerance limits (ter Braak and Van Dam 1989; ter Braak and Looman 1995). This approach is usually based on modern diatom samples and associated water chemistry (providing a calibration training set) with the aim of reconstructing past changes in conditions (Battarbee *et al.* 2001).



**Figure 1.4:** The JJ periphytometer, an artificial substrate collection device used for diatom sampling. Glass slides are shown secured within a customised plexiglass base. Associated specifications are also provided in the schematic diagram (units are in mm). Source (John 1998).

Inference models are widely used to determine changes in salt loads over time through the examination of fossil diatoms in sedimentary records (Gasse *et al.* 1997; Gell 1998; De Seve 1999; Kashima 2003). These analyses calculate predicted values for salinity using the observed dataset and derive species preferences from the weighted mean (optima) and standard deviation (tolerance) of taxa (Birks 1995; ter Braak and Looman 1995; Fritz *et al.* 1999). Transfer functions tend to cover large time scales spanning thousands of years, and more recently techniques have been refined to examine decadal variability within aquatic systems (Fritz *et al.* 1991; Anderson 1993; Battarbee 2000). However, further investigation is required into their application for predicting future trends in the salinity of lakes and streams in localised areas (John 2003). Additional research may prove that diatom-based inference models, in conjunction with water quality data, form a significant part of biomonitoring strategies for the problem of salinisation.

### **1.6 Objectives of the Research**

Although several publications on diatom distribution in response to secondary salinisation are available throughout the world and in Australia, there has been no research to establish the salinity tolerance limits of species in the south-west of Western Australia. This study has been undertaken to address these issues through the identification of diatom assemblages indicative of a wide range of conditions from freshwater to hypersaline, covering a much broader scope than any of the previously published literature. The data provide the basis for the development of a statistically robust inference model, with the potential to be integrated into paleolimnological studies or future biomonitoring programs. Specific objectives have been outlined below, established to complement the extensive data collected for the project.

- Examine and compare water quality and peripheral vegetation of representative lakes and streams in the south-west of Western Australia, in relation to salinity.
- Explore the relationship between water quality variables and diatoms, and determine the factors responsible for influencing species composition.



- Investigate diatom distribution from water bodies with a wide range of salinity and evaluate the effect of increasing concentration on community structure.
- Study the composition of hypersaline wetlands and explore species-environment relations and possible indicators of these conditions.
- Develop a diatom-based transfer function for salinity using the calibration dataset of south-west sites, providing specific optima and tolerance limits of taxa.
- Explore the use of diatoms as indicators of salinisation, providing suitable examples of representative taxa from the different salinity categories.

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## Chapter 2: Study Sites

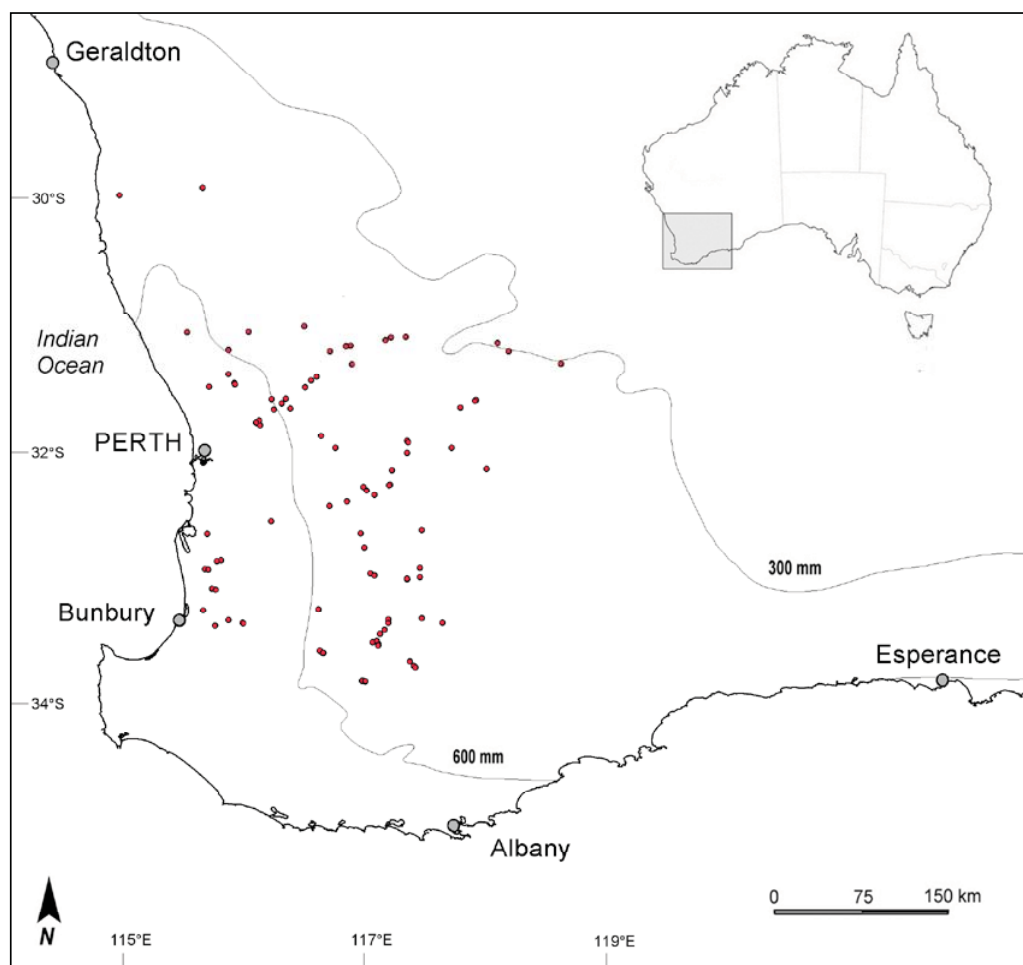
### 2.1 Introduction

A total of 95 lakes and streams from the south-west of Western Australia were sampled, located within a 400 km radius from the Perth metropolitan region (**Figure 2.1**). Sites occurred in the south-west drainage division (Schofield *et al.* 1988), which can be further separated into 7 water resource regions (Western Australian Water Resources Council 1984), five of which were covered in this study (**Figure 2.2**). Water bodies were sampled once only between 1999 and 2002, mostly in spring during the peak inundation period, following winter rainfall. The temporary nature of aquatic systems in the area allowed for the inclusion of both flowing (rivers and streams) and standing waters (lakes and wetlands).

A Magellan® GPS 2000 satellite navigational instrument recorded site coordinates as degrees longitude and latitude (**Appendix 2.1**) and the program ArcView Version 3.3 (ESRI Inc. 2002) was used to derive the map. Each sampling location was photographed and catalogued, with examples of representative water bodies displayed in **Figure 2.3**. Sites were allocated codes ranging from freshwater (SW01) to hypersaline (SW95), for the purpose of statistical analyses – with SW referring to the south-west. This system has been consistently used throughout the chapters in the thesis.

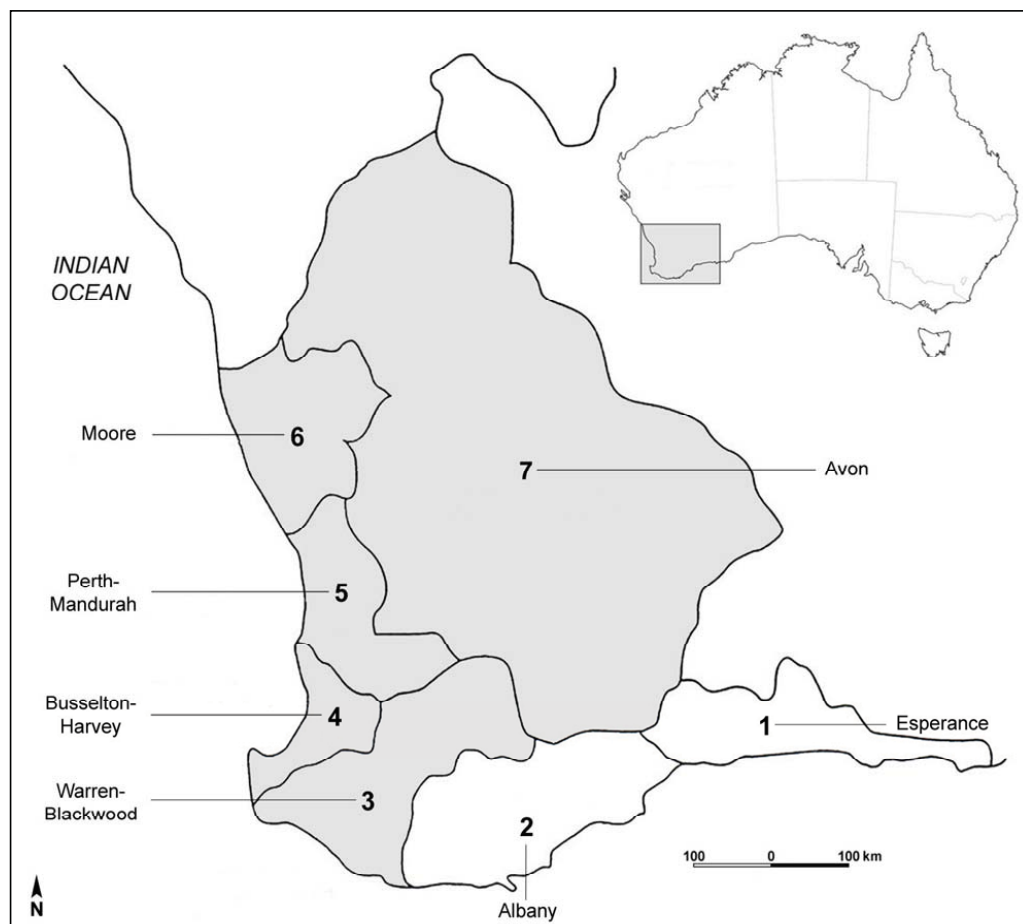
### 2.2 Description of Study Sites

As the focus of the study was on salinity, sites were separated according to Hammer's (1986) classification regime. Although some of the wetlands are naturally salt-affected (primary salinity), all have been influenced by secondary salinisation attributable to anthropogenic factors changing the hydrological balance (Williams 1996). A brief description of each salinity category is provided, with further information on the water resource region and associated hydrology of sites presented in **Appendix 2.1**.



**Figure 2.1:** The location of the 95 study sites (indicated by ●) in the south-west region of Western Australia. The 300 and 600 mm annual isohyets approximately define the wheatbelt area.





**Figure 2.2:** South-west drainage division showing the water resource regions covered in this study (shaded grey). Source (Western Australian Water Resources Council 1984).

### 2.2.1 Freshwater sites

Freshwaters sampled included 28 sites, displaying salinity concentrations of less than 3 ppt. They were mainly located in high rainfall areas that receive more than 600 mm per year, and have greater freshwater flushing compared to the other salinity groups. For this reason both flowing waters such as Hamilton River (SW05; **Figure 2.3a**) and Serpentine River (SW24) and standing waters including Lake Moyanup (SW16) and Lake Nangar (SW17) tend to remain permanently inundated. Freshwater lakes found near the coast of the south-west have been altered by impoundments (reservoirs) to serve agricultural, industrial and domestic requirements (Williams 1983). Examples of this are Harvey Dam (SW06) and Lake Navarino (SW18, **Figure 2.3b**). Many sites in these highly populated zones have also been exposed to nutrient input and possible eutrophication directly related to land-use practices (John 1998; Jakowyna 2000).

### 2.2.2 Hyposaline sites

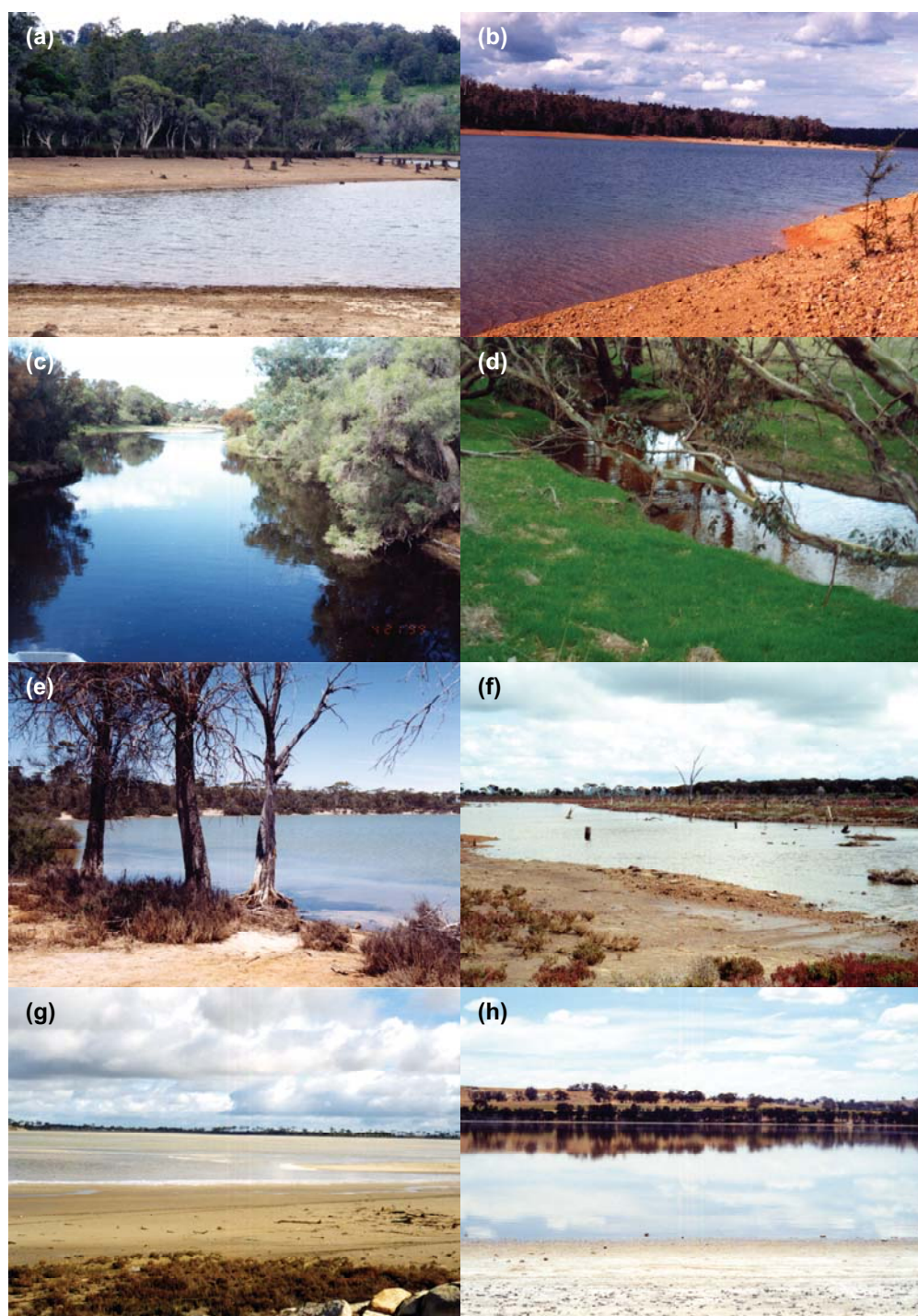
A total of 31 sites were hyposaline, ranging in salinity from 3-20 ppt, with most located in the Avon River catchment, the largest drainage division of the south-west. Rainfall in this area varies markedly from 1200 mm annually near the coast, steadily decreasing inland to approximately 400 mm (Avon River System Management Committee and the Waterways Commission 1993). Some of the sites that are part of this extensive river system include Avon River: Gwambygine Pool (SW33) and Avon River: Katrine Bridge (SW34, **Figure 2.3c**), with Boyagin Creek (SW38, **Figure 2.3d**) and Jimperding Brook (SW46) forming smaller tributary streams. A number of hyposaline wetlands including Towerinning (SW55) and Wannamal Lake (SW57) were previously considered to be freshwater, and currently have remedial measures in place with the aim of reducing salinity levels (Halse *et al.* 1993; Cale *et al.* 2004). Land in the Avon region has been extensively cleared for agriculture (approximately 75 %), causing erosion, salt loading and sedimentation of lakes and streams (Powell 1998; Weaving 1999; National Land and Water Resources Audit 2001).

### 2.2.3 Mesosaline sites

The mesosaline group contained 16 sites with salinities between 20 and 50 ppt. They were generally located further inland, in an area that experiences between 300 and 600 mm of annual rainfall, referred to as the wheatbelt (Cale *et al.* 2004). This is one of the most acutely salinised parts of the south-west (Halse *et al.* 2003; Timms 2005), with water bodies becoming seasonally inundated over winter and spring and drying out over summer, concentrating salts in the water column (McComb and Lake 1990). Standing waters are more susceptible (Davis 2004), with sites such as Lake Mears (SW66) and Yenyening Lakes (SW75) undergoing rehabilitation for salinity problems. For wetlands such as Ardath Lake (SW60, **Figure 2.3e**), acidification is an additional environmental management issue (Halse *et al.* 2003; Cale *et al.* 2004). Flowing waters are also influenced by salinisation, with examples including Hotham River (SW63) and Salt River (SW71, **Figure 2.3f**) classified as mesosaline. Many of these sites may also be subject to hypersaline conditions for certain periods, the degree to which is largely dependent on localised evaporation rates (McComb and Lake 1990; Boulton and Brock 1999).

### 2.2.4 Hypersaline sites

There were 20 hypersaline sites (greater than or equal to 50 ppt), all of which were located in the wheatbelt. These lakes are characterised by their shallow, temporary nature and are generally dominated by sodium chloride, following an ionic spectrum similar to that of seawater (Schofield *et al.* 1988; Boulton and Brock 1999; Pinder *et al.* 2005). They are subjected to extremely high salt concentrations, reaching saturation levels with the onset of the drying phase of their hydrocycle (Williams 1983). This group contains many large open water bodies greater than 500 ha in size, such as Dumbleyung Lake (SW80, **Figure 2.3g**) and Lake Champion (SW85). It also consists of chains of wetlands such as Flagstaff (SW81) and Queerearrup (SW94, **Figure 2.3h**) from the Beaufort River system and Norring (SW91) and Parkeyerring (SW92), which are part of the Wagin Lakes (Pen 1997). These sites are located in the southern wheatbelt area, experiencing elevated salinities due to intensive agriculture (Cale *et al.* 2004). Acidification is another degradation concern associated with hypersaline lakes, attributed to natural causes and in some cases acidic drainage from the surrounding catchment (Halse *et al.* 2003).



**Figure 2.3:** Selected site photos of water bodies from the south-west, classified according to Hammer's (1986) salinity categories. Freshwater sites: (a) Hamilton River and (b) Lake Navarino. Hyposaline sites: (c) Avon River: Katrine Bridge and (d) Boyagin Creek. Mesosaline sites: (e) Ardath Lake and (f) Salt River. Hypersaline sites: (g) Dumbleyung Lake and (h) Queerearrup Lake.

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## Chapter 3: The Relationship of Salinity to Water Quality and Peripheral Vegetation in Lakes and Streams of South-West Western Australia

### 3.1 Abstract

A total of 95 lakes and streams from the south-west of Western Australia were sampled to investigate the physico-chemical environment in relation to salinity. Salt concentrations showed a wide variation from freshwater (0.04 ppt) through to hypersaline (156.80 ppt), and pH ranged from acidic (2.90) to alkaline (10.51). Dissolved oxygen levels were recorded from 1.11 to 18.67 mgL<sup>-1</sup>, water temperature from 6.30 to 28.10 °C and peripheral vegetation ranged from sparse to dense cover. Analysis of variance (ANOVA) showed that salinities were significantly higher in wetlands located further inland in the wheatbelt region ( $p < 0.05$ ). There were also significant differences between salinity categories in comparison to pH, dissolved oxygen, water temperature and the density of peripheral vegetation ( $p < 0.05$ ). Principal components analysis (PCA) of the data captured almost 60 % of the variation over 2 dimensions, with dissolved oxygen, salinity and pH displaying a strong inverse correlation to site distribution. The results from this study indicate that the hydrocycle has a considerable influence on the salt loads of lakes and streams in the south-west, attributable to rainfall and clearing practices. The environmental variables of freshwaters were dependent on localised catchment conditions, however hypersaline wetlands were characterised by low dissolved oxygen, high water temperatures and a lack of peripheral vegetation. In addition, the installation of drainage networks appears to be associated with acidification problems in some wheatbelt areas. The temporary nature of aquatic systems in the south-west means that it is difficult to establish long-term trends in water quality, with data from this study contributing to baseline information available for future management purposes.

### 3.2 Introduction

One of the most serious environmental issues affecting lakes and streams in the south-west region of Western Australia is secondary salinisation (Ghassemi *et al.* 1995; Beresford 2001). The salinity concentration of these water bodies is steadily rising, and can be attributed to changes in hydrology brought about by the replacement of native vegetation with annual crops and pastures used in agriculture (Alexandra and Eyre 1993; George *et al.* 1999; Frost *et al.* 2001). In the salt-affected

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wheatbelt area for example, wetlands such as Parkeyerring and Norring Lakes from the Wagin system provide valuable waterbird habitats, currently under threat from degradation (Halse *et al.* 1993; Pen 1997). Similarly, the once freshwater Avon River is experiencing elevated salinity levels caused by the intrusion of hypersaline water from surrounding streams (Schofield *et al.* 1988; Avon River System Management Committee and the Waterways Commission 1993).

Freshwater systems located in high rainfall zones further south are also at risk from human-induced salinisation (Schofield *et al.* 1988; Avon River System Management Committee and the Waterways Commission 1993; Williams 1999; 2001). The Collie River is an important water resource that has been used for both agricultural and domestic purposes. However, land-clearing practices have led to a substantial rise in salinity causing a decline in potability, decreasing the productivity of irrigation areas (Imberger 1981; Loh and Stokes 1981; Lymbery *et al.* 2003). In order to prevent further deterioration of the river and its tributaries, regulations have been put in place to protect the integrity of the system (Hillman 1981; Steering Committee for Research on Land Use and Water Supply 1989). One problem associated with the conservation of the Collie River as well as other water bodies at risk of secondary salinisation, is the lack of basic water quality data that could be used to predict future changes for management purposes (Schofield *et al.* 1988).

The environmental conditions of lakes and streams in the south-west vary significantly according to chemical, physical and biological factors (Meybeck and Helmer 1996). In freshwater systems, pH is an important variable that has the ability to alter ecosystem function and dynamics, with processes such as effluent discharge or the build-up of organic matter potentially causing acidic or alkaline conditions (Chapman and Kimstach 1996; Boulton and Brock 1999; Fellenberg 2000). The level of dissolved oxygen in freshwaters is affected by photosynthetic rates (Williams 1983; Connell 2005) and water temperature is largely dependent on seasonal variations (Chapman and Kimstach 1996). Rising water temperatures are also associated with an increase in salinity, a common problem in the shallow, temporary water bodies of Western Australia (Boulton and Brock 1999). However, in comparison to freshwaters, the ionic concentration of salt lakes is a key feature that can be a major determinant of the surrounding environment (Williams 1998a).



The hypersaline nature of lakes from inland regions directly influences the interaction of a number of different variables. For example, the solubility of oxygen in water decreases significantly at elevated salinities and is linked to temperature as well as other chemical characteristics (Baird 1999; Manahan 2005). High salinity concentrations can influence pH, which is also related to geology and run-off from the surrounding catchment (McComb and Lake 1990; Fellenberg 2000). Increasing salt loads and prolonged inundation regimes have led to the degradation of riparian vegetation, common throughout the wheatbelt (Cramer and Hobbs 2002; Lymbery *et al.* 2003; Pinder *et al.* 2005). Monitoring programs that include the measurement of environmental variables from water bodies in the south-west provide much needed information, with most of the literature currently available being restricted to localised areas (Froend *et al.* 1987; Froend and McComb 1991) or focussing on biodiversity aspects (Halse *et al.* 1993; Kay *et al.* 2001; Pinder *et al.* 2005).

The objective of this chapter was to investigate water quality, in relation to salinity from lakes and streams in the south-west of Western Australia. Data were analysed according to geography and the nature of the water body, to determine differences in salt loads. Physico-chemical data were also examined to identify patterns based on varying salinity concentrations. Increased understanding of secondary salinised systems allows long-term trends to be established and effective management protocols to be implemented, along with the development of appropriate rehabilitation measures that target specific catchment areas.

### 3.3 Materials and Methods

#### 3.3.1 Environmental variables

Environmental variables were measured *in-situ* from the surface waters of each of the 95 study sites (**Figure 3.1**), conducted at the deepest accessible point to ensure stable sampling conditions. A WP-81 TPS® was used to determine pH, salinity (ppt) electrical conductivity ( $\text{mScm}^{-1}$ ) and water temperature ( $^{\circ}\text{C}$ ), with dissolved oxygen ( $\text{mgL}^{-1}$ ) recorded using an YSI® 550 DO Meter. These meters were calibrated prior to sampling in accordance with standard calibration techniques. Vegetative complexes were also observed at each location and a score was allocated from 1 to 5 based on density. A score of 1 indicated little or no vegetative cover where as a

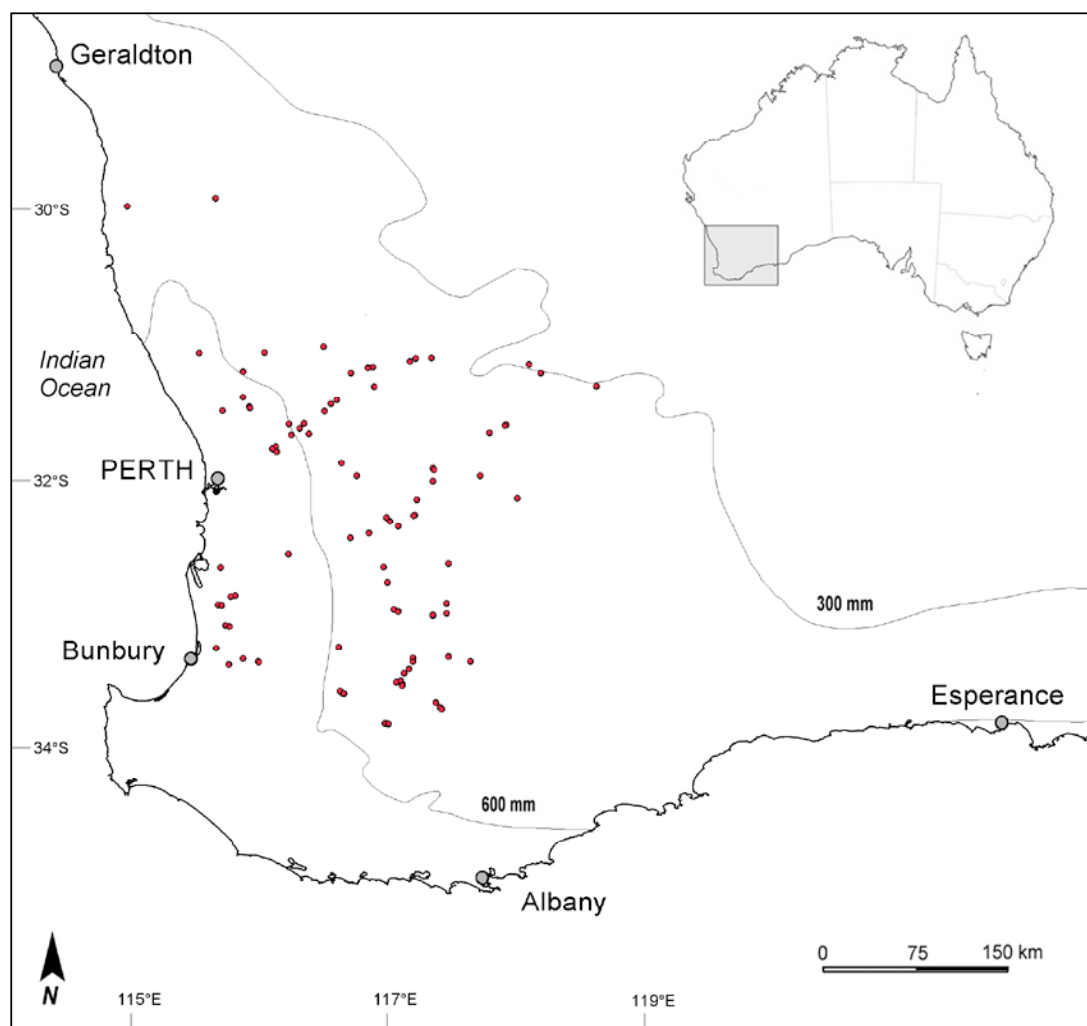
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rating of 5 indicated dense upper and understorey vegetative cover. The final dataset of physico-chemical variables used in this chapter and throughout the thesis excluded electrical conductivity, which was removed due to its collinear relationship with salinity.

### 3.3.2 Data analysis

Prior to statistical analyses, data were examined using the Kolmogorov-Smirnov test for normality and Levene's test for homoscedasticity. Based on these preliminary investigations, salinity data were  $\log_{10}$  transformed and pH was square-root transformed to reduce skewness. One-way analysis of variance (ANOVA) was then performed on environmental factors in relation to geography (south-west and wheatbelt areas), water body type (flowing and standing waters) and Hammer's four salinity categories (Hammer 1986). Differences between means were considered significant if the probability level (p) was less than 0.05, with significant values indicated at  $<0.05$ ,  $<0.01$  and  $<0.001$ . Tukey's post hoc multiple comparisons test was employed when ANOVA found significant differences were present. In instances where the assumption of equality of variance was not met, Games-Howell post hoc testing was implemented. All univariate statistical analyses were completed in the software package SPSS 11.0 Version 11.0.4 (SPSS Inc. 2005).

Principal components analysis (PCA) was used to identify the underlying structure in the environmental data. The objective of this correlation-based technique is to reduce dimensionality whilst still retaining important characteristics. Prior to the ordination procedure all variables were standardised and salinity was  $\log_{10}$  transformed to improve its representation. Results of the PCA have been reported in terms of the proportion of variance explained over the first two principal component axes (PC1 and PC2) and the associated correlation coefficients (McCune and Grace 2002). Multivariate analysis was performed in the statistical program PRIMER 5 (Plymouth Marine Laboratory 2002).



**Figure 3.1:** The location of the 95 study sites (indicated by ●) in the south-west region of Western Australia. The 600 and 300 mm annual isohyets approximately define the wheatbelt area.

### 3.4 Results

#### 3.4.1 Univariate analysis

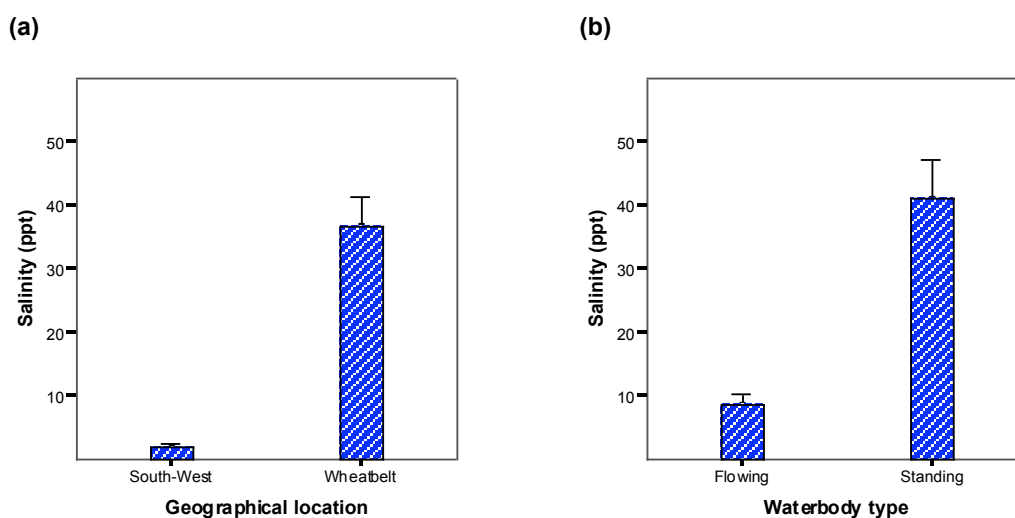
The water quality of lakes and streams in the south-west displayed considerable variability, with summary statistics provided in **Table 3.1**, and the complete dataset presented in **Appendix 3.1**. The minimum salinity concentration recorded was 0.04 ppt, with a maximum reading of 156.80 ppt. The pH varied from highly acidic (2.90) to strongly alkaline (10.51). Dissolved oxygen ranged from 0.11 to 18.67 mgL<sup>-1</sup> and water temperature from 6.30 to 28.10 °C. Observations of peripheral vegetation density included the allocation of scores from 1 (little cover) to 5 (dense cover).

**Table 3.1:** Summary statistics for environmental variables from the 95 study sites in the south-west. The arithmetic mean is shown for salinity (ppt), dissolved oxygen mgL<sup>-1</sup>, temperature (°C) and vegetation (score) and the geometric mean is indicated for pH. The median values are also displayed for all variables.

Statistic	Salinity	pH	Dissolved oxygen	Temperature	Vegetation score
Minimum	0.04	2.90	1.11	6.30	1
Maximum	156.80	10.51	18.67	28.10	5
Mean	27.43	7.86	7.40	17.69	2
Median	10.30	8.07	7.70	18.60	2

The salinity of lakes and streams was examined in relation to geographical location as well as water body type. Monitoring sites were separated into south-west (>600 mm average annual rainfall) and wheatbelt areas (300-600 mm average annual rainfall), with 25 and 70 sites in each of the respective regions. One-way ANOVA showed that the mean salinity was significantly higher ( $p < 0.001$ ) in the inland wheatbelt zone (backtransformed mean=36.57 ppt), compared to the remainder of the south-west (backtransformed mean=1.83 ppt, **Figure 3.2a**).

Sites were also classified into flowing and standing waters, with approximately equal group sizes of 40 and 55 respectively. The mean salinity of lakes and wetlands (standing waters) was significantly greater ( $p < 0.05$ ) than that of rivers and streams (flowing waters), with backtransformed means of 41.08 and 8.66 ppt (**Figure 3.2b**).



**Figure 3.2:** Backtransformed mean salinities for (a) geographic location, south-west and wheatbelt sites and (b) water body type, flowing and standing waters. Error bars represent +1.0 standard error of the mean.

To determine the relationship of environmental factors including pH, dissolved oxygen, temperature and vegetation density to Hammer's (1986) salinity regime, analysis of variance was performed. This procedure determined that there was a significant difference between the mean pH of the four different salinity categories ( $p < 0.001$ , **Figure 3.3a**). Games-Howell post hoc testing ( $p < 0.01$ ) indicated that the pH of both freshwater and hypersaline water bodies was significantly lower (backtransformed value of 7.57 and 6.97) than hyposaline lakes and streams (backtransformed value of 8.85). In contrast, the mesosaline sites showed no significant difference in relation to the other groups ( $p > 0.05$ ), with a backtransformed mean pH of 8.56.

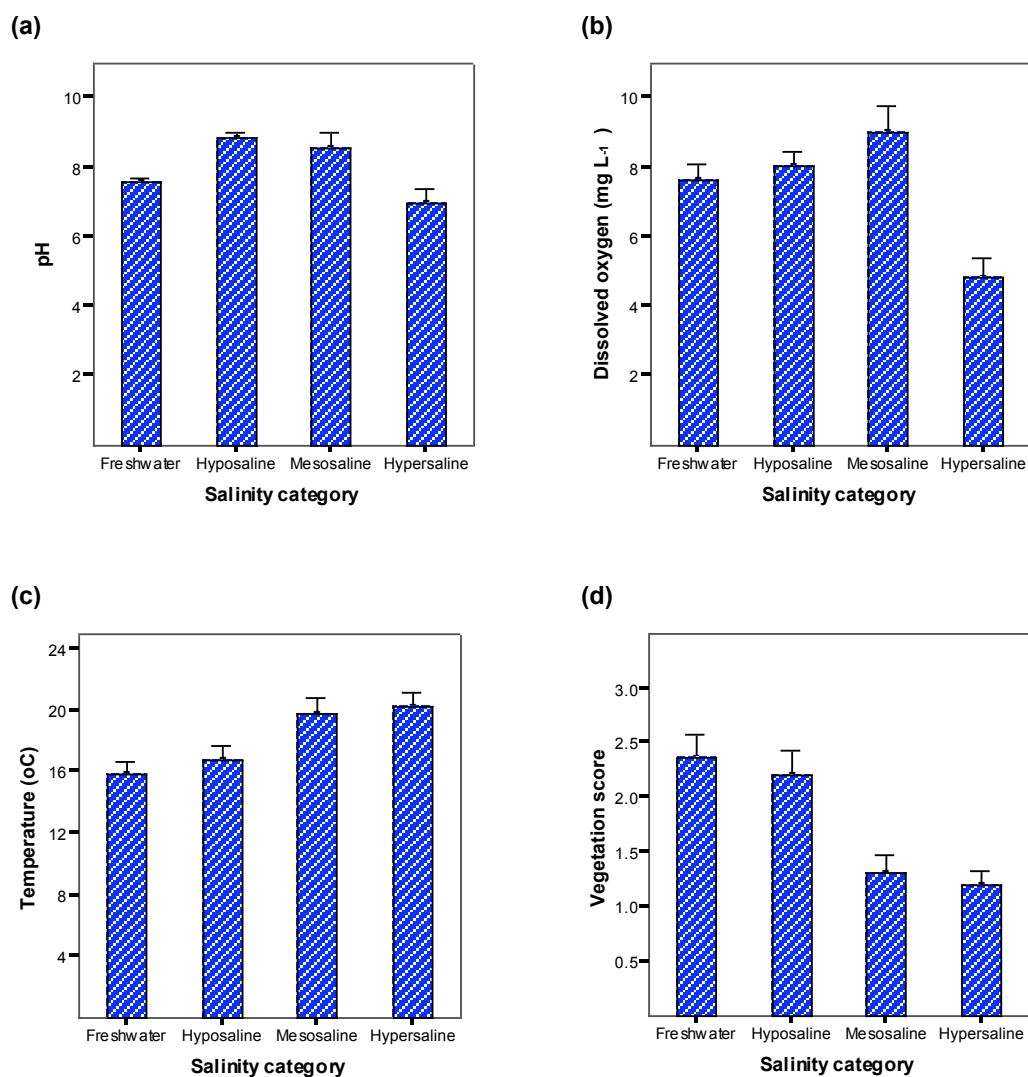
The mean dissolved oxygen level was significantly different over the range of salinity concentrations according to ANOVA results ( $p < 0.001$ , **Figure 3.3b**). Further analysis with Tukey's testing concluded that there was no significant difference ( $p > 0.05$ ) between the mean dissolved oxygen of the freshwater, hyposaline and mesosaline sites (means of 7.62, 8.03 and 8.99  $\text{mgL}^{-1}$  respectively). However, hypersaline water bodies displayed a significantly lower mean dissolved oxygen concentration (mean = 4.84  $\text{mgL}^{-1}$ ) in comparison to the other categories ( $p < 0.01$ ).

Significantly different means were apparent for the environmental variables of water temperature ( $p < 0.01$ , **Figure 3.3c**) and peripheral vegetation density ( $p < 0.001$ , **Figure 3.3d**). Tukey's testing ( $p > 0.05$ ) found that freshwater and hyposaline mean temperatures were similar being 15.79 and 16.75 °C respectively. These results were significantly lower ( $p < 0.05$ ) than the mesosaline and hypersaline groups, which also had like means of 19.76 and 20.18 °C ( $p > 0.05$ ). There was no significant difference ( $p > 0.05$ ) between hyposaline and mesosaline water temperatures, with intermediate means of 16.75 and 19.76 °C.

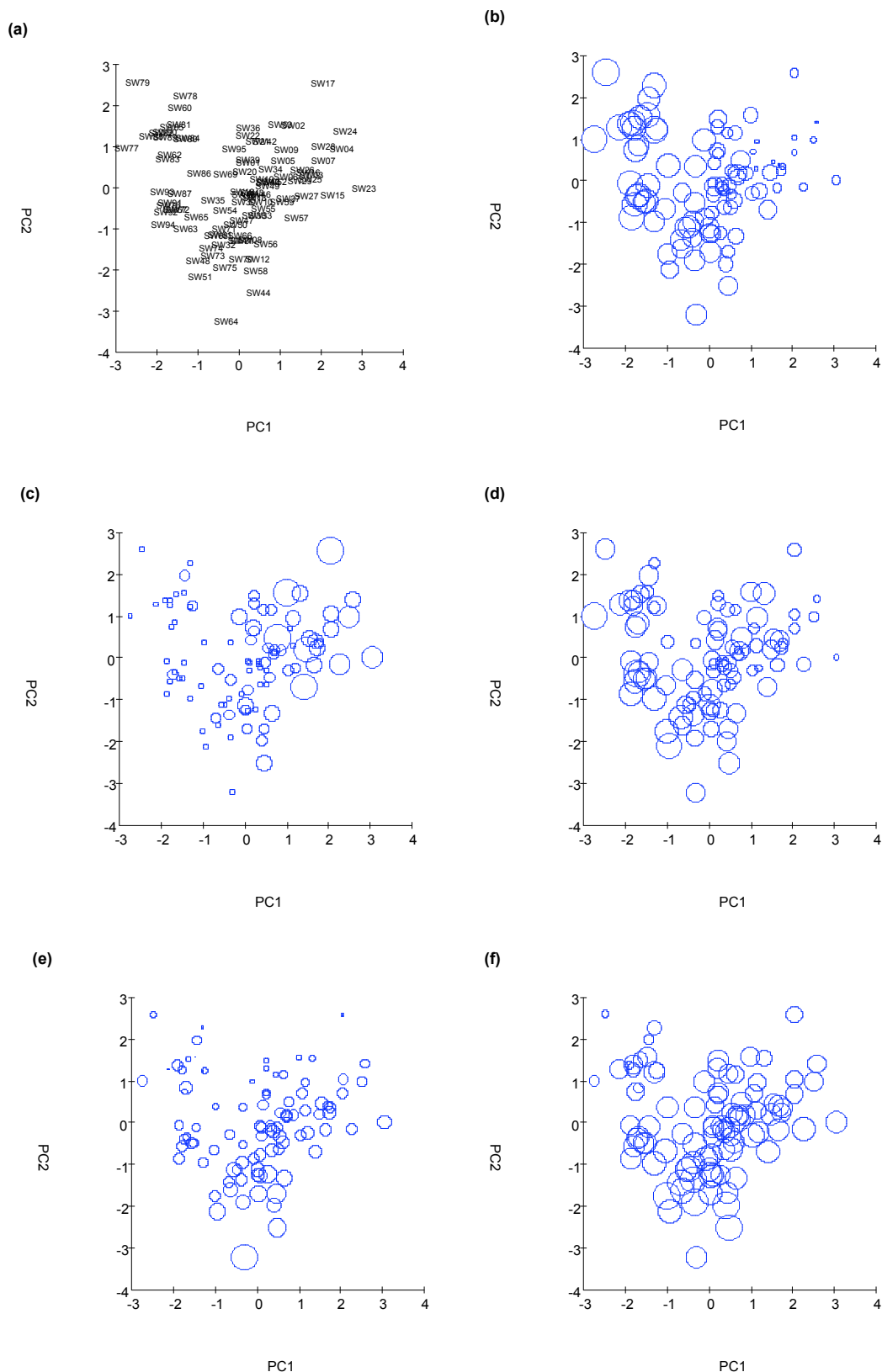
The mean vegetation density was not significantly different in the lower salinity ranges (freshwater and hyposaline mean scores  $\approx 2$ ) based on Games-Howell post hoc testing ( $p > 0.05$ ). However, these scores were significantly higher than those from elevated salinity concentrations (mesosaline and hypersaline mean scores  $\approx 1$ ), which had reduced vegetative cover ( $p < 0.05$ ).

#### 3.4.2 Multivariate analysis

Ordination of the 95 study sites was carried out using principal components analysis (PCA) and effectively determined the main patterns in the environmental data (**Figure 3.4a**). A total of 58.20 % of the variation was captured in the 2 dimensional approximation, with 32.10 % accounted for in PC axis 1 and 26.10 % in PC axis 2. Based on this analysis, plots of individual variables were generated, with circles overlaid to show the gradient for each factor. Smaller circles indicate a lower recorded value for each variable, whereas larger circles represent a higher measurement. Salinity displayed a strong inverse relationship with PC axis 1, with a correlation coefficient of -0.68 (**Figure 3.4b**). Vegetation density and temperature were also correlated to PC axis 1 although to a lesser extent, with correlation coefficients of 0.53 and -0.45 (**Figure 3.4c-d**). The environmental variables of dissolved oxygen and pH showed a strong negative relationship with PC axis 2, with correlation coefficients of -0.70 and -0.66 respectively (**Figure 3.4e-f**).



**Figure 3.3:** Means and standard error bars for the environmental variables of (a) pH (backtransformed values displayed), (b) dissolved oxygen ( $\text{mg L}^{-1}$ ), (c) temperature ( $^{\circ}\text{C}$ ) and (d) vegetation scores based on Hammer's (1986) salinity categories. The y-axis scale is different for each variable. Error bars represent +1.0 standard error of the mean.



**Figure 3.4:** 2-dimensional PCA ordination of environmental variables from the 95 study sites. (a) PCA plot showing site distribution using site codes. PCA plots with circles overlaid proportional in diameter to (b) salinity concentration (ppt,  $\log_{10}$  transformed), (c) vegetation score, (d) temperature ( $^{\circ}\text{C}$ ), (e) dissolved oxygen level ( $\text{mgL}^{-1}$ ) and (f) pH. Plots are shown in order of correlation to PC axes



### 3.5 Discussion

#### 3.5.1 Salinity in the south-west

The results indicated that the salinity levels of water bodies increased further inland, with a mean salinity of 36.57 ppt in the wheatbelt, compared to 1.83 ppt in the remainder of the south-west, attributed to anthropogenic and environmental conditions (Williams 2001). It has been well-documented that in high rainfall areas receiving between 1200 and 600 mm per year there is little or no salinisation, with very little salt being stored in the soil profile (Halse *et al.* 2003). Systems that occur in the upper end of this range will also continue to remain fresh, regardless of the proportion of catchment cleared (Schofield *et al.* 1988). In contrast, the wheatbelt area, characterised by low rainfall (<600 mm/yr) and high evaporation rates is severely salt-affected with impacts occurring most rapidly in the western parts due to intensive agriculture (Halse *et al.* 2003; Halse 2004). However currently, one of the most important factors contributing to the problems encountered in this region is the deliberate or accidental discharge of saline groundwater from drainage and pumping schemes used to repair salinised land (Lymbery *et al.* 2003). These activities have an influence on both lakes and streams, with the degree of salinisation related to the level of connectivity within a given catchment (Williams 1999).

#### 3.5.2 Salinity of standing and flowing waters

Salinity was found to be significantly higher in standing waters (mean=41.08 ppt), as opposed to flowing waters (mean=8.66 ppt), which remove salts from the system via flushing (Davis 2004). Freshwater rivers and their tributaries were once again found in high rainfall zones, with examples such as Hamilton (SW05) and Serpentine (SW24) Rivers recording salinities of less than 1 ppt. The Collie River ranged from below 0.5 to greater than 3 ppt (Sites SW04 and SW42) and over the last 10 years has had an average of 1.5 ppt. Although still freshwater, these readings are considered to be higher than those from the previous decade (Mayer *et al.* 2004), with salt loading related to the intensity of land use (Lymbery *et al.* 2003). Further inland in the wheatbelt all major rivers are salinised and currently export 2-30 times the amount of salt that their catchments receive annually (Halse *et al.* 2003). The Avon, a previously freshwater resource (Water and Rivers Commission and Avon Waterways Committee 2002), is now considered permanently hyposaline with all six sites (SW30-SW35) found to be within this category. Moore River, also located in

the northern wheatbelt had a maximum salinity of close to 10 ppt, with previous studies showing an average of around 7 ppt (Mayer *et al.* 2004). Although salinity appears to be increasing for most rivers and streams in the south-west, further research is required, as there are few catchments that have long records and annual ranges are largely dependent on rainfall and associated flow volumes (Schofield *et al.* 1988; Mayer *et al.* 2004).

Lake systems in comparison, especially those in the wheatbelt, rarely receive freshwater flushing (except in high rainfall events) and therefore tend to lose water only through evaporation leading to the concentration of salts (McComb and Lake 1990; Davis 2004). This was supported by the results, which found that the majority of wetlands in the region were categorised as either mesosaline or hypersaline. Widely fluctuating salinities are characteristic of these water bodies, with annual and interannual variability influenced by the hydrocycle (Williams 1983; Cale *et al.* 2004). Lake Yealering (SW90), affected by secondary salinisation had a salinity close to 100 ppt, however has previously recorded values ranging from freshwater (<1 ppt) through to almost saturation point (>250 ppt) (Jaensch and Lane 1992). Yenyening Lakes (SW75) and Lake Mears (SW66) from the Avon catchment receive saline run-off from agriculture and have in the past varied from mesosaline (close to 20 ppt) (Strehlow *et al.* 2005; Taukulis and John 2006), to hypersaline (up to 85 ppt) (Blinn *et al.* 2004; Strehlow *et al.* 2005), with both sites being in the former category for this study. Parkeyerring Lake (SW92), which forms part of the Wagin Lakes system has displayed approximate concentrations of between 25 ppt (Blinn *et al.* 2004) and in excess of 50 ppt (Cale *et al.* 2004) and on this occasion had a salinity of almost 100 ppt. Norring Lake (SW91) and Queerearrup Lake (SW94), also situated in this chain of wetlands recorded salinities of close to 100 ppt, most likely due to numerous inflows from surrounding drainage channels (Cale *et al.* 2004). The highly unstable conditions demonstrated by temporary water bodies highlight the difficulties associated with determining long-term trends in salt loads, which are believed to be steadily rising (Halse *et al.* 2003). This problem is further compounded by anthropogenic impacts including the installation of complex drainage networks that not only increase salinity concentrations, but also affect other environmental variables such as pH (Davis 2004; Halse 2004).

### 3.5.3 Salinity in relation to water quality and peripheral vegetation

The mean pH for the different salinity categories was generally within the range of most natural waters; between 6.0 and 8.5 (Chapman and Kimstach 1996). Freshwaters, with a mean pH close to neutral (Cheal *et al.* 1993; Boulton and Brock 1999) included Lake Navarino (SW18) and Lake Brockman (SW14), as well as Brunswick River (SW03) and Harvey River (SW07). These water bodies had a pH range of approximately 7 to 8, showing no apparent effects from nutrient rich run-off or organic acids that may alter the balance (Cheal *et al.* 1993; Boulton and Brock 1999). In comparison, the hyposaline and mesosaline sites had the highest mean pH (8.85 and 8.56 respectively), with these values indicating the influence of potential eutrophication as well as increased salinity concentrations, both possible causes of alkaline conditions (Chapman and Kimstach 1996; Boulton and Brock 1999). Smaller tributaries of the Avon such as Dale River (SW43) and Mortlock River (SW68) have been subjected to nutrient loading and increased salinities due to agriculture and catchment clearing (Avon River System Management Committee and the Waterways Commission 1993) and both recorded high pH values (>8). The mean pH of hypersaline sites was lower (6.97), with a number of acidic wetlands present in this group. The problem of acidity in hypersaline wheatbelt lakes appears to be increasing with many systems including Lake Champion (SW85) and Baandee Lake (SW77), which recorded pH values of less than 6.5, most likely attributable to acidic groundwater (Kay *et al.* 2001; Halse *et al.* 2003). Overall, however the relationship between pH and other factors is complex, being influenced by chemical and biological processes (Hammer 1986; Williams 1998b). This is especially relevant when taking into account the one-off sampling regime undertaken for this study, which also affects dissolved oxygen and water temperatures.

Dissolved oxygen levels were significantly lower in hypersaline sites with a mean of less than 5 mgL<sup>-1</sup>, a concentration considered to be critically limiting to the function and survival of aquatic biota (Chapman and Kimstach 1996). However, it is important to note that both seasonal and diel variations can occur, mainly related to the activities of photosynthetic organisms (Connell 2005). Reduced oxygen concentrations or even anaerobic conditions may arise from the decay of plant material in surface sediments (Williams 1983). One of the fundamental features of salt-affected waters is their reduced ability to dissolve oxygen, with the level of

solubility dependent on factors such as temperature and depth (Williams 1998b; Baird 1999; Manahan 2005). Results showed that significantly higher water temperatures occurred in hypersaline sites such as Cowcowing Lakes (SW79), Lake Yealering (SW90) and Kevills Lake (SW83), which displayed temperatures close to 20 °C and dissolved oxygen concentrations that were below critical levels (<5 mgL<sup>-1</sup>). Changes in temperature are also related to seasonal and diurnal differences (Vesilind 1975; Chapman and Kimstach 1996; Connell 2005), however the high temperatures recorded from these wetlands are generally attributable to their shallow exposed nature (Williams 1998a).

ANOVA showed that the density of riparian vegetation surrounding lakes and streams with elevated salinities was lower than that of freshwaters. However, overall the mean vegetation score for all salinity categories was low (mean scores  $\leq 2$ ), with the peripheral vegetation of water bodies in the south-west having been subjected to a high degree of disturbance (Halse *et al.* 1993; Davis and Froend 1999). A number of freshwater systems including the Collie River and its tributaries (SW04 and SW42) are currently being revegetated in order to conserve remaining potable water sources (Mayer *et al.* 2004). In comparison, Moore River (SW20 and SW51) with almost 80 % of its catchment area cleared has incurred considerable hydrological changes including increased salinity and variable flow rates (Mayer *et al.* 2004; Taylor 2004). Many wetlands in the wheatbelt that once supported dense vegetative stands along foreshore regions are now supersaturated with salt as they dry (Lane and McComb 1988). For example, Coomelberrup Lake (SW78) has suffered from large amounts of saline water entering the system, resulting in extensive tree deaths (Pen 1997; Cale *et al.* 2004). Even systems that are classified as part of nature reserves, such as Norring (SW91) and Parkeyerring (SW92) Lakes only provide for narrow belts of fringing vegetation and remnant bush (Pen 1997). A significant illustration of the effects of salinisation on riparian plant communities is Lake Dumbleyung (SW80), once freshwater and now completely devoid of upland vegetation causing problems such as siltation and eutrophication (Pen 1997).

One factor that affects the function of lakes and streams that was not monitored in this study is nutrient concentration, which is directly related to modification of the surrounding catchment area (Schofield and Davies 1996). For example, many

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freshwater systems including Harvey River (SW07) and Serpentine River (SW24) have moderate levels of total phosphorus due to intensive horticultural and livestock grazing practices, with increasing trends in the tributaries of both rivers being cause for concern (Jakowyna 2000). The Avon River (SW30-35) is experiencing similar problems, attributed to fertiliser usage, which is adversely affecting water quality (Avon River System Management Committee and the Waterways Commission 1993; Water and Rivers Commission and Avon Waterways Committee 2002). A number of salt-affected wetlands such as Yenyening Lakes (SW75), and Lake Yealering (SW90) are also under threat from eutrophication as a result of ongoing agricultural activities (Jaensch and Lane 1992). It may be considered useful in future studies to take into account the nutrient levels of these water bodies, which can influence environmental variables such as pH and dissolved oxygen (Archer 2001; Connell 2005; Manahan 2005).

#### *3.5.4 Salinity and multivariate analysis*

Multivariate analyses found that although salinity was one of the key factors associated with site distribution, pH and dissolved oxygen also displayed a high correlation. In addition, the relationship between these variables becomes more apparent at elevated salinities (Williams 1998a;b). In this study, the nature of the sampling regime did not take into account seasonal, temporal and spatial variations. However, the interpretation of water quality results is determined by specific objectives and should be seen in the broader context of resource management (Meybeck *et al.* 1992). In this case the focus was to encompass a wide range of salinities over a large geographical area, with data from the research contributing substantially to the understanding of temporary water bodies in the south-west. It also provides the basis for long-term monitoring strategies for systems affected by the problem of salinisation.

### 3.6 Conclusion

The increased salinity of water bodies in the wheatbelt, compared to freshwaters in other parts of the south-west, can be attributed to a number of factors including low rainfall, extensive land clearing and the installation of drainage networks. In particular, wetlands are clearly more susceptible to rising salt loads due to the lack of freshwater flushing and evaporation. The discharge from deep drainage channels and nutrient rich run-off from agricultural land also appears to be influencing pH, with a number of hypersaline waters being acidic. The shallow exposed nature of these temporary lakes causes a reduction in the solubility of dissolved oxygen as a result of increased salinities and elevated water temperatures, further concentrating salts in the system. This is exacerbated by the degradation and subsequent death of peripheral vegetation, related to increased salinity and waterlogging. The limited availability of physico-chemical data for lakes and streams affected by or at risk of secondary salinisation has led to difficulties in establishing long-term trends, with this study contributing to baseline information that can be used in management or rehabilitation efforts in the region.

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**Appendix 3.1:** Environmental variables used in the ANOVA analyses including geographic location (south-west and wheatbelt regions), water body type (flowing and standing waters) and salinity categories (based on Hammer's 1986 classification system). Water quality data is also indicated including salinity (ppt), pH, dissolved oxygen (mgL<sup>-1</sup>), temperature (°C) and peripheral vegetation score (allocated values from 1: little or no vegetative cover to 5: high density of upper and understorey vegetative cover).

Code	Site name	Geographic location	Waterbody type	Salinity category	Salinity	pH	Oxygen	Temperature	Vegetation score
SW01	Bailup Rd Lake	South-West	Standing	Freshwater	1.47	7.19	6.35	19.60	2
SW02	Blindroo Wetland A	South-West	Standing	Freshwater	0.04	6.63	4.40	23.70	3
SW03	Brunswick River	South-West	Flowing	Freshwater	0.24	7.74	8.16	11.20	2
SW04	Collie River: Honeymoon Pool	South-West	Flowing	Freshwater	0.37	7.40	7.42	11.10	4
SW05	Hamilton River	South-West	Flowing	Freshwater	0.28	6.94	7.53	11.60	1
SW06	Harvey Dam	South-West	Standing	Freshwater	0.15	7.33	8.19	14.00	1
SW07	Harvey River	South-West	Flowing	Freshwater	0.30	7.38	7.93	11.90	3
SW08	Katanning Creek A	Wheatbelt	Flowing	Freshwater	2.52	7.83	12.50	16.90	1
SW09	Knapping Wetland	South-West	Standing	Freshwater	0.15	7.10	5.90	22.30	3
SW10	Kojonup Brook A	Wheatbelt	Flowing	Freshwater	0.95	7.71	9.42	15.40	1
SW11	Kojonup Brook B	Wheatbelt	Flowing	Freshwater	2.29	7.75	8.80	14.10	1
SW12	Kojonup Brook C	Wheatbelt	Flowing	Freshwater	2.43	8.21	13.55	21.30	2
SW13	Kojonup Creek	Wheatbelt	Flowing	Freshwater	1.50	7.80	8.84	15.10	1
SW14	Lake Brockman	South-West	Standing	Freshwater	0.08	7.08	8.30	20.60	1
SW15	Lake Kulimup	South-West	Standing	Freshwater	0.43	9.18	8.00	15.60	4
SW16	Lake Moyanup	South-West	Standing	Freshwater	0.07	8.23	6.39	20.80	3
SW17	Lake Nangar	South-West	Standing	Freshwater	0.68	6.61	2.41	14.80	5
SW18	Lake Navarino	South-West	Standing	Freshwater	0.96	7.59	8.04	19.10	1
SW19	Mistake Creek	Wheatbelt	Flowing	Freshwater	2.52	7.97	7.13	15.60	2
SW20	Moore River: Regans Ford	South-West	Flowing	Freshwater	2.68	6.95	7.71	19.00	2
SW21	Narrogin Brook A	Wheatbelt	Flowing	Freshwater	1.44	7.62	4.09	16.30	2
SW22	Narrogin Brook B	Wheatbelt	Flowing	Freshwater	2.53	7.62	3.42	16.40	2
SW23	Red Swamp Brook	South-West	Flowing	Freshwater	0.67	8.39	10.14	6.30	4
SW24	Serpentine River	South-West	Flowing	Freshwater	0.10	7.09	6.28	8.60	3
SW25	Toodyay/Bailup Rd Lake	South-West	Standing	Freshwater	0.76	7.97	8.33	13.10	3
SW26	Toodyay Rd Lake	Wheatbelt	Standing	Freshwater	0.14	7.06	8.31	19.90	3
SW27	Utah Rd Lake	South-West	Standing	Freshwater	0.73	8.61	8.51	15.10	3
SW28	Wallsend Lake	South-West	Standing	Freshwater	0.18	6.92	7.32	12.70	3
SW29	Arthur River: Moodiarrup Bridge	Wheatbelt	Flowing	Hypersaline	5.38	8.42	7.60	19.30	5
SW30	Avon River South	Wheatbelt	Flowing	Hypersaline	4.67	8.61	7.70	14.30	1
SW31	Avon River: Boyagarra Pool	Wheatbelt	Flowing	Hypersaline	10.30	9.02	10.60	14.30	1
SW32	Avon River: Brookton	Wheatbelt	Flowing	Hypersaline	16.80	10.15	8.22	22.50	2
SW33	Avon River: Gwambygine Pool	Wheatbelt	Flowing	Hypersaline	3.85	9.33	8.10	11.80	1
SW34	Avon River: Katrine Bridge	Wheatbelt	Flowing	Hypersaline	15.80	8.00	6.60	22.00	5
SW35	Avon River: Mears Five Mile Pool	Wheatbelt	Flowing	Hypersaline	13.63	8.39	7.00	23.00	2
SW36	Bollonine Brook	Wheatbelt	Flowing	Hypersaline	9.32	7.72	3.01	11.60	2
SW37	Boyagerring Brook	Wheatbelt	Flowing	Hypersaline	4.96	8.28	9.51	8.30	2
SW38	Boyagin Creek	Wheatbelt	Flowing	Hypersaline	3.69	8.54	9.30	13.40	1
SW39	Brockman River	South-West	Flowing	Hypersaline	5.14	7.80	5.26	20.50	3
SW40	Cockerding Brook	Wheatbelt	Flowing	Hypersaline	12.79	8.22	7.66	9.10	2
SW41	Collie Changerup Rd Lake	Wheatbelt	Standing	Hypersaline	15.20	9.72	9.20	18.60	2
SW42	Collie River: Collie	South-west	Flowing	Hypersaline	3.65	7.19	5.46	11.50	2
SW43	Dale River South	Wheatbelt	Flowing	Hypersaline	3.44	8.30	7.60	12.70	1
SW44	Douglas Rd Lake	Wheatbelt	Standing	Hypersaline	15.50	10.36	13.10	22.20	3
SW45	Ibis Lake	Wheatbelt	Standing	Hypersaline	12.52	8.06	8.35	10.50	1
SW46	Jimperding Brook	Wheatbelt	Flowing	Hypersaline	3.20	7.82	8.69	16.40	2
SW47	Lake Indoon	Wheatbelt	Standing	Hypersaline	3.05	8.84	8.53	22.20	2
SW48	Lake Mears Overflow	Wheatbelt	Standing	Hypersaline	15.90	10.43	8.34	24.70	1
SW49	Lake Nambung	South-West	Standing	Hypersaline	5.71	10.31	3.93	12.10	2
SW50	Mackie River: Marley Pool Bridge	Wheatbelt	Flowing	Hypersaline	11.33	9.57	7.90	14.70	1
SW51	Moore River East: New Norcia	Wheatbelt	Flowing	Hypersaline	9.56	9.31	11.62	26.60	1
SW52	Murray River	South-West	Flowing	Hypersaline	4.33	8.07	7.84	10.90	2
SW53	Needoonga Lake	South-West	Standing	Hypersaline	5.39	7.66	3.31	20.70	5
SW54	North Rd Lake	Wheatbelt	Standing	Hypersaline	18.30	9.64	6.20	19.80	2
SW55	Towerrining Lake	Wheatbelt	Standing	Hypersaline	5.83	8.56	8.98	14.20	2
SW56	Walyormouring Lake Inflow	Wheatbelt	Flowing	Hypersaline	7.88	8.97	11.36	19.80	3
SW57	Wannamal Lake	South-West	Standing	Hypersaline	11.07	9.33	9.32	19.90	5
SW58	Williams Darkan Rd Lake	Wheatbelt	Standing	Hypersaline	5.11	10.51	10.60	20.20	2
SW59	Yornaning Dam	Wheatbelt	Standing	Hypersaline	3.33	9.07	7.96	11.30	2
SW60	Arдах Lake	Wheatbelt	Standing	Mesosaline	48.50	3.81	6.40	21.10	2
SW61	Cowcowing Lakes B	Wheatbelt	Standing	Mesosaline	36.80	10.11	7.70	15.20	1
SW62	Hines Hill Lake B	Wheatbelt	Standing	Mesosaline	23.70	4.47	8.88	23.60	1
SW63	Hotham River	Wheatbelt	Flowing	Mesosaline	21.20	9.60	6.63	24.70	1
SW64	Katanning Creek B	Wheatbelt	Flowing	Mesosaline	24.00	8.32	18.67	20.00	1
SW65	Kevill's Lake Overflow	Wheatbelt	Standing	Mesosaline	30.50	9.15	6.90	20.30	1
SW66	Lake Mears	Wheatbelt	Standing	Mesosaline	20.90	9.17	9.65	22.30	3
SW67	Lime Lake Rd West Lake	Wheatbelt	Standing	Mesosaline	44.80	8.01	7.70	23.60	1
SW68	Mortlock River	Wheatbelt	Flowing	Mesosaline	20.00	8.89	9.60	18.00	1
SW69	Nomans Lake	Wheatbelt	Standing	Mesosaline	36.60	8.15	6.12	10.30	1
SW70	Nomans Lake Inflow	Wheatbelt	Flowing	Mesosaline	31.20	9.27	11.85	17.10	2
SW71	Salt River	Wheatbelt	Flowing	Mesosaline	28.00	9.06	9.10	14.70	1
SW72	Shackleton Lake	Wheatbelt	Standing	Mesosaline	49.70	8.61	6.70	22.70	1
SW73	Wagin Lake	Wheatbelt	Standing	Mesosaline	21.60	9.50	10.20	19.80	1
SW74	Yarra Yarra Lakes Overflow	Wheatbelt	Standing	Mesosaline	20.90	10.28	7.84	24.90	2
SW75	Yenyening Lakes	Wheatbelt	Standing	Mesosaline	20.70	10.48	9.90	17.80	1
SW76	Ashwell Rd Lake	Wheatbelt	Standing	Hypersaline	79.20	8.05	7.00	28.10	2
SW77	Baandee Lake	Wheatbelt	Standing	Hypersaline	122.90	4.35	7.49	28.10	1
SW78	Coomelberrup Lake	Wheatbelt	Standing	Hypersaline	87.20	5.74	2.37	12.30	1
SW79	Cowcowing Lakes A	Wheatbelt	Standing	Hypersaline	156.80	2.90	4.80	20.80	1
SW80	Dumbleyung Lake	Wheatbelt	Standing	Hypersaline	154.10	8.64	1.14	13.10	1
SW81	Flagstaff Lake	Wheatbelt	Standing	Hypersaline	130.00	7.78	1.11	14.30	1
SW82	Hines Hill Lake A	Wheatbelt	Standing	Hypersaline	50.50	3.65	8.38	21.90	1
SW83	Kevills Lake	Wheatbelt	Standing	Hypersaline	59.40	7.22	4.47	21.50	1
SW84	Lake Brown	Wheatbelt	Standing	Hypersaline	50.20	6.55	4.36	21.00	2
SW85	Lake Champion	Wheatbelt	Standing	Hypersaline	73.70	6.21	3.71	17.90	1
SW86	Lake Dowerin	Wheatbelt	Standing	Hypersaline	77.90	8.56	4.60	14.10	1
SW87	Lake Koombekine	Wheatbelt	Standing	Hypersaline	71.40	8.57	5.60	20.10	1
SW88	Lake Ninan	Wheatbelt	Standing	Hypersaline	109.40	7.54	1.54	22.10	1
SW89	Lake Wallambin	Wheatbelt	Standing	Hypersaline	66.30	5.37	5.90	20.40	1
SW90	Lake Yealering	Wheatbelt	Standing	Hypersaline	96.20	7.26	2.11	19.00	1
SW91	Norring Lake	Wheatbelt	Standing	Hypersaline	95.60	8.45	6.40	22.00	1
SW92	Parkeyerring Lake	Wheatbelt	Standing	Hypersaline	92.80	8.03	7.80	23.60	1
SW93	Pink Lake	Wheatbelt	Standing	Hypersaline	121.10	7.79	6.40	22.40	1
SW94	Queerarrup Lake	Wheatbelt	Standing	Hypersaline	87.20	8.30	8.30	25.20	1
SW95	Toolbin Lake Inflow	Wheatbelt	Flowing	Hypersaline	51.30	8.48	3.37	15.70	3

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## Chapter 4: The Response of Diatom Community Structure to Varying Salinity from Lakes and Streams in South-West Western Australia

### 4.1 Abstract

The community structure of diatoms in relation to varying salinity concentration was investigated from the dataset of 95 lakes and streams in the south-west. Diatoms were sampled using an artificial substrate collector (JJ periphytometer) to ensure uniformity and that collection was indicative of ambient conditions. A total of 217 taxa were identified, with the highest diversity observed in freshwater sites (up to 33 species) and limited to less than 15 in hypersaline waters. NMDS ordination of the diatom data determined that significant differences were apparent according to geography, water body type and salinity categories. The BIOENV procedure showed that of the variables measured, salinity was most related to diatom community structure ( $\rho_w=0.54$ ) and may be responsible for shifts in species dominance. SIMPER analysis reported a number of discriminating taxa between sites located in the wheatbelt compared to the remainder of the south-west. The highest dissimilarity was observed between salinity categories and specifically between diatom assemblages from freshwater and hypersaline sites. Taxa such as *Achnantheidium minutissimum* and *Gomphonema parvulum* were frequently encountered and may be considered indicative of freshwaters. In comparison, *Amphora coffeaeformis* and *Nitzschia ovalis* were most commonly associated with hypersaline water bodies. These species may be used as potential indicators of the different salinity ranges for lakes and streams at risk of salinisation. The study provides the basis for monitoring programs that incorporate the use of diatoms, with the potential to be implemented into future management strategies that assess the biodiversity of secondary salinised systems in the south-west.

### 4.2 Introduction

The physico-chemical environment of lakes and streams in the south-west was discussed in detail in the previous chapter. This section of the thesis aims to investigate the relationship between abiotic factors, focussing on salinity and their relationship to diatoms, used as biological monitors.

Secondary salinisation of rivers and wetlands in the south-west of Western Australia has the potential to cause irreversible damage to the structure and function of aquatic communities (Williams 1999). Agricultural activities such as the installation of drainage networks may alter complex hydroperiods, increasing ionic concentrations and adversely affecting biodiversity (Pannell *et al.* 2001; Williams 2001; Lymbery *et al.* 2003). Diatoms have an integral role in the primary production of fresh and saline waters (Patrick 1994; John 2003) and are sensitive indicators that can be used to assess salinity changes through shifts in species composition (Snoeijs 1999). This feature means that they are a useful tool for monitoring the influence salinisation in lakes and streams of the south-west.

It has been well established that a qualitative relationship exists between diatom community structure and salinity concentration (Foged 1978; John 1983; Gell and Gasse 1990; Roberts and McMinn 1999; Witkowski *et al.* 2000). Investigations by Czarnecki and Blinn (1978), Archibald (1983), and Potapova and Charles (2003) have highlighted the importance of ionic composition and species assemblages in rivers and tributaries from the United States and South Africa. Similarly, studies by Gasse (1986) and Ehrlich (1995) in East Africa and Israel have shown a strong association between diatoms and salinity from wetland systems.

The influence of salinity on species diversity has also been widely documented (Ehrlich and Ortal 1979; Daemen *et al.* 1982; John 1988; van der Molen 1998). Studies by Blinn (1993) in North America showed that species numbers were highest in low conductivity lakes and Clavero *et al.* (2000) found a clear trend of decreasing diversity in hypersaline waters from Mexico. This inverse relationship between diatoms and salinity was also demonstrated from water bodies in Canada (Hammer *et al.* 1983; Wilson *et al.* 1994) and northern Egypt (Compère 1994). Research in eastern Australia by Blinn and Bailey (2001) and Gell *et al.* (2002) found that elevated salt loads led to reduced diatom diversity and a shift to more salt tolerant species from rivers and wetlands.

In Western Australia, there has been little research into diatom assemblages from varying salinities that encompass both standing and flowing waters. A number of studies have focussed on the Perth metropolitan area (John 1983; 1988; John and

Helleren 1998; Siva *et al.* 1998; John 2000) and primary salinised lakes located in the arid interior (John 1998a). In terms of secondary salinised systems, Blinn *et al.* (2004) analysed the diatom distribution from 56 wetlands in the wheatbelt, and John (1998b) assessed river health using diatoms as biomonitors in 46 rivers systems from the south-west. More recently, Taukulis and John (2006) sampled diatom communities from 51 inland water bodies, however the scope of this research was limited. In comparison, this study integrates a much larger number of lakes and streams that cover a broad geographical area and include a wide range of habitats from fresh to hypersaline waters.

The objectives of this chapter were to investigate patterns in diatom diversity from the south-west and to determine the influence of environmental variables, specifically salinity, on community structure. Potential indicator species of different salinity ranges were also examined. This study is the most extensive conducted on salt-affected water bodies of the south-west and attempts to provide a comprehensive database of diatom taxa from the region.

### **4.3 Materials and Methods**

#### *4.3.1 Environmental data*

Physico-chemical variables were measured from the 95 study sites (**Figure 4.1**), with a more detailed description presented in the previous chapter. Measurements of pH, salinity (ppt), dissolved oxygen ( $\text{mgL}^{-1}$ ), water temperature ( $^{\circ}\text{C}$ ) and peripheral vegetation density were recorded from surface waters and the results collated with the diatom dataset.

#### *4.3.2 Collection and preparation of diatom samples*

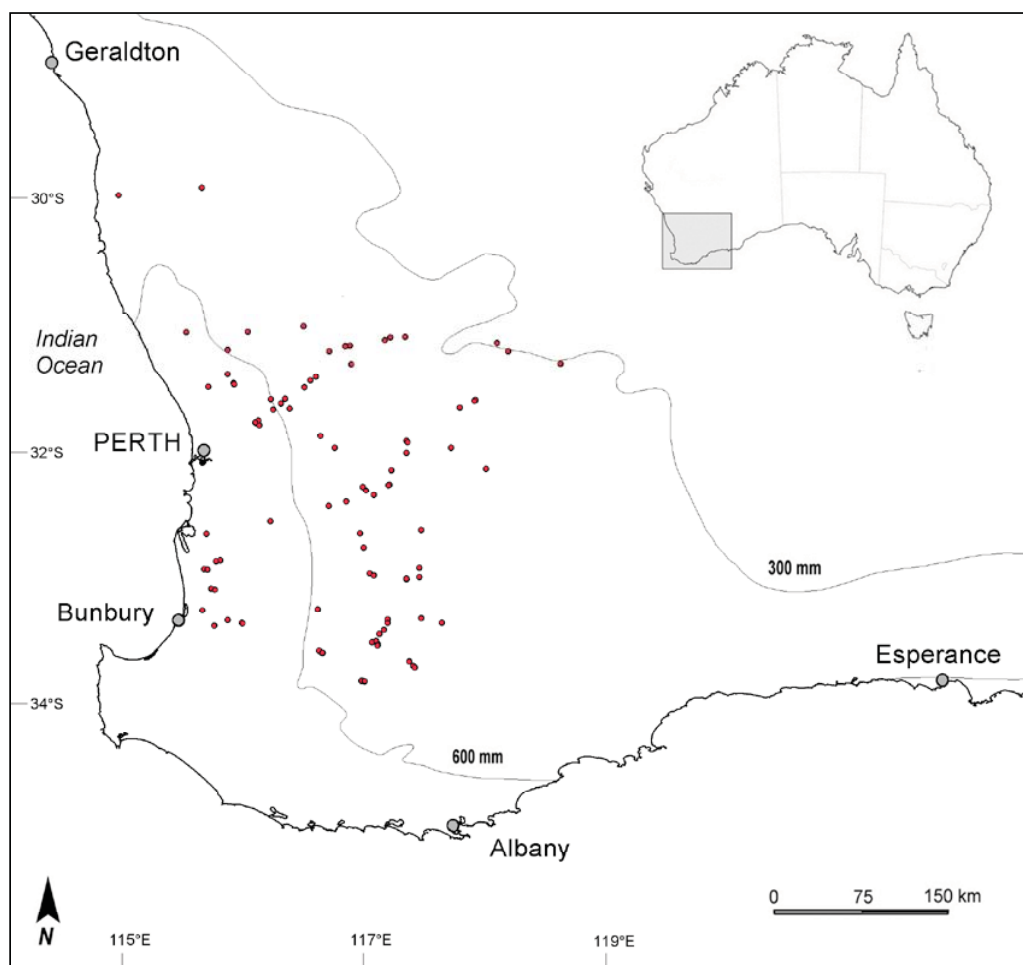
An artificial substrate collector; the JJ periphytometer (John 1998b) was employed for diatom collection at each site, ensuring that species assemblages reflected ambient conditions (Taukulis and John 2006). This device provided a uniform surface for colonisation by diatoms from periphytic, benthic, epiphytic and planktonic habitats. The periphytometer, containing 10 glass microscopy slides was secured to an appropriate structure in the water column (generally wooden stakes or tree branches) and left well immersed. A 14-day exposure period was sufficient for a climax population of diatoms to be reached, attaching to the glass slides (John

1998b). Once retrieved, the slides were transferred into 100 mL vials containing approximately 25 mL of deionised water and preserved with Lugol's iodine solution, in readiness for permanent slide preparation.

Slide making was carried out following techniques described by John (1983), to remove organic material from the samples and allow the siliceous cell wall structure of the diatoms to be viewed under a light microscope. The 10 slides from each site were scraped using a single edged razor blade and between 10-25 mL of sample placed into 100 mL Pyrex glass beakers. The equivalent volume of 70 % nitric acid (molecular weight=63.012 g/mol) was then added and the material digested (acid-cleaned) on a hotplate set to 80 °C in a fume cupboard. Once the solution had evaporated over a period of 2-3 hours, approximately 10 mL of sample remained, which was poured into centrifuge tubes to remove traces of nitric acid. Centrifugation was undertaken using a BHG Roto Uni II Centrifuge for a total of 5 minutes: thirty seconds on low (1500 rpm) and the remaining time on high-speed rotation (3500 rpm). After each centrifuge the supernatant was decanted and the diatoms were resuspended with deionised water.

Sterilised coverslips (22 × 22 mm) were placed on the hotplate and a volume of between 50 and 1000 µL of the cleaned sample (dependent on density of diatoms) was transferred with a Nichipet 5000DG digital micro-pipettor. If necessary the sample was diluted using deionised water. A drop of 70 % ethanol was added to ensure that the diatom frustules were evenly dispersed and the solution was left to evaporate. Once dry, the inverted coverslips were placed onto clean glass slides (76.2 × 25.4 mm) containing the toluene-based mounting medium Naphrax® (with a high refractive index of 1.74). Slides were then returned to the hotplate and removed from the heat when the solvent and air bubbles had dissipated. After cooling, the mounting medium solidified, with the completed slides providing a permanent record for identification purposes. A total of three replicates were made for each of the study sites and these voucher slides were then deposited at the International Diatom Herbarium, Department of Environmental Biology, Curtin University of Technology in Perth, Western Australia.





**Figure 4.1:** The location of the 95 study sites (indicated by ●) in the south-west region of Western Australia. The 600 and 300 mm annual isohyets approximately define the wheatbelt area.

#### 4.3.3 Enumeration and taxonomy of diatoms

The enumeration of diatoms was undertaken using an Orion BM-LUX-4 light microscope (and associated ocular graticule) at 1000× total magnification to determine species composition. A minimum of 300 diatom valves were counted from each site along horizontal transects, however for very sparse samples this number was reduced to 100 valves, considered adequate for routine analysis (Battarbee *et al.* 2001). Results were then converted to percentage abundances for statistical analyses.

Over 400 photomicrographs of representative species were taken with a light microscope (Olympus VANOX-S AH-2) and camera attachment (Olympus C-35AD2) on Kodak Technical Pan Black and White film. Total magnifications ranged from 200× to 1000×, and included a 10 µm scale to enable the dimensions of each taxon to be accurately established. Specialised literature used for identification was mainly: Hustedt (1930); Chohnoky (1966b; 1966a); Patrick and Reimer (1966); Schoeman (1973); Schoeman and Archibald (1976b; 1976a); Czarnecki and Blinn (1978); Foged (1978; 1979); Archibald (1983); John (1983; 1994; 1998b; 2000); Hustedt and Jensen (1985); Gasse (1986); Krammer and Lange Bertalot (1986); Osada and Kobayasi (1988), Holland and Clark (1989); Round *et al.* (1990); Lange-Bertalot and Moser (1994); Cumming *et al.* (1995); Ehrlich (1995); Hartley *et al.* (1996); Snoeijs and Balashova (1998); Roberts and McMinn (1999); Camburn and Charles (2000); Witkowski *et al.* (2000); Lange-Bertalot *et al.* (2003); Siver *et al.* (2005). The taxonomic nomenclature used in this thesis is based on Round *et al.* (1990) and Fourtanier and Kociolek (1999), with updated use of genera names applied where possible.

#### 4.3.4 Analysis of diatom community structure

Univariate indices including the number of species (S) and Shannon diversity [ $H' = -\sum P_i \log_e(P_i)$ , where  $P_i$  is the proportion of the observations found in category  $i$ ] (Shannon and Weaver 1964), were calculated. Patterns in diatom community structure were assessed using non-metric multi-dimensional scaling (referred to as NMDS), which allowed the data to be viewed in two dimensions (Kruskal and Wish 1978). Stress values of less than 0.2 were considered informative and appropriate for interpretation (Clarke and Warwick 2001). Species abundances were square-root

transformed and similarities between sites calculated with the Bray-Curtis coefficient (Bray and Curtis 1957). Analysis of similarities (ANOSIM) (Clarke and Green 1988), a non-parametric permutation technique, was used to identify differences between diatom assemblages and site groupings including geographical location, waterbody type and salinity category. Up to 999 random permutations estimated the probability of observed results (p-value), with levels of significance reported as  $p < 0.05$ ,  $< 0.01$  and  $< 0.001$ . The degree of separation (R statistic) for pairwise comparisons was also examined where values close to one indicated the complete separation of groups and values approaching zero implied little or no segregation (Clarke and Warwick 2001). The BIOENV procedure (Clarke and Ainsworth 1993) was employed to determine environmental variables that best reflected diatom community structure, measured using the weighted Spearman rank correlation coefficient ( $\rho_w$ ). Similarity Percentage (SIMPER) analysis established the diatom species that were most responsible for contributing to differences in the data. Species were then ranked in order of their average percentage contribution to the total average dissimilarity between the site groupings (Clarke and Warwick 2001). The results provide an indication of the first five taxa with the most contribution from this procedure. All statistical analyses were performed in PRIMER 5 (Plymouth Marine Laboratory 2002).

## 4.4 Results

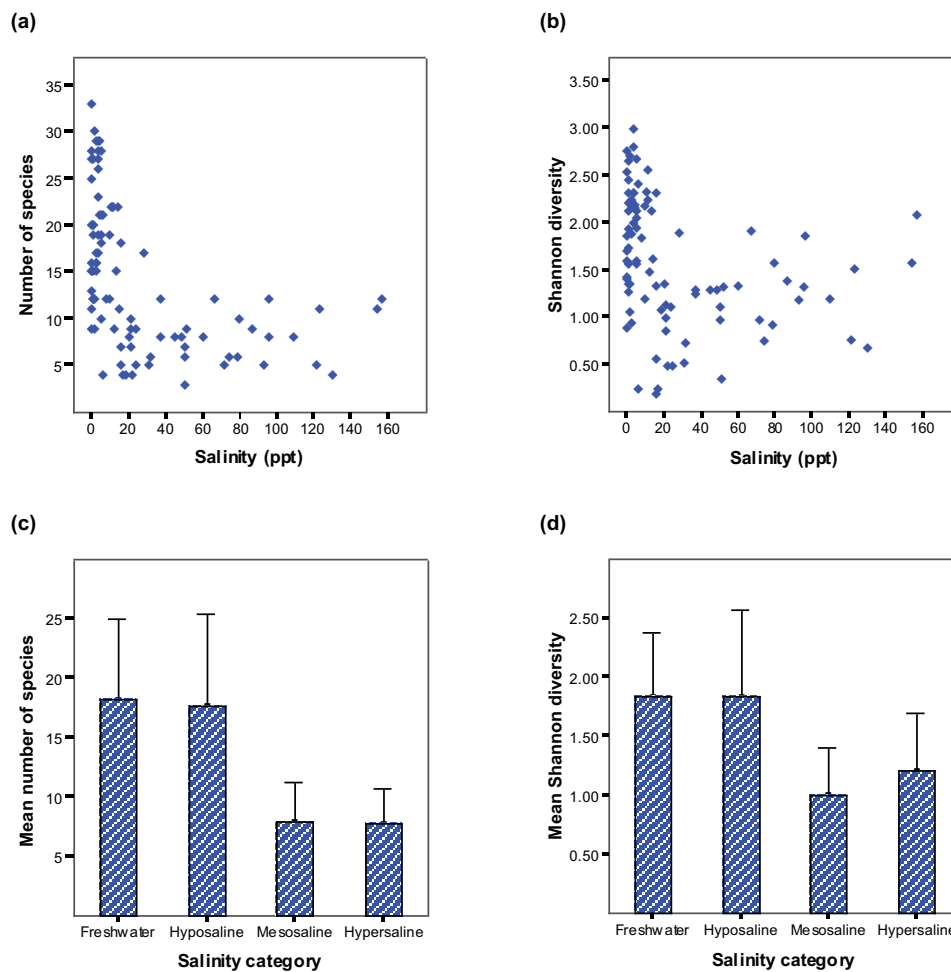
### 4.4.1 Diversity indices

A total of 217 taxa consisting of 56 genera were identified from the 95 lakes and streams in the south-west. The most dominant genera in descending order were *Nitzschia* (35 species), *Navicula* (31 species) and *Amphora* (13 species). Commonly occurring species included *Amphora* sp. aff. *luciae* and *Amphora coffeaeformis*, recorded from over 40 study sites (**Table 4.1**). This was followed by *Cocconeis placentula*, *Navicula cryptocephala* and *Tabularia tabulata*, found in more than 30 sites (**Table 4.1**). The complete list of species names and their authorities along with mean relative abundances have been provided in **Appendix 4.1**.

**Table 4.1:** Diatom taxa occurring in greater than or equal to 10 south-west study sites, with an abundance of greater than or equal to 1 %, in order from most common to least common species.

Taxon	Number of occurrences	Taxon	Number of occurrences
<i>Amphora</i> sp. aff. <i>luciae</i>	44	<i>Navicula cincta</i>	14
<i>Amphora coffeaeformis</i>	42	<i>Navicula</i> sp. aff. <i>salinicola</i>	14
<i>Cocconeis placentula</i>	36	<i>Nitzschia</i> sp. aff. <i>rostellata</i>	14
<i>Navicula cryptocephala</i>	32	<i>Stauroneis pachycephala</i>	14
<i>Tabularia tabulata</i>	32	<i>Achnanthydium oblongella</i>	13
<i>Nitzschia palea</i>	27	<i>Amphora</i> sp. aff. <i>coffeaeformis</i>	13
<i>Nitzschia frustulum</i>	26	<i>Brachysira styriaca</i>	13
<i>Nitzschia microcephala</i>	26	<i>Entomoneis tenuistriata</i>	13
<i>Achnanthydium minutissimum</i>	24	<i>Navicula salinarum</i>	13
<i>Cyclotella meneghiniana</i>	24	<i>Navicula</i> sp. aff. <i>agrestis</i>	13
<i>Navicula tripunctata</i>	24	<i>Nitzschia</i> sp. aff. <i>fontifuga</i>	13
<i>Planothidium lanceolatum</i>	24	<i>Synedra ulna</i>	13
<i>Bacillaria paxillifer</i>	22	<i>Fallacia tenera</i>	12
<i>Navicella pusilla</i>	21	<i>Craticula halophila</i>	12
<i>Nitzschia ovalis</i>	19	<i>Pleurosigma elongatum</i>	12
<i>Thalassiosira weissflogii</i>	19	<i>Cyclotella stelligera</i>	11
<i>Haslea spicula</i>	17	<i>Mastogloia pumila</i>	11
<i>Navicula perminuta</i>	17	<i>Navicula</i> sp. aff. <i>incertata</i>	11
<i>Entomoneis paludosa</i>	16	<i>Proschkinia</i> sp. aff. <i>complanata</i>	11
<i>Nitzschia sigma</i>	16	<i>Tryblionella hungarica</i>	11
<i>Gomphonema parvulum</i>	15	<i>Amphora veneta</i>	10
<i>Nitzschia aurariae</i>	15	<i>Encyonopsis microcephala</i>	10
<i>Achnanthydium reidensis</i>	14	<i>Tryblionella apiculata</i>	10
<i>Hantzschia</i> sp. aff. <i>baltica</i>	14		

Overall there was an inverse relationship between species numbers and salinity concentration, with the highest number of taxa (33) observed in less than 1 ppt and the lowest (three) from greater than 20 ppt. As salinity concentrations approached 50 ppt species numbers declined sharply, and above this value were limited to less than 15 (**Figure 4.2a**). Shannon diversity displayed a similar trend with a maximum index value of 2.99 and minimum of 0.19 (**Figure 4.2b**). According to Hammer's (1986) salinity classification system the mean number of species and Shannon diversity (**Figure 4.2c-d**) was also greatest in the freshwater (mean=18.11 and 1.84) and hyposaline categories, (mean=17.55 and 1.83) and decreased markedly in the mesosaline (mean=7.88 and 1.00) and hypersaline groups (mean=7.75 and 1.20). **Appendix 4.2** presents the number of species and Shannon diversities recorded from the study sites with the minimum, maximum and mean values according to salinity regime.



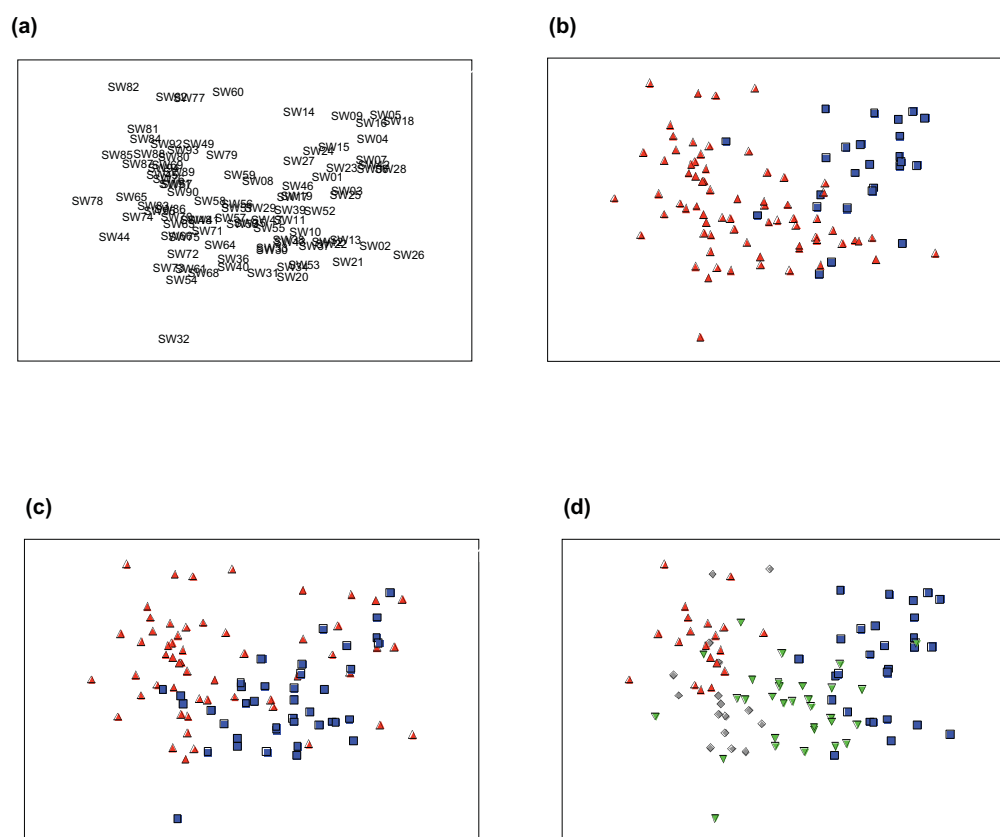
**Figure 4.2:** Diversity indices for diatom taxa from the south-west study sites. (a) Number of species and (b) Shannon diversity in relation to salinity concentration. (c) Mean number of species and (d) mean Shannon diversity based on Hammer's (1986) salinity categories, where error bars represent  $\pm 1.0$  standard error of the mean. The y-axis scale is different for each of the indices presented.

#### 4.4.2 NMDS, ANOSIM and BIOENV analysis

The NMDS ordination of sites based on similarities in diatom community structure generated an acceptable stress level (0.16) and revealed discrete patterns according to geography, water body type and salinity categories (**Figure 4.3a-d**). Sites were clearly separated into the wheatbelt area and the south-west (**Figure 4.3b**). This was confirmed by ANOSIM testing, which indicated a significant difference ( $p < 0.01$ , **Table 4.2**) in diatom assemblages from the two regions, supported by a moderate R statistic (0.51). Sites also clustered into standing and flowing waters (**Figure 4.3c**), with ANOSIM showing a significant difference ( $p < 0.01$ , **Table 4.2**). However, the associated R value was low ( $R = 0.18$ ) pointing to there being little variation between diatom communities from the two water body types based on collections with the periphytometer. ANOSIM determined that in relation to species composition and Hammer's (Hammer 1986) salinity regime all categories were significantly different ( $p < 0.01$ , **Table 4.2**). Freshwater and hypersaline sites had the most distinct diatom communities and the highest R value of 0.91, followed by the freshwater and mesosaline groups with an R value of 0.81. In contrast, there was little segregation between categories closest together, demonstrated by hyposaline and mesosaline waters displaying the lowest R statistic of 0.28. These findings can be seen on the NMDS plot with salinity categories superimposed (**Figure 4.3d**).

**Table 4.2:** Summary of one-way analysis of similarities (ANOSIM) performed on square-root diatom data. Pairwise comparisons are shown for geographical location (south-west and wheatbelt regions), water body type (standing and flowing waters) and Hammer's (1986) salinity categories. Bold type indicates R statistics greater than 0.50, and significant p-values (\*) are also presented.

Factor	Pairwise comparison	R statistic	Probability (p)
Geographical location	south-west, wheatbelt	0.42	<0.01*
Waterbody type	standing, flowing	0.18	<0.01*
Salinity category	freshwater, hyposaline	0.38	<0.01*
	freshwater, mesosaline	<b>0.81</b>	<0.01*
	freshwater, hypersaline	<b>0.91</b>	<0.01*
	hyposaline, mesosaline	0.28	<0.01*
	hyposaline, hypersaline	<b>0.51</b>	<0.01*
	mesosaline, hypersaline	0.27	<0.01*



**Figure 4.3:** Multi-dimensional scaling (NMDS) ordination of the 95 study sites from the south-west based on square-root transformed diatom abundance data. (a) NMDS plot showing site distribution using site codes (b) NMDS plot with geographical locations superimposed where ■ are south-west and ▲ indicate wheatbelt sites. (c) NMDS plot with water body types superimposed where ▲ are standing waters and ■ represent flowing waters. (d) NMDS plot with salinity categories superimposed where ■ are freshwater <3 ppt sites, ▼ are hyposaline 3-20 ppt sites, ◆ are mesosaline 20-50 ppt sites and ▲ are hypersaline  $\geq 50$  ppt sites. Stress level=0.16.

The BIOENV procedure (**Table 4.3**), determined that salinity was the best explanatory variable in relation to the diatom data and had the highest correlation coefficient of 0.62. This was closely followed by the combination of salinity and pH, with a correlation of 0.59. Salinity also featured in the remaining combinations of environmental variables that reflected community structure.

**Table 4.3:** Summary of BIOENV results, with variable combinations that provide the highest Spearman rank correlation coefficients ( $\rho_w$ ) between environmental ( $\log_{10}$  salinity) and square-root diatom data. Bold type indicates overall optimum, only those combinations with a correlation coefficient greater than 0.40 have been shown.

Number of variables	Best variable combination	Correlation ( $\rho_w$ )
1	<b>salinity</b>	<b>0.62</b>
2	salinity, pH	0.59
3	salinity, pH, temperature	0.50
4	salinity, pH, temperature, vegetation	0.44
5	salinity, pH, dissolved oxygen, temperature, vegetation	0.40

#### 4.4.3 SIMPER results

SIMPER analysis was used to identify the dominant species contributing to differences according to geography, water body type and salinity regime (**Table 4.4 and 4.5**). There was a high mean dissimilarity (94.02) between diatom communities based on location, with *Amphora* sp. aff. *luciae* and *Amphora coffeaeformis* abundant in wheatbelt sites and *Achnantheidium minutissimum* dominant in the south-west. The latter was also the most consistent taxa (a good discriminating species) in relation to these geographical regions, with a ratio of greater than 1.00 (**Table 4.4**). Comparisons between water body types also showed a relatively high dissimilarity (91.02), with *Amphora coffeaeformis* typical of standing waters and *Nitzschia frustulum* associated with flowing waters. However, *Amphora* sp. aff. *luciae* was a common taxon in both. The most consistent species (with a ratio of close to 1.00) based on water body type was *Amphora coffeaeformis* (**Table 4.4**).



**Table 4.4:** Summary of similarity percentage (SIMPER) analysis showing species contributing to the greatest differences in diatom community structure based on geographical location and water body type. The average abundance of species in each group has been provided and the mean dissimilarity values indicate the degree of differences between the composition for each group. Consistency ratios demonstrate the usefulness of each species in discriminating between groups (higher values are more consistent). The % contribution of each species to the total dissimilarity as well as the cumulative % contribution is also given.

Taxon	Mean abundance		Consistency ratio	Contributing %	Cumulative %
	south-west	wheatbelt			
1 <i>Amphora</i> sp. aff. <i>luciae</i>	0.92	15.79	0.77	4.91	4.91
2 <i>Amphora coffeaeformis</i>	3.98	12.33	0.77	4.87	9.79
3 <i>Achnanthydium minutissimum</i>	8.80	0.05	1.21	4.10	13.89
4 <i>Bacillaria paxillifer</i>	7.12	0.78	0.59	2.63	16.52
5 <i>Brachysira styriaca</i>	4.94	0.04	0.63	2.26	18.78
<i>mean dissimilarity:</i>		94.02			
Taxon	Mean abundance		Consistency ratio	Contributing %	Cumulative %
	flowing	standing			
1 <i>Amphora</i> sp. aff. <i>luciae</i>	11.17	12.40	0.84	5.95	5.95
2 <i>Amphora coffeaeformis</i>	1.74	16.23	0.90	5.50	11.45
3 <i>Nitzschia frustulum</i>	5.82	0.47	0.69	2.76	14.21
4 <i>Nitzschia ovalis</i>	0.00	6.78	0.50	2.69	16.90
5 <i>Tabularia tabulata</i>	3.97	0.30	0.75	2.30	19.20
<i>mean dissimilarity:</i>		91.02			

In relation to salinity categories, the highest level of mean dissimilarity (98.78) was found between the freshwater and hypersaline sites, following patterns observed in the NMDS and ANOSIM analyses. Differences were largely attributable to *Achnanthydium minutissimum* in freshwaters and *Amphora coffeaeformis* and *Nitzschia ovalis* in the hypersaline range (**Table 4.5**). The lowest mean dissimilarity (80.51) occurred between the mesosaline and hypersaline sites, demonstrated by the presence of *Amphora coffeaeformis*, which was abundant in the two groups (**Table 4.5**). Ratio values above 1.00 were shown by *Achnanthydium minutissimum*, *Amphora coffeaeformis* and *A. sp. aff. luciae*, considered to be some of the most consistent taxa overall.

**Table 4.5:** Summary of similarity percentage (SIMPER) analysis showing species contributing to the greatest differences in diatom community structure based on Hammer's (1986) salinity categories. The average abundance of the species in each group has been provided and the mean dissimilarity values indicate the degree of differences between the composition for each group. Consistency ratios demonstrate the usefulness of each species in discriminating between groups (higher values are more consistent). The % contribution of each species to the total dissimilarity as well as the cumulative % contribution is also given.

Taxon	Mean abundance	Mean abundance	Consistency ratio	Contributing %	Cumulative %
	freshwater	hyposaline			
1 <i>Amphora</i> sp. aff. <i>luciae</i>	0.27	12.22	0.75	4.21	4.21
2 <i>Achnanthidium minutissimum</i>	7.43	0.49	1.01	3.29	7.50
3 <i>Nitzschia frustulum</i>	5.41	3.46	0.84	2.97	10.46
4 <i>Amphora coffeaeformis</i>	0.32	6.40	0.54	2.62	13.08
5 <i>Bacillaria paxillifer</i>	3.70	4.07	0.66	2.52	15.61
	<b>mean dissimilarity: 90.51</b>				
	freshwater	mesosaline			
1 <i>Amphora</i> sp. aff. <i>luciae</i>	0.27	36.80	1.20	9.70	9.70
2 <i>Amphora coffeaeformis</i>	0.32	14.06	0.79	4.73	14.44
3 <i>Achnanthidium minutissimum</i>	7.43	0.00	1.02	3.67	18.11
4 <i>Nitzschia palea</i>	4.47	0.00	1.02	2.88	20.99
5 <i>Gomphonema parvulum</i>	6.93	0.00	0.51	2.78	23.77
	<b>mean dissimilarity: 97.61</b>				
	freshwater	hypersaline			
1 <i>Amphora coffeaeformis</i>	0.32	26.50	1.63	8.29	8.29
2 <i>Nitzschia ovalis</i>	0.00	16.14	0.88	5.62	13.91
3 <i>Achnanthidium minutissimum</i>	7.43	0.00	1.02	3.54	17.45
4 <i>Amphora</i> sp. aff. <i>luciae</i>	0.27	7.67	0.77	3.24	20.70
5 <i>Navicula</i> sp. aff. <i>incertata</i>	0.00	7.73	0.66	3.09	23.79
	<b>mean dissimilarity: 98.78</b>				
	hyposaline	mesosaline			
1 <i>Amphora</i> sp. aff. <i>luciae</i>	12.22	36.80	1.19	9.99	9.99
2 <i>Amphora coffeaeformis</i>	6.40	14.06	0.83	6.15	16.14
3 <i>Cocconeis placentula</i>	3.52	2.56	0.98	3.23	19.36
4 <i>Nitzschia</i> sp. aff. <i>archibaldii</i>	2.61	5.28	0.65	3.16	22.53
5 <i>Cylindrotheca closterium</i>	6.06	1.50	0.45	2.98	25.51
	<b>mean dissimilarity: 87.19</b>				
	hyposaline	hypersaline			
1 <i>Amphora coffeaeformis</i>	6.40	26.50	1.44	8.33	8.33
2 <i>Nitzschia ovalis</i>	0.00	16.14	0.86	6.23	14.55
3 <i>Amphora</i> sp. aff. <i>luciae</i>	12.22	7.67	0.90	5.72	20.28
4 <i>Navicula</i> sp. aff. <i>incertata</i>	0.00	7.73	0.65	3.42	23.70
5 <i>Proschkinia</i> sp. aff. <i>complanata</i>	3.15	6.23	0.43	3.12	26.82
	<b>mean dissimilarity: 91.15</b>				
	mesosaline	hypersaline			
1 <i>Amphora</i> sp. aff. <i>luciae</i>	36.80	7.67	1.21	13.35	13.35
2 <i>Amphora coffeaeformis</i>	14.06	26.50	1.52	11.26	24.61
3 <i>Nitzschia ovalis</i>	3.14	16.14	0.92	8.81	33.42
4 <i>Navicula</i> sp. aff. <i>salinicola</i>	5.39	5.74	0.68	5.30	38.72
5 <i>Navicula</i> sp. aff. <i>incertata</i>	1.20	7.73	0.72	5.16	43.88
	<b>mean dissimilarity: 80.51</b>				

## 4.5 Discussion

### 4.5.1 Species indices

The total number of taxa identified from the south-west sites (217) was comparatively high, with previous studies from the region recording between 72 and 200 species (John and Hellen 1998; Blinn *et al.* 2004; Taukulis and John 2006). The majority of diatoms were representative of fresh and hyposaline waters, with the highest number (33) found at Harvey Dam (SW06), a protected resource used for industrial and domestic purposes (Schofield *et al.* 1988; Jakowyna 2000). Similar numbers were also observed at freshwater sites including Bailup Rd Lake (SW01) and Mistake Creek (SW19) and from hyposaline locations such as Dale River South (SW43) and Murray River (SW52). It has been established that these ranges are generally productive, with Blinn *et al.* (2004) reporting over 30 taxa from salinities less than 3 ppt in the wheatbelt of Western Australia and Gell and Gasse (1990) finding species numbers were greatest in concentrations less than 20 ppt from eastern Australia. In these conditions, there tends to be an even distribution and a high number of species, rather than the dominance of a few diatoms, which may result from environmental or anthropogenic changes to the system (Patrick and Reimer 1966).

As salt loads increased, the well-documented relationship of reduced diversity became apparent (Williams 1981; Hammer 1986; Halse *et al.* 2003). This was demonstrated by a marked decline in species numbers from the mesosaline and hypersaline water bodies. The lowest number of taxa (three) occurred in the hypersaline Hines Hill Lake A (SW82) and the mesosaline Lake Mears (SW66). Hines Hill Lake A was both acidic (pH<4) and saline at the time of sampling, creating an environment that very few biota are able to tolerate (Halse *et al.* 2003; Davis 2004; Timms 2005). Low species numbers (five or less) were also common in the hypersaline category, with examples such as Flagstaff and Wagin Lakes being part of a wetland chain that consistently exhibit high ionic concentrations (Pen 1997; Cale *et al.* 2004). Studies in the wheatbelt by Taukulis and John (2006) found that species numbers were limited to less than 10 in salinities greater than 40 ppt. Also in this region, Blinn *et al.* (2004) showed that taxa numbers were restricted to below 15 in concentrations above 80 ppt. The correlation between salinity and diversity

however, is not strictly linear (Williams 1998), with characteristics such as surrounding land use and hydroperiod influencing water chemistry and species composition (Davis 2004; Halse 2004).

#### 4.5.2 *Multivariate differences in species composition*

NMDS and ANOSIM results showed a significant difference between diatom assemblages located in the wheatbelt compared to the remainder of the south-west, most likely due to the increased salinities of inland waters. It has been well-documented that areas receiving less than 600 mm of annual rainfall have been severely salt-affected (Halse *et al.* 2003), reflected in the dominance of halotolerant taxa recorded from this region. Similar findings have also been observed for other groups of aquatic biota including macroinvertebrates (Kay *et al.* 2001; Pinder *et al.* 2005), as well as submerged and riparian vegetation communities (Davis *et al.* 2003; Lymbery *et al.* 2003). Increases in soil and water salinity are occurring throughout the wheatbelt, causing a shift to more salt-tolerant biota and leading to an overall reduction in the diversity of lakes and streams (Halse *et al.* 2003; Timms 2005).

Diatom composition, although shown to be significantly different in standing and flowing waters, had a very low R separation value, supporting the inclusion of the two types of water bodies. This may also reflect sampling methods, with the JJ periphytometer providing a uniform substrate (John 1998b). Research by Ehrlich (1995) on diatoms from Israel integrated streams, rivers, lakes and artificial pools finding freshwater and saline species common to both. Blinn and Bailey (2001) also reported similar results on the composition of salt-affected lakes and streams in Victoria, most likely related to the efficient dispersal mechanisms of diatoms (Blinn 1995). Generally however, there has been little work carried out on taxa from standing and flowing waters, with this study indicating that salinised wetlands and rivers tend to have comparable assemblages.

The most significant difference from the ANOSIM analysis was between diatom composition and Hammer's (1986) salinity classification system, with the greatest separation amongst freshwater and hypersaline sites. In comparison, the higher salinity categories (mesosaline and hypersaline waters) showed the least amount of

difference, with species displaying much broader tolerance limits. A number of authors have also reported the persistence of some taxa over a wide spectrum of salinities (Gell and Gasse 1990; Compère 1994; Clavero *et al.* 2000). This finding is further supported by Williams (1998; 2001) noting that aquatic biota typical of freshwaters tend to have lower tolerance levels, compared to those inhabiting saline conditions.

The BIOENV procedure further suggests that salinity was the overriding factor influencing diatom community structure, attributable to the wide range of concentrations included in the study. Salinity has also been reported as one of the most important determinants in river systems from South Africa (van der Molen 1998) and the United States (Potapova and Charles 2003) as well as from lakes in Canada (Hammer *et al.* 1983) and Australia (Blinn 1995; Gell *et al.* 2002; Taukulis and John 2006). Although considered to be a defining variable of diatom distribution patterns, changes in species abundance and composition may be a result of a combination of environmental factors (Snoeijs 1999).

Salinity and pH were also shown to be influential variables and may be due to an overall decrease in the pH of hypersaline waters. It is important to recognise that factors not taken into account in this study such as acidification, eutrophication and pesticide contamination may also be responsible for diatom composition (Dixit *et al.* 1987; Peterson *et al.* 1997; Siregar and Nakahara 2000; Sonneman *et al.* 2001; Sienkiewicz *et al.* 2006). The occurrence of taxa including *Bacillara paradoxa*, *Nitzschia palea* and *Nitzschia frustulum* for example, may be indicative of impacts such as nutrient loading and intensive agriculture (Schoeman 1973; John 1998b; van der Molen 1998; Blinn and Bailey 2001; Sylvestre *et al.* 2001). Although nutrient concentration was not measured, it has been established that rivers and tributaries from the Avon catchment are susceptible to increased levels via fertiliser run-off (Avon River System Management Committee and the Waterways Commission 1993; Water and Rivers Commission and Avon Waterways Committee 2002). This aspect may require further research, in order to determine the specific affects of land use related factors on diatom community structure.

#### 4.5.3 Discriminating taxa

According to SIMPER results diatoms including *Achnantheidium minutissimum*, *Bacillaria paxillifer* and *Brachysira styriaca*, were distinct to south-west water bodies and are taxa commonly associated with low salinities and anthropogenic influences. Both *Achnantheidium minutissimum* and *Brachysira styriaca* have been related to relatively pristine sites with low conductivities by Gell and Gasse (1990); Cumming *et al.* (1995) and John (2000). In contrast, *Bacillaria paxillifer* has been linked to habitats subjected to intensive agriculture and nutrient enrichment (John 1998b; Blinn and Bailey 2001), reflecting the degradation of wetlands in the south-west (Davis and Froend 1999). Assemblages that distinguished inland wheatbelt regions included *A. sp. aff. luciae*, a taxon capable of withstanding elevated salt loads (Witkowski *et al.* 2000) and *Amphora coffeaeformis*, a well-known and widespread saline species (Ehrlich 1995; Sylvestre *et al.* 2001). These diatoms indicate the extent of salinisation in lakes and streams throughout the area, with the majority of aquatic biota inhabiting these waters considered to be halotolerant (Nielsen *et al.* 2003; Pinder *et al.* 2005; Strehlow *et al.* 2005).

Differences between flowing and standing waters were also significant, however it is clear that both have been affected by increasing salt loads attributable to surrounding agricultural practices (Boulton and Brock 1999; Williams 1999). This is supported by species such as *Amphora sp. aff. luciae* being relatively abundant in streams and wetlands, with the salinisation of flowing and standing waters closely related (Williams 2001). In addition, the temporary nature of rivers and streams in this area causes water to stagnate, resulting in characteristics similar to that of lake systems. Taxa that discriminated between the water body types were *Nitzschia frustulum* and *Tabularia tabulata*, common in flowing waters, and also capable of tolerating elevated salinities (Ehrlich 1995; John 1998b; Blinn and Bailey 2001; Blinn *et al.* 2004). In comparison, *Amphora coffeaeformis* and *Nitzschia ovalis* were associated with standing waters, and are known to dominate in extreme hypersalinity (Compère 1994; Blinn *et al.* 2004), reflecting higher salt concentrations in standing waters.

Freshwater diatom species were completely separated from higher salinity levels including the mesosaline and hypersaline groups, although there was some overlap in community structure with the hyposaline category. For example, species such as

*Bacillaria paxillifer*, *Nitzschia frustulum* and *N. palea* occurred in both of the lower range groups. These species have been previously reported as tolerant of increased salinity levels in the south-western United States (Czarnecki and Blinn 1978), Israel (Ehrlich 1995) and Brazil (Sylvstre *et al.* 2001) and from the east and south-west coasts of Australia (John 1998b; Blinn and Bailey 2001). However, the freshwater category had a number of abundant taxa discriminating salinities less than 3 ppt including *Achnantheidium minutissimum* and *Gomphonema parvulum*. Both have been documented from freshwater habitats throughout the world, including North America (Blinn 1993), Israel (Ehrlich 1995), East Africa (Gasse 1986) and New Zealand (Foged 1979). Also in Australia, they have been identified as common taxa in fresher ranges (<3 ppt) by Gell and Gasse (1990) and Blinn and Bailey (2001), and have been highly correlated to freshwater sites from the south-west by Blinn *et al.* (2004) and Taukulis and John (2006). The preference of *Achnantheidium minutissimum* and *Gomphonema parvulum* for waters with low ionic composition means they may be considered as potential indicators of freshwater, or undisturbed sites that are not influenced by secondary salinisation in south-west lakes and streams.

There were some comparable species assemblages in the mesosaline and hyposaline groups, which also had the lowest dissimilarity overall, including *Amphora* sp. aff. *luciae*, *Amphora coffeaeformis*, *Navicula* sp. aff. *salinicola* and *N.* sp. aff. *incertata* and *Nitzschia ovalis*. Changes however, were noted in the relative abundance of these taxa as salinity increased to over 50 ppt. For example, *Amphora* sp. aff. *luciae* was dominant in mesosaline waters, compared to *Nitzschia ovalis* in hypersaline waters and *Amphora coffeaeformis* was highly abundant in both. *Amphora coffeaeformis* has been found to tolerate habitats with high salinities (above 50 ppt) in parts of Africa (Gasse 1986; Compère 1994), North America (Blinn 1993), and Canada (Hammer *et al.* 1983; Cumming *et al.* 1995). This taxon is also widespread throughout Australia from the east coast (Gell and Gasse 1990; Blinn and Bailey 2001) to the arid central region (Blinn 1991) and the south-west, where it has been observed as one to the most dominant species inhabiting Western Australian salt lakes (John 1998b; Blinn *et al.* 2004; Taukulis and John 2006). Also in the south-west, Taukulis and John (2006) and Blinn *et al.* (2004) have identified hypersaline species including *Navicula* sp. aff. *salinicola* and *Nitzschia ovalis*, taxa also found in salt lakes from Western

Victoria, Egypt and East Africa (Gasse 1986; Gell and Gasse 1990; Compère 1994). *Navicula* sp. aff. *incertata* has only previously been reported from wetlands on the east coast, however it has been associated with similar environments to *Amphora coffeaeformis* (Gell and Gasse 1990; Gell *et al.* 2002). The presence of these characteristic taxa together with their high tolerances to increasing salt loads, means that they may be considered useful as indicators of salinisation.

#### 4.5.4 Comparisons in community structure

Artificial substrates were the preferred method of diatom sampling for this study as they integrate ambient water quality conditions and provide a representation of diatoms found growing in periphytic, benthic, epiphytic and planktonic habitats (Taukulis and John 2006). The use of artificial substrates also avoids the problem of old valves, with living diatoms colonising the substrata during the exposure period (John 1998b). This technique aims to reduce the confusion that may occur from using sediment samples, which can often incorporate diatom communities over seasons, especially in shallow fluctuating environments (Fritz *et al.* 1993; Gasse *et al.* 1997). It is important to provide uniformity when integrating lakes and streams for the purpose of biomonitoring and artificial substrates are extremely valuable in this type of comparative research, providing a means of standardising habitats (Aloi 1990).

The method of diatom collection can also account for differences in diatom composition found in similar regions such as the wheatbelt. For example, *Hantzschia amphioxys*, *Nitzschia hybrida* and *Tryblionella hungarica* were important taxa recorded from some of the most severely salinised wetlands by Blinn *et al.* (2004), however were rarely observed in samples from this study. Reasons for this may include the habitat preferences of these genera, with *Hantzschia* and *Nitzschia* in particular being associated with damp soil and eroded sediments (John 2000). In contrast, *Amphora coffeaeformis* was characteristic of hypersaline waters in both studies showing the resilience of this taxon and high adaptive ability in salt-affected wetlands.

The results from this study provide the most comprehensive database on diatom assemblages and their relationship to secondary salinisation in the south-west. In



general, species composition was most similar to that reported from inland areas of Western Australia (Blinn *et al.* 2004; Taukulis and John 2006) and parts of eastern Australia (Gell and Gasse 1990; Gell *et al.* 2002). The efficient dispersal mechanisms of diatoms have most likely led to their uniform distribution throughout Australian water bodies (Blinn *et al.* 2004), related to the temporary nature of many lake systems that contain widely dispersed species and few endemics (Williams and Kokkinn 1988). Many of the diatom taxa identified from lakes in the south-west are also common in Africa, however some hypersaline species require further taxonomic investigation. Although preliminary, discriminating taxa identified from this research have the potential to be used as indicators for future long-term biomonitoring studies that incorporate diatoms, for those systems that may be subjected to secondary salinisation.

#### **4.6 Conclusion**

Diatom community structure from lakes and streams in the south-west was investigated to determine their response to varying salinity levels. Species numbers were greatest in freshwater, and restricted to less than 15 in hypersaline sites. Multivariate analyses confirmed that salinity was the main factor that best explained diatom composition. A number of taxa were identified as discriminators between groups according to geography, water body type and salinity regime. For example, *Brachysira styriaca* was indicative of pristine sites located further south, and *Tabularia tabulata* had a high abundance in streams with low salinities. The most important indicator species were those that discriminated between the different salinity categories. *Achnantheidium minutissimum* and *Gomphonema parvulum* were characteristic of freshwater, in contrast *Amphora coffeaeformis* and *Nitzschia ovalis* dominated hypersaline conditions. Taxa such as *Amphora* sp. aff. *luciae* and *Navicula* sp. aff. *incertata* were also abundant in salinities above 50 ppt and together with the aforementioned species may be considered indicators of secondary salinity. This study provides baseline data on the distribution of diatoms along a salinity gradient from a range of lakes and streams throughout the south-west. Specifically, diatoms associated with increasing salt loads have the potential to be used in future biomonitoring programs, for the assessment of water bodies at risk of salinisation.

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#### 4.7 References

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**Appendix 4.1:** List of diatom taxa (with authorities), identified from the 95 study sites in the south-west of Western Australia. Number of occurrences (No occ), maximum abundance (Max abun) and mean relative abundance (Mean abun, zeroes removed) are shown in relation to Hammer's (1986) salinity categories.

Taxon	Authority	Salinity category	No occ	Max abun	Mean abun
<i>Achnanthes brevipes</i>	Agardh	Mesosaline	1	0.32	0.32
<i>Achnanthyidium binodis</i>	Kützing	Freshwater, Hyposaline	8	5.45	1.65
<i>Achnanthyidium hungaricum</i>	Grunow	Freshwater	2	6.41	4.66
<i>Achnanthyidium minutissimum</i>	(Kützing) Czarnecki	Freshwater, Hyposaline	24	36.28	9.31
<i>Achnanthyidium oblongella</i>	Oestrup	Freshwater, Hyposaline	13	17.63	3.56
<i>Achnanthyidium ploenensis</i>	Hustedt	Freshwater, Hyposaline, Mesosaline, Hypersaline	9	1.26	0.72
<i>Achnanthyidium reidensis</i>	Foged	Freshwater, Hyposaline	14	73.79	8.98
<i>Achnanthyidium</i> sp. aff. <i>hungaricum</i>	Grunow	Freshwater	2	4.99	3.97
<i>Amphora acutiuscula</i>	Kützing	Freshwater, Hyposaline	5	4.53	2.18
<i>Amphora australiensis</i>	John	Freshwater, Hyposaline	5	1.24	0.80
<i>Amphora coffeaeformis</i>	(Agardh) Kützing	Freshwater, Hyposaline, Mesosaline, Hypersaline	42	95.03	22.91
<i>Amphora fontinalis</i>	Hustedt	Freshwater	4	14.83	5.93
<i>Amphora holsatica</i>	Hustedt	Freshwater, Hyposaline, Mesosaline	7	18.78	3.90
<i>Amphora ovalis</i>	(Kützing) Kützing	Freshwater, Hyposaline	2	2.03	1.17
<i>Amphora paraveneta</i>	Lange-Bertalot	Hyposaline, Mesosaline, Hypersaline	5	8.33	3.41
<i>Amphora</i> sp. A		Hypersaline	1	59.11	59.11
<i>Amphora</i> sp. aff. <i>atacamae</i>	Frenguelli	Hyposaline	1	0.32	0.32
<i>Amphora</i> sp. aff. <i>coffeaeformis</i>	(Agardh) Kützing	Freshwater, Hyposaline	13	26.01	6.81
<i>Amphora</i> sp. aff. <i>luciae</i>	Cholnoky	Freshwater, Hyposaline, Mesosaline, Hypersaline	44	90.26	25.65
<i>Amphora suburgida</i>	Hustedt	Freshwater, Hyposaline	7	16.51	4.92
<i>Amphora veneta</i>	Kützing	Freshwater, Hyposaline, Mesosaline, Hypersaline	10	43.99	8.70
<i>Bacillaria paxillifer</i>	(O.F. Müller) Hendey	Freshwater, Hyposaline, Hypersaline	22	77.87	10.57
<i>Brachysira brebissonii</i>	Ross	Freshwater	2	0.96	0.64
<i>Brachysira styriaca</i>	(Grunow) Ross	Freshwater, Hyposaline	13	46.71	9.74
<i>Brachysira vitrea</i>	(Grunow) Ross	Freshwater	6	3.54	1.64
<i>Caloneis silicula</i> forma <i>intermedia</i>	Hustedt	Freshwater	1	1.61	1.61
<i>Caloneis ventricosa</i>	(Ehrenberg) Meister	Hypersaline	1	1.32	1.32
<i>Campylodiscus bicostatus</i>	W. Smith	Hyposaline	1	0.31	0.31
<i>Chaetoceros muelleri</i>	Hustedt	Mesosaline	2	29.64	22.16
<i>Cocconeis distans</i>	Gregory	Hyposaline, Hypersaline	5	4.70	2.16
<i>Cocconeis placentula</i>	(Ehrenberg) Hustedt	Freshwater, Hyposaline, Mesosaline, Hypersaline	36	29.87	5.55
<i>Cocconeis placentula</i> var. <i>euglypta</i>	(Ehrenberg) Cleve	Hyposaline, Mesosaline	6	50.16	21.12
<i>Craticula cuspidata</i>	(Kützing) Mann	Freshwater	1	0.63	0.63
<i>Craticula halophila</i>	(Grunow) Mann	Freshwater, Hyposaline	12	36.39	11.70
<i>Ctenophora pulchella</i>	(Ralfs ex Kützing) Williams & Round	Freshwater, Hyposaline	8	12.39	2.81
<i>Cyclotella atomus</i>	Hustedt	Freshwater, Hyposaline	3	0.63	0.51
<i>Cyclotella meneghiniana</i>	Kützing	Freshwater, Hyposaline, Mesosaline, Hypersaline	24	30.75	3.02
<i>Cyclotella stelligera</i>	Cleve & Grunow	Freshwater, Hyposaline	11	60.97	11.15
<i>Cyclotella striata</i>	(Kützing) Grunow	Hyposaline	2	7.76	4.04
<i>Cylindrotheca closterium</i>	(Ehrenberg) Lewin & Reimann	Hyposaline, Mesosaline	9	94.23	23.53
<i>Cymbella cistula</i>	(Ehrenberg) Kirchner	Freshwater	1	0.33	0.33
<i>Cymbella cymbiformis</i> var. <i>nonpunctata</i>	Fontell	Freshwater	1	0.64	0.64
<i>Cymbella similis</i>	Krasske	Freshwater	1	0.58	0.58
<i>Cymbella tumida</i>	(Brébisson) Van Heurck	Freshwater	2	0.32	0.31
<i>Cymbella turgida</i>	(Gregory) Cleve	Freshwater	1	1.93	1.93
<i>Denticula subtilis</i>	Grunow	Hyposaline	1	0.31	0.31
<i>Diadensis confervacea</i>	Kützing	Freshwater	1	16.50	16.50
<i>Diploneis chersonensis</i>	(Grunow) Cleve	Hyposaline	3	0.96	0.53
<i>Diploneis ovalis</i>	(Hilse ex Rabenhorst) Cleve	Hyposaline	4	0.93	0.69
<i>Diploneis smithii</i>	(Brébisson ex Smith) Cleve	Freshwater, Hyposaline	2	0.32	0.32
<i>Diploneis subovalis</i>	Cleve	Freshwater, Hyposaline	5	1.81	0.60
<i>Encyonema delicatulum</i>	(Kützing) Mann	Freshwater, Hyposaline	6	38.15	9.72
<i>Encyonema gracile</i>	Ehrenberg	Freshwater	3	2.82	2.01
<i>Encyonema minutum</i>	(Hilse ex Rabenhorst) Mann	Freshwater	7	4.81	1.18
<i>Encyonopsis microcephala</i>	(Grunow) Krammer	Freshwater, Hyposaline	10	56.57	12.02
<i>Entomoneis costata</i>	(Hustedt) Reimer	Freshwater	1	0.96	0.96
<i>Entomoneis paludosa</i>	(W. Smith) Reimer	Freshwater, Hyposaline	16	7.69	2.21
<i>Entomoneis pseudoduplex</i>	Osada & Kobayasi	Mesosaline, Hypersaline	4	6.95	2.36
<i>Entomoneis</i> sp. aff. <i>paludosa</i>	(W. Smith) Reimer	Hypersaline	5	77.34	15.98
<i>Entomoneis tenuistriata</i>	John	Hyposaline, Mesosaline, Hypersaline	13	20.30	4.66
<i>Eunotia bilunaris</i>	(Ehrenberg) Mills	Freshwater	3	31.09	10.79
<i>Eunotia camelus</i>	Ehrenberg	Freshwater	3	0.96	0.75
<i>Eunotia flexuosa</i>	(Brébisson) Kützing	Hyposaline	1	1.27	1.27
<i>Eunotia pectinalis</i>	(O.F. Müller) Rabenhorst	Freshwater, Hyposaline	4	5.09	2.90
<i>Eunotia pectinalis</i> var. <i>minor</i>	(Kützing) Rabenhorst	Freshwater	3	3.81	1.80
<i>Eunotia pectinalis</i> var. <i>recta</i>	Mayer ex Patrick	Freshwater	1	0.32	0.32
<i>Fallacia auriculata</i>	(Hustedt) Mann	Freshwater, Hyposaline	5	5.44	1.53
<i>Fallacia pygmaea</i>	(Kützing) Stickle & Mann	Hyposaline, Mesosaline	3	0.32	0.31
<i>Fallacia tenera</i>	(Hustedt) Mann	Freshwater, Hyposaline	12	15.69	2.60
<i>Fragilaria famelica</i>	(Kützing) Lange-Bertalot	Hyposaline	2	50.73	30.87
<i>Fragilaria rumpens</i>	(Kützing) Carlson	Freshwater	1	0.32	0.32
<i>Fragilaria vaucheriae</i>	(Kützing) Petersen	Freshwater	7	69.01	26.05
<i>Fragilariforma virescens</i>	Ralfs	Freshwater	1	9.45	9.45

**Appendix 4.1 (continued):** List of diatom taxa (with authorities), identified from the 95 study sites in the south-west of Western Australia. Number of occurrences (No occ), maximum abundance (Max abun) and mean relative abundance (Mean abun, zeroes removed) are shown in relation to Hammer's (1986) salinity categories.

Taxon	Authority	Salinity category	No occ	Max abun	Mean abun
<i>Frustulia magaliesmontana</i>	Cholnoky	Freshwater	5	2.82	1.29
<i>Frustulia vulgaris</i>	(Thwaites) De Toni	Freshwater	3	4.82	1.91
<i>Gomphonema acuminatum</i>	Ehrenberg	Freshwater	1	0.32	0.32
<i>Gomphonema affine</i>	Kützing	Hyposaline	1	0.31	0.31
<i>Gomphonema affine</i> var. <i>insigne</i>	(Gregory) Andrews	Freshwater	1	0.33	0.33
<i>Gomphonema auritum</i>	Braun	Freshwater, Hyposaline	4	6.45	2.17
<i>Gomphonema parvulum</i>	(Kützing) Kützing	Freshwater, Hyposaline	15	72.82	13.38
<i>Gomphonema truncatum</i>	Ehrenberg	Freshwater	1	0.58	0.58
<i>Gomphonema undulatum</i>	Hustedt	Freshwater, Hyposaline	4	1.25	0.70
<i>Gyrosigma kutzingii</i>	(Grunow) Cleve	Freshwater, Hyposaline	5	4.81	1.78
<i>Gyrosigma spencerii</i>	(W. Smith) Cleve	Hyposaline, Mesosaline	4	1.63	0.88
<i>Hantzschia amphioxys</i>	(Ehrenberg) Grunow	Hyposaline, Hypersaline	4	1.99	0.97
<i>Hantzschia</i> sp. aff. <i>baltica</i>	Simonsen	Hyposaline, Mesosaline, Hypersaline	14	10.26	3.55
<i>Hantzschia</i> sp. aff. <i>barckhausenii</i>	Lange-Bertalot & Metzeltin	Hypersaline	2	14.96	9.27
<i>Hantzschia</i> sp. aff. <i>weyprechti</i>	Grunow	Hyposaline	1	0.99	0.99
<i>Haslea spicula</i>	(Hickie) Bukhtiyarova	Freshwater, Hyposaline, Mesosaline, Hypersaline	17	4.34	1.46
<i>Hippodonta capitata</i>	(Ehrenberg) Lange-Bertalot	Freshwater	4	11.07	3.70
<i>Hyalodiscus lentiginosus</i>	John	Hyposaline	1	0.32	0.32
<i>Hyalodiscus scoticus</i>	(Kützing) Grunow	Freshwater	1	1.25	1.25
<i>Luticola mutica</i>	(Kützing) Mann	Freshwater, Hypersaline	2	1.95	1.13
<i>Maryana martyi</i>	(Héribaud) Round	Hypersaline	2	12.85	7.42
<i>Mastogloia baltica</i>	Grunow	Freshwater	3	0.88	0.51
<i>Mastogloia braunii</i>	Grunow	Mesosaline	1	0.88	0.88
<i>Mastogloia halophila</i>	John	Hyposaline	1	0.62	0.62
<i>Mastogloia pumila</i>	(Grunow) Cleve	Hyposaline, Mesosaline, Hypersaline	11	40.31	5.47
<i>Mastogloia reimeri</i>	John	Hypersaline	1	3.58	3.58
<i>Mastogloia smithii</i>	Thwaites	Freshwater, Hyposaline	2	0.61	0.45
<i>Melosira nummuloides</i>	(Dillwyn) Agardh	Freshwater, Hyposaline	2	22.51	15.82
<i>Melosira varians</i>	Agardh	Freshwater	1	0.29	0.29
<i>Navicella pusilla</i>	(Grunow ex. A. Schmidt) Krammer	Freshwater, Hyposaline, Mesosaline, Hypersaline	21	43.59	3.36
<i>Navicella accomoda</i>	Hustedt	Freshwater	1	1.88	1.88
<i>Navicula cincta</i>	(Ehrenberg) Kützing	Freshwater, Hyposaline, Mesosaline	14	15.06	1.87
<i>Navicula cruciculoides</i>	Brockman	Freshwater	1	0.63	0.63
<i>Navicula cryptocephala</i>	Kützing	Freshwater, Hyposaline, Mesosaline	32	31.82	3.94
<i>Navicula cryptocephala</i> var. <i>exilis</i>	(Kützing) Grunow	Hyposaline	1	2.25	2.25
<i>Navicula elegans</i>	W. Smith	Freshwater, Hypersaline	2	2.27	1.28
<i>Navicula elegantoides</i>	Hustedt	Hyposaline	1	0.91	0.91
<i>Navicula notha</i>	Wallace	Freshwater, Hyposaline	2	22.19	12.03
<i>Navicula perminuta</i>	Grunow	Freshwater, Hyposaline	17	31.72	8.38
<i>Navicula pseudofrickia</i>	Patrick	Freshwater	1	0.31	0.31
<i>Navicula punctulata</i>	Smith	Hyposaline	2	0.32	0.32
<i>Navicula radiosa</i>	Kützing	Freshwater, Hyposaline	8	5.45	2.08
<i>Navicula ramosissima</i>	(Agardh) Cleve	Hyposaline	1	4.63	4.63
<i>Navicula recens</i>	Lange-Bertalot	Hyposaline	2	2.44	2.15
<i>Navicula rhyngocephala</i>	Kützing	Freshwater	1	0.33	0.33
<i>Navicula salinarum</i>	Grunow	Freshwater, Hyposaline, Hypersaline	13	58.54	6.30
<i>Navicula schroeteri</i>	Meister	Freshwater	1	1.76	1.76
<i>Navicula</i> sp. A		Hypersaline	1	4.47	4.47
<i>Navicula</i> sp. aff. <i>agrestis</i>	Hustedt	Freshwater, Hyposaline, Mesosaline	13	32.61	9.92
<i>Navicula</i> sp. aff. <i>arvensis</i>	Hustedt	Hypersaline	1	27.37	27.37
<i>Navicula</i> sp. aff. <i>cryptocephala</i>	Kützing	Mesosaline, Hypersaline	2	35.43	18.17
<i>Navicula</i> sp. aff. <i>duerrenbergiana</i>	Hustedt	Mesosaline, Hypersaline	2	9.51	7.31
<i>Navicula</i> sp. aff. <i>ignota</i>	Krasske	Hypersaline	1	0.95	0.95
<i>Navicula</i> sp. aff. <i>incertata</i>	Lange-Bertalot	Mesosaline, Hypersaline	11	74.29	15.80
<i>Navicula</i> sp. aff. <i>salinicola</i>	Hustedt	Hyposaline, Mesosaline, Hypersaline	14	60.81	15.44
<i>Navicula subrhyngocephala</i>	Hustedt	Freshwater, Hyposaline	5	2.62	1.32
<i>Navicula symmetrica</i>	Patrick	Hyposaline	1	1.29	1.29
<i>Navicula tenelloides</i>	Hustedt	Mesosaline, Hypersaline	6	25.71	9.88
<i>Navicula tripunctata</i>	(O.F. Müller) Bory	Freshwater, Hyposaline, Mesosaline	24	19.36	4.55
<i>Navicula tripunctata</i> var. <i>schizemoides</i>	(Van Heurck) Patrick	Hyposaline, Hypersaline	2	18.89	9.76
<i>Navicula viridula</i>	Kützing	Freshwater, Hyposaline	8	1.88	0.61
<i>Neidium affine</i>	(Ehrenberg) Pfitzer	Freshwater	1	0.32	0.32
<i>Nitzschia agnata</i>	Hustedt	Freshwater, Hyposaline	4	17.03	6.05
<i>Nitzschia aurariae</i>	Cholnoky	Freshwater, Hyposaline	15	18.86	3.66
<i>Nitzschia desertorum</i>	Hustedt	Freshwater	3	12.31	7.00
<i>Nitzschia dissipata</i>	(Kützing) Grunow	Freshwater, Hyposaline	2	0.32	0.31
<i>Nitzschia epithemoides</i>	Grunow	Hyposaline, Mesosaline	3	2.93	1.50
<i>Nitzschia fasciculata</i>	Grunow	Freshwater, Hyposaline	3	3.16	1.57
<i>Nitzschia frustulum</i>	(Kützing) Grunow	Freshwater, Hyposaline	26	42.58	9.95
<i>Nitzschia gracilis</i>	Hantzsch	Freshwater, Hyposaline	4	4.69	3.31
<i>Nitzschia hybrida</i>	Grunow	Hyposaline	2	6.67	3.80
<i>Nitzschia linearis</i>	W. Smith	Freshwater	4	11.63	5.48
<i>Nitzschia lorenziana</i>	Grunow	Freshwater	1	0.32	0.32

**Appendix 4.1 (continued):** List of diatom taxa (with authorities), identified from the 95 study sites in the south-west of Western Australia. Number of occurrences (No occ), maximum abundance (Max abun) and mean relative abundance (Mean abun, zeroes removed) are shown in relation to Hammer's (1986) salinity categories.

Taxon	Authority	Salinity category	No occ	Max abun	Mean abun
<i>Nitzschia microcephala</i>	Grunow	Freshwater, Hyposaline	26	13.23	2.67
<i>Nitzschia obtusa</i>	W. Smith	Freshwater, Hyposaline	7	1.29	0.67
<i>Nitzschia ovalis</i>	Arnott	Mesosaline, Hypersaline	19	89.63	19.63
<i>Nitzschia palea</i>	W. Smith	Freshwater, Hyposaline	27	49.84	5.23
<i>Nitzschia paleacea</i>	Grunow	Freshwater, Hyposaline	9	17.59	4.25
<i>Nitzschia paleaeformis</i>	Hustedt	Freshwater, Mesosaline	3	12.54	7.40
<i>Nitzschia pellucida</i>	Grunow	Hyposaline, Mesosaline, Hypersaline	6	5.14	2.05
<i>Nitzschia prolongata</i>	Hustedt	Hyposaline	1	56.19	56.19
<i>Nitzschia punctata</i>	(W. Smith) Grunow	Hyposaline	1	0.32	0.32
<i>Nitzschia punctata</i> forma <i>minor</i>	Hustedt	Mesosaline, Hypersaline	6	4.30	0.98
<i>Nitzschia romana</i>	Grunow	Freshwater, Hyposaline	6	10.90	2.44
<i>Nitzschia scalaris</i>	(Ehrenberg) Smith	Hyposaline	1	0.64	0.64
<i>Nitzschia scalpelliformis</i>	(Grunow) Grunow	Freshwater, Hyposaline	5	2.25	0.88
<i>Nitzschia sigma</i>	(Kützing) W. Smith	Freshwater, Hyposaline, Mesosaline	16	3.57	1.02
<i>Nitzschia</i> sp. aff. <i>archibaldii</i>	Lange-Bertalot	Hyposaline, Mesosaline	9	52.99	18.37
<i>Nitzschia</i> sp. aff. <i>capitellata</i>	Hustedt	Freshwater, Hyposaline	3	10.20	7.78
<i>Nitzschia</i> sp. aff. <i>fontifuga</i>	Cholnoky	Freshwater, Hyposaline	13	8.18	3.23
<i>Nitzschia</i> sp. aff. <i>frustulum</i>	(Kützing) Grunow	Hyposaline	1	0.32	0.32
<i>Nitzschia</i> sp. aff. <i>marginata</i>	Hustedt	Mesosaline, Hypersaline	2	2.56	2.42
<i>Nitzschia</i> sp. aff. <i>microcephala</i>	Grunow	Mesosaline	1	43.09	43.09
<i>Nitzschia</i> sp. aff. <i>rostellata</i>	Hustedt	Hyposaline, Mesosaline, Hypersaline	14	69.67	10.93
<i>Nitzschia</i> sp. aff. <i>vitrea</i> var. <i>salinarum</i>	Grunow	Hyposaline	1	1.46	1.46
<i>Nitzschia subinflata</i>	Hustedt	Freshwater	1	3.45	3.45
<i>Nitzschia vidovichii</i>	Grunow	Freshwater	2	0.64	0.47
<i>Pinnularia gibba</i>	Ehrenberg	Hyposaline	1	1.99	1.99
<i>Pinnularia microstauron</i>	(Ehrenberg) Cleve	Mesosaline, Hypersaline	3	13.54	4.95
<i>Pinnularia</i> sp. aff. <i>subcapitata</i>	Gregory	Mesosaline	1	20.07	20.07
<i>Pinnularia subcapitata</i>	Gregory	Freshwater	2	0.59	0.45
<i>Placoneis elginensis</i>	(Gregory) Cox	Freshwater	1	2.25	2.25
<i>Planolithidium delicatulum</i>	(Kützing) Round & Bukhtiyarova	Freshwater, Hyposaline	7	6.77	2.79
<i>Planolithidium dubium</i>	(Grunow) Round & Bukhtiyarova	Hyposaline, Hypersaline	5	4.48	1.95
<i>Planolithidium frequentissimum</i>	(Lange-Bertalot) Round & Bukhtiyarova	Hypersaline	2	47.57	24.11
<i>Planolithidium haikiamum</i>	(Grunow) Round & Bukhtiyarova	Hyposaline	6	7.83	3.64
<i>Planolithidium lanceolatum</i>	(Brébisson) Round & Bukhtiyarova	Freshwater, Hyposaline, Mesosaline	24	15.91	4.17
<i>Pleurosigma elongatum</i>	W. Smith	Freshwater, Hyposaline, Hypersaline	12	8.02	1.27
<i>Pleurosigma salinarum</i>	Grunow	Hyposaline, Mesosaline	3	0.64	0.42
<i>Proschkina</i> sp. aff. <i>complanata</i>	(Grunow) Mann	Hyposaline, Mesosaline, Hypersaline	11	96.43	22.88
<i>Pseudostaurosira brevistriata</i>	(Grunow) Williams & Round	Freshwater, Hyposaline	7	42.58	14.41
<i>Rhopalodia gibberula</i>	(Ehrenberg) O.F. Müller	Freshwater, Hyposaline	6	5.10	2.29
<i>Rhopalodia gibberula</i> var. <i>globosa</i>	Hustedt	Mesosaline	1	0.32	0.32
<i>Rhopalodia gibberula</i> var. <i>timsahensis</i>	(O.F. Müller) Freguelli	Hyposaline	1	4.31	4.31
<i>Rhopalodia musculus</i>	(Kützing) O.F. Müller	Freshwater, Hyposaline, Mesosaline	4	3.88	2.07
<i>Sellaphora pupula</i>	(Kützing) Mereschowsky	Freshwater	2	0.87	0.76
<i>Sellaphora pupula</i> forma <i>capitata</i>	(Skvortsov & Meyer) Poulin	Freshwater	1	0.64	0.64
<i>Sellaphora pupula</i> var. <i>rectangularis</i>	(Gregory) Mereschowsky	Freshwater	1	0.31	0.31
<i>Seminavis ventricosa</i>	(Gregory) M. Garcia-Baptista	Hyposaline	3	0.91	0.62
<i>Stauroneis anceps</i>	Ehrenberg	Freshwater	1	0.32	0.32
<i>Stauroneis anceps</i> forma <i>linearis</i>	(Ehrenberg) Hustedt	Freshwater	1	0.29	0.29
<i>Stauroneis dubitabilis</i>	Hustedt	Hyposaline	1	0.29	0.29
<i>Stauroneis pachycephala</i>	Cleve	Freshwater, Hyposaline	14	3.52	1.07
<i>Stauroneis phoenicenteron</i>	(Nitzsch) Ehrenberg	Freshwater	1	0.32	0.32
<i>Staurosira construens</i>	Ehrenberg	Freshwater, Hyposaline	3	0.94	0.52
<i>Staurosira contruens</i> var. <i>venter</i>	(Ehrenberg) Hamilton	Freshwater, Hyposaline	9	13.06	3.70
<i>Surirella angusta</i>	Kützing	Freshwater	1	0.64	0.64
<i>Surirella fluvicygnorum</i>	John	Freshwater	1	0.31	0.31
<i>Surirella ovalis</i>	Brébisson	Freshwater, Hyposaline	4	0.64	0.40
<i>Surirella</i> sp. A		Freshwater	1	5.13	5.13
<i>Surirella tenera</i>	Gregory	Freshwater	1	0.32	0.32
<i>Synedra acus</i>	Kützing	Freshwater	1	0.31	0.31
<i>Synedra levigata</i> var. <i>hyalina</i>	Grunow	Freshwater	1	7.19	7.19
<i>Synedra</i> sp. aff. <i>radians</i>	Kützing	Hyposaline, Mesosaline	2	80.83	51.70
<i>Synedra ulna</i>	(Nitzsch) Ehrenberg	Freshwater, Hyposaline	13	13.44	3.06
<i>Tabellaria flocculosa</i>	(Rabhenhorst) Kützing	Freshwater	4	9.25	4.21
<i>Tabularia tabulata</i>	(Agardh) Snoeijis	Freshwater, Hyposaline, Mesosaline	32	61.86	5.47
<i>Thalassiosira lacustris</i>	(Grunow) Hasle	Hyposaline	1	0.31	0.31
<i>Thalassiosira weissflogii</i>	(Grunow) Fryxell & Hasle	Freshwater, Hyposaline, Hypersaline	19	19.36	4.04
<i>Tryblionella acuminata</i>	W. Smith	Hyposaline	3	0.64	0.51
<i>Tryblionella apiculata</i>	Gregory	Freshwater, Hyposaline, Hypersaline	10	4.97	1.28
<i>Tryblionella hungarica</i>	Grunow	Freshwater, Hyposaline, Mesosaline	11	2.48	0.80
<i>Tryblionella tryblionella</i>	(Hantzsch) Proschkina-Lavrenko	Freshwater	1	0.94	0.94
<i>Tryblionella victorae</i>	Grunow	Hyposaline	1	0.32	0.32

**Appendix 4.2:** Species numbers and Shannon diversity values from the 95 study sites in the south-west of Western Australia, based on Hammer's (1986) salinity regime. The mean number of species have been indicated for each category as well as the minimum and maximum values, where (a) is freshwater, (b) hyposaline, (c) mesosaline and (d) is hypersaline sites.

**(a)**

Code	Site name	Salinity category	Number of species	Shannon diversity
SW01	Bailup Rd Lake	Freshwater	30	2.71
SW02	Blindroo Wetland A	Freshwater	11	1.41
SW03	Brunswick River	Freshwater	28	2.45
SW04	Collie River: Honeymoon Pool	Freshwater	27	2.30
SW05	Hamilton River	Freshwater	20	2.12
SW06	Harvey Dam	Freshwater	33	2.76
SW07	Harvey River	Freshwater	27	2.66
SW08	Katanning Creek A	Freshwater	14	1.53
SW09	Knapping Wetland	Freshwater	13	1.43
SW10	Kojonup Brook A	Freshwater	12	1.56
SW11	Kojonup Brook B	Freshwater	15	1.88
SW12	Kojonup Brook C	Freshwater	16	2.24
SW13	Kojonup Creek	Freshwater	9	1.35
SW14	Lake Brockman	Freshwater	15	1.70
SW15	Lake Kulinup	Freshwater	12	1.26
SW16	Lake Moyanup	Freshwater	16	1.85
SW17	Lake Nangar	Freshwater	19	1.92
SW18	Lake Navarino	Freshwater	12	1.74
SW19	Mistake Creek	Freshwater	29	2.22
SW20	Moore River: Regans Ford	Freshwater	16	0.94
SW21	Narrogin Brook A	Freshwater	12	1.06
SW22	Narrogin Brook B	Freshwater	17	2.19
SW23	Red Swamp Brook	Freshwater	20	1.35
SW24	Serpentine River	Freshwater	15	1.60
SW25	Toodyay/Bailup Rd Lake	Freshwater	15	1.58
SW26	Toodyay Rd Lake	Freshwater	9	0.89
SW27	Utah Rd Lake	Freshwater	20	2.21
SW28	Wallsend Lake	Freshwater	25	2.53
		<b>MEAN</b>	<b>18.11</b>	<b>1.84</b>
		<b>MINIMUM</b>	<b>9.00</b>	<b>0.89</b>
		<b>MAXIMUM</b>	<b>33.00</b>	<b>2.76</b>

**(b)**

Code	Site name	Salinity category	Number of species	Shannon diversity
SW29	Arthur River: Moodiarrup Bridge	Hyposaline	19	1.95
SW30	Avon River South	Hyposaline	21	1.60
SW31	Avon River: Boyagarra Pool	Hyposaline	22	2.33
SW32	Avon River: Brookton	Hyposaline	4	0.25
SW33	Avon River: Gwambygne Pool	Hyposaline	23	2.15
SW34	Avon River: Katrine Bridge	Hyposaline	18	2.31
SW35	Avon River: Mears Five Mile Pool	Hyposaline	22	1.61
SW36	Bollonine Brook	Hyposaline	12	1.19
SW37	Boyagerring Brook	Hyposaline	18	2.05
SW38	Boyagin Creek	Hyposaline	28	2.99
SW39	Brockman River	Hyposaline	28	2.67
SW40	Cockerding Brook	Hyposaline	15	2.12
SW41	Collie Changerup Rd Lake	Hyposaline	11	1.33
SW42	Collie River: Collie	Hyposaline	26	2.31
SW43	Dale River South	Hyposaline	29	2.79
SW44	Douglas Rd Lake	Hyposaline	5	0.19
SW45	Ibis Lake	Hyposaline	9	1.48
SW46	Jimperding Brook	Hyposaline	27	2.00
SW47	Lake Indoon	Hyposaline	19	2.31
SW48	Lake Mears Overflow	Hyposaline	7	0.56
SW49	Lake Nambung	Hyposaline	4	0.25
SW50	Mackie River: Marley Pool Bridge	Hyposaline	22	2.55
SW51	Moore River East: New Norcia	Hyposaline	19	2.17
SW52	Murray River	Hyposaline	29	2.18
SW53	Needoonga Lake	Hyposaline	21	2.12
SW54	North Rd Lake	Hyposaline	4	1.07
SW55	Towerrining Lake	Hyposaline	21	2.41
SW56	Walyormouring Lake Inflow	Hyposaline	12	1.84
SW57	Wannamal Lake	Hyposaline	22	2.23
SW58	Williams Darkan Rd Lake	Hyposaline	10	1.56
SW59	Yornaning Dam	Hyposaline	17	2.18
		<b>MEAN</b>	<b>17.55</b>	<b>1.83</b>
		<b>MINIMUM</b>	<b>4.00</b>	<b>0.19</b>
		<b>MAXIMUM</b>	<b>29.00</b>	<b>2.99</b>

**Appendix 4.2 (continued):** Species numbers and Shannon diversity values from the 95 study sites in the south-west of Western Australia, based on Hammer's (1986) salinity regime. The mean number of species have been indicated for each category as well as the minimum and maximum values, where (a) is freshwater, (b) hyposaline, (c) mesosaline and (d) is hypersaline sites.

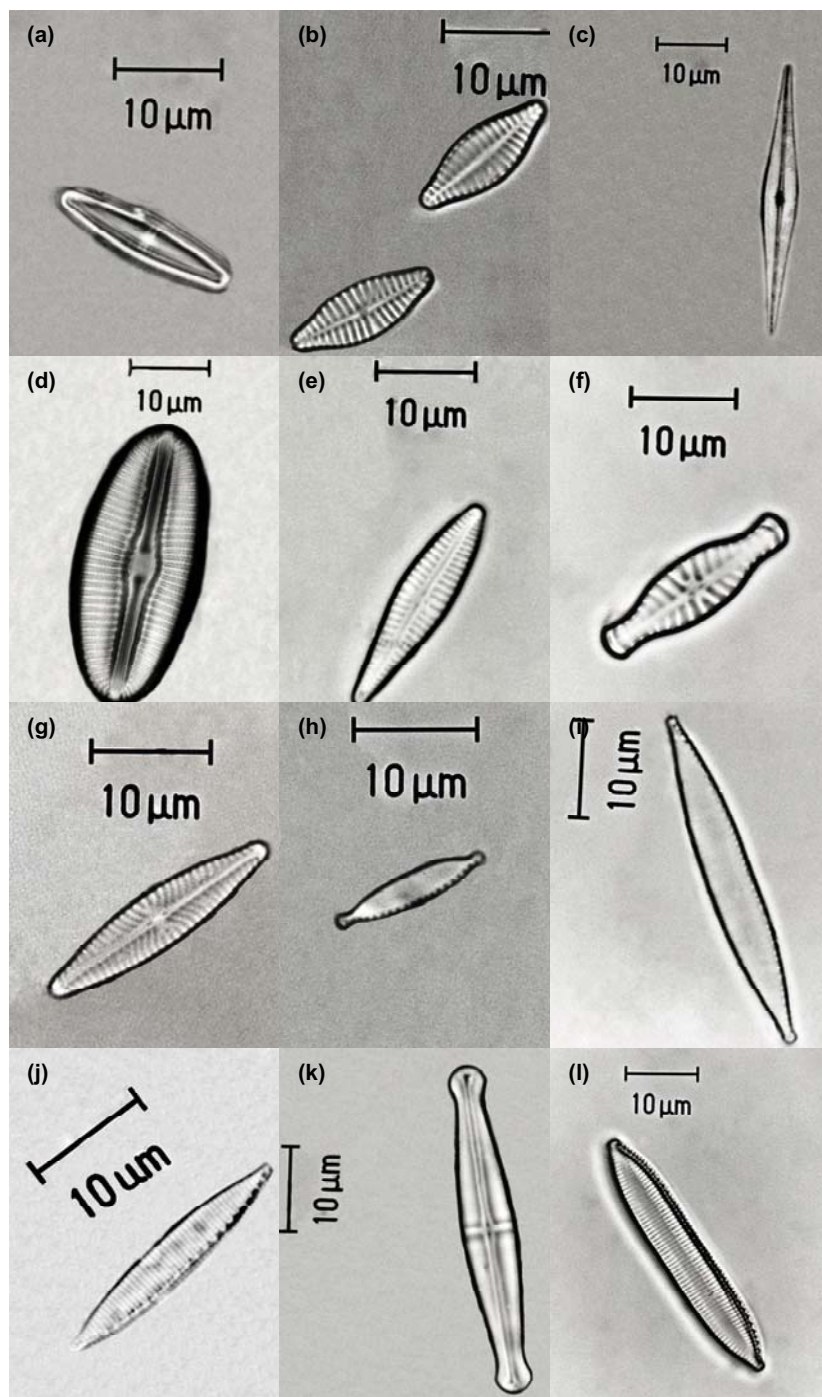
(c)

Code	Site name	Salinity category	Number of species	Shannon diversity
SW60	Ardath Lake	Mesosaline	8	1.28
SW61	Cowcowing Lakes B	Mesosaline	8	1.24
SW62	Hines Hill Lake B	Mesosaline	5	1.11
SW63	Hotham River	Mesosaline	9	1.12
SW64	Katanning Creek B	Mesosaline	9	0.49
SW65	Kevill's Lake Overflow	Mesosaline	5	0.52
SW66	Lake Mears	Mesosaline	3	0.41
SW67	Lime Lake Rd West Lake	Mesosaline	8	1.29
SW68	Mortlock River	Mesosaline	8	1.36
SW69	Nomans Lake	Mesosaline	12	1.28
SW70	Nomans Lake Inflow	Mesosaline	6	0.72
SW71	Salt River	Mesosaline	17	1.89
SW72	Shackleton Lake	Mesosaline	7	0.97
SW73	Wagin Lake	Mesosaline	4	0.48
SW74	Yarra Yarra Lakes Overflow	Mesosaline	10	0.85
SW75	Yenyening Lakes	Mesosaline	7	0.98
		<b>MEAN</b>	<b>7.88</b>	<b>1.00</b>
		<b>MINIMUM</b>	<b>3.00</b>	<b>0.41</b>
		<b>MAXIMUM</b>	<b>17.00</b>	<b>1.89</b>

(d)

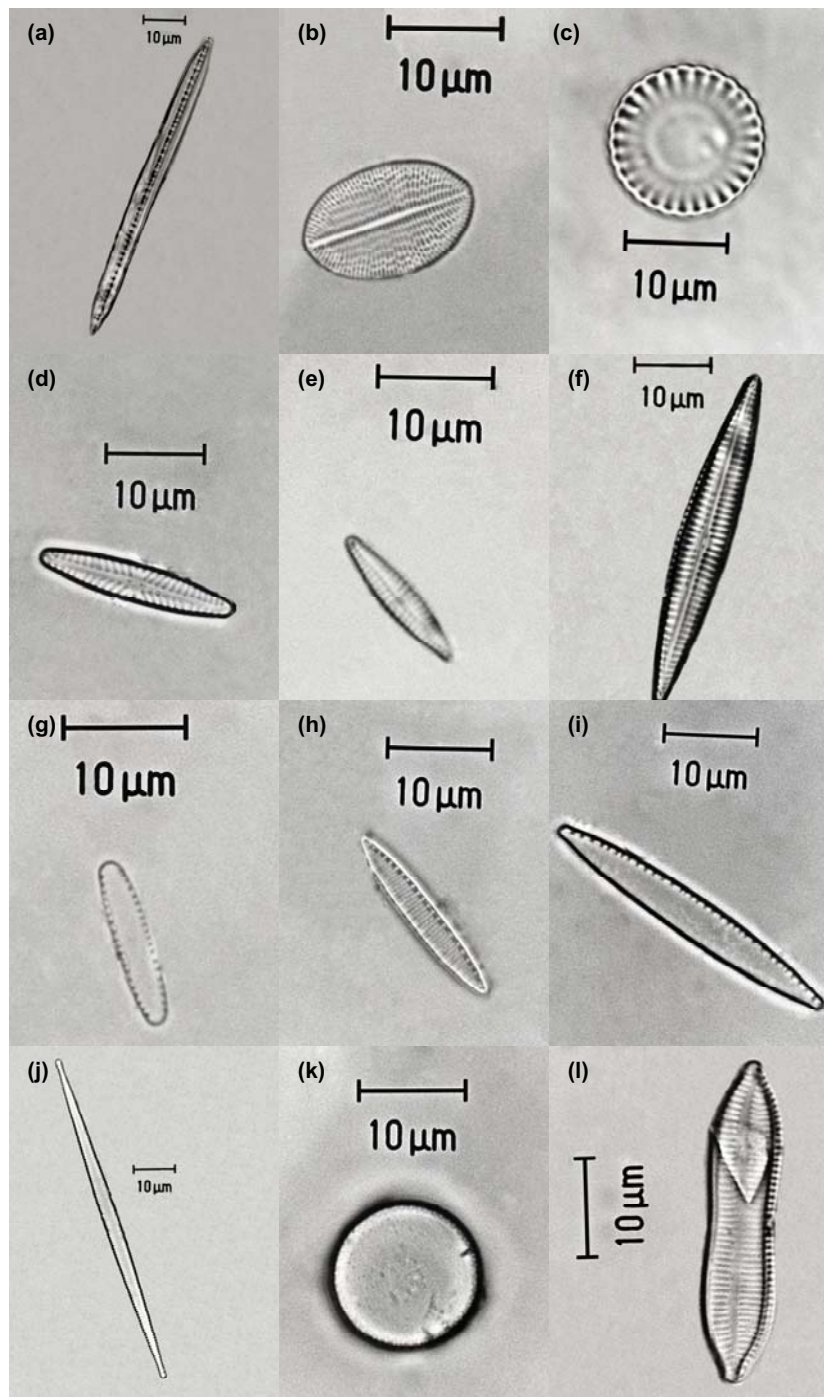
Code	Site name	Salinity category	Number of species	Shannon diversity
SW76	Ashwell Rd Lake	Hypersaline	10	1.57
SW77	Baandee Lake	Hypersaline	11	1.51
SW78	Coomelberrup Lake	Hypersaline	5	0.29
SW79	Cowcowing Lakes A	Hypersaline	12	2.08
SW80	Dumbleyung Lake	Hypersaline	11	1.58
SW81	Flagstaff Lake	Hypersaline	4	0.67
SW82	Hines Hill Lake A	Hypersaline	3	0.35
SW83	Kevills Lake	Hypersaline	8	1.33
SW84	Lake Brown	Hypersaline	6	1.11
SW85	Lake Champion	Hypersaline	6	0.74
SW86	Lake Dowerin	Hypersaline	6	0.91
SW87	Lake Koombekine	Hypersaline	5	0.97
SW88	Lake Ninan	Hypersaline	8	1.20
SW89	Lake Wallambin	Hypersaline	12	1.91
SW90	Lake Yealering	Hypersaline	12	1.85
SW91	Norring Lake	Hypersaline	8	1.31
SW92	Parkeyerring Lake	Hypersaline	5	1.18
SW93	Pink Lake	Hypersaline	5	0.77
SW94	Queerearrup Lake	Hypersaline	9	1.38
SW95	Toolibin Lake Inflow	Hypersaline	9	1.32
		<b>MEAN</b>	<b>7.75</b>	<b>1.20</b>
		<b>MINIMUM</b>	<b>3.00</b>	<b>0.29</b>
		<b>MAXIMUM</b>	<b>12.00</b>	<b>2.08</b>

**Appendix 4.3:** Diatom plates of common freshwater taxa from the south-west study sites. Photomicrographs were taken at 1000× total magnification, with 10 μm scales indicated. (a) *Achnantheidium minutissimum*, (b) *Achnantheidium reidensis*, (c) *Brachysira styriaca*, (d) *Diploneis subovalis*, (e) *Gomphonema parvulum*, (f) *Hippodonta capitata*, (g) *Navicula cryptocephala*, (h) *Nitzschia microcephala*, (i) *Nitzschia palea*, (j) *Nitzschia romana*, (k) *Stauroneis pachycephala* and (l) *Tryblionella hungarica*.

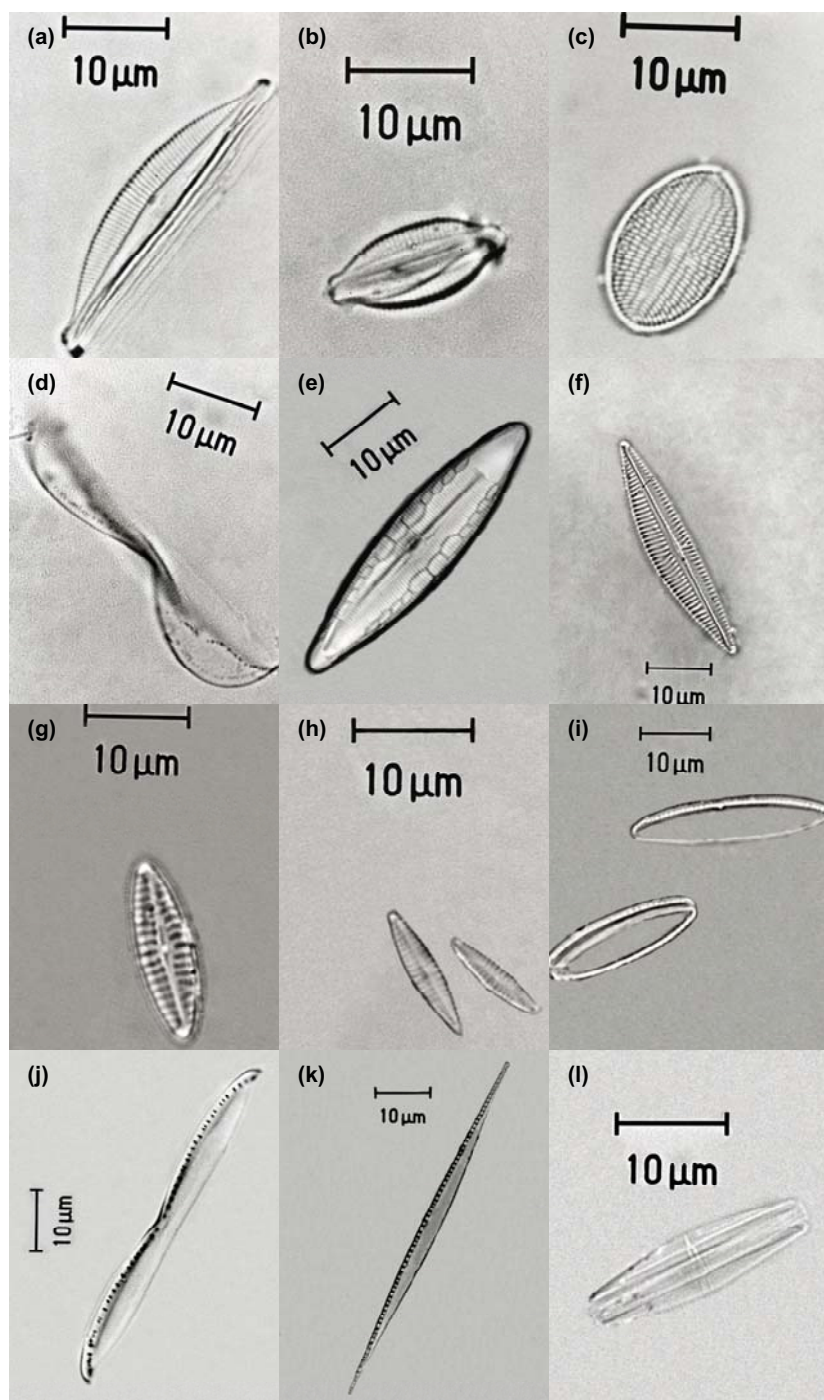




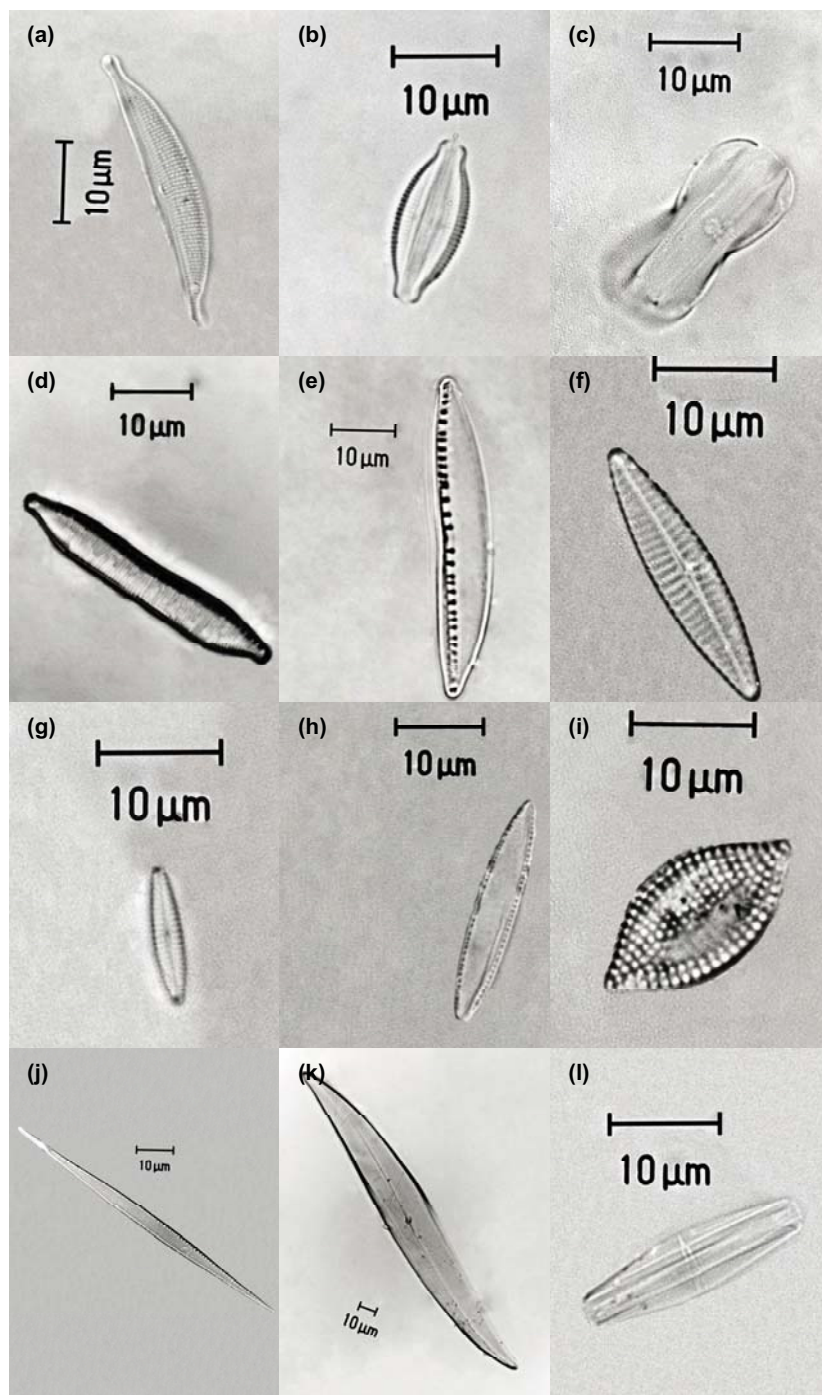
**Appendix 4.3 (continued):** Diatom plates of common hyposaline taxa from the south-west study sites. Photomicrographs were taken at 1000× total magnification, with 10 μm scales indicated. (a) *Bacillaria paxillifer*, (b) *Cocconeis placentula*, (c) *Cyclotella meneghiniana*, (d) *Navicula cincta*, (e) *Navicula perminuta*, (f) *Navicula tripunctata*, (g) *Nitzschia aurariae*, (h) *Nitzschia frustulum*, (i) *Nitzschia palea*, (j) *Tabularia tabulata*, (k) *Thalassiosira weissflogii* and (l) *Tryblionella apiculata*.



**Appendix 4.3 (continued):** Diatom plates of common mesosaline taxa from the south-west study sites. Photomicrographs were taken at 1000× total magnification, with 10 μm scales indicated. Diatom plates of common hyposaline taxa from the south-west study sites. Micrographs were taken at 1000× total magnification, with 10 μm scales indicated. (a) *Amphora coffeaeformis*, (b) *Amphora* sp. aff. *luciae*, (c) *Cocconeis placentula*, (d) *Entomoneis tenuistriata*, (e) *Mastogloia pumila*, (f) *Navicella pusilla*, (g) *Navicula* sp. aff. *incertata*, (h) *Navicula* sp. aff. *salinicola*, (i) *Nitzschia ovalis*, (j) *Nitzschia pellucida*, (k) *Nitzschia* sp. aff. *rostellata* and (l) *Proschkinia* sp. aff. *complanata*.



**Appendix 4.3 (continued):** Diatom plates of common hypersaline taxa from the south-west study sites. Photomicrographs were taken at 1000 $\times$  total magnification, with 10  $\mu\text{m}$  scales indicated. (a) *Amphora coffeaeformis*, (b) *Amphora* sp. aff. *luciae*, (c) *Entomoneis* sp. aff. *paludosa*, (d) *Hantzschia amphioxys*, (e) *Hantzschia* sp. aff. *baltica*, (f) *Navicula* sp. aff. *incertata*, (g) *Navicula* sp. aff. *salinicola*, (h) *Nitzschia ovalis*, (i) *Nitzschia punctata* forma *minor*, (j) *Nitzschia* sp. aff. *rostellata*, (k) *Pleurosigma elongatum* and (l) *Proschkinia* sp. aff. *complanata*.



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## Chapter 5: Diatom Assemblages in Hypersaline Water bodies of Varying pH from the Wheatbelt Region of Western Australia

### 5.1 Abstract

In this chapter diatom community structure was investigated from hypersaline wetlands, forming a subset of the larger sample from the south-west. These water bodies totalled 20 in number and were located in the inland wheatbelt area of Western Australia. The salinity of sites ranged from 50.20 to 156.80 ppt and had a pH of between 2.90 and 8.64. The number of diatom taxa identified was relatively high (47), however only 11 species were shown to dominate these conditions. Commonly occurring diatoms that may be considered potential indicator species of hypersalinity included *Amphora coffeaeformis*, *Hantzschia* sp. aff. *baltica* and *Nitzschia ovalis*. Analysis of similarities (ANOSIM) showed that there was no observable difference between diatom assemblages between 50 and 100 ppt and above 100 ppt and that generally, taxa displayed a wide tolerance to both salinity and pH. NMDS ordination and BIOENV was conducted on the data and determined that pH was the most highly correlated variable to species composition ( $\rho_w=0.54$ ). The impacts of rising salt loads and the increasing acidity of many wheatbelt lakes is of concern, with the need for further baseline studies to determine adverse affects on aquatic biota.

### 5.2 Introduction

The distribution pattern of diatoms along a salinity gradient was investigated in the previous chapter. This section continues this theme, focussing on species assemblages from hypersaline wetlands. The response of diatoms to extreme salinity concentrations is an important area of research, with many wetlands in the wheatbelt subjected to increased salt loads and waterlogging, with concomitant adverse impacts on species diversity (Pinder *et al.* 2005). In addition, the acidification of wetlands is associated with salinisation in the region, further threatening biodiversity (Halse *et al.* 2003).

Salinity is an important factor influencing the distribution of diatom assemblages (Hammer 1986; Blinn 1993; Snoeijs 1999), however in hypersaline waters ( $\geq 50$  ppt) the relationship between species diversity and salt loading becomes less clear. One of

the reasons for this is that the tolerance level of biota inhabiting waters in this range is generally very broad and other environmental variables may instead become the major determinants of community structure (Williams 1998). The response of diatoms to varying salinity concentrations has been established in a number of studies around the world (Cholnoky 1966; Ehrlich and Ortal 1979; Gasse 1986; John 1988). However, there is some degree of confusion in relation to morphological differences that can occur within hypersaline communities (Archibald 1983; Gasse 1986).

Diatoms indicative of hypersalinity are generally considered to be ubiquitous and the majority of salt tolerant taxa have been well-documented (Gasse 1986; Gell and Gasse 1990; Compère 1994; Ehrlich 1995). For example, *Amphora coffeaeformis* is one of the most common species associated with saline waters and has been identified from regions including South Africa (Archibald 1983), Egypt (Compère 1994), North America (Blinn 1993) and Canada (Wilson *et al.* 1994). This taxon has also been frequently observed in eastern Australia (Gell *et al.* 2002) and from inland lakes and streams in south Western Australia (John 1998; Blinn *et al.* 2004). Although many saline diatoms are cosmopolitan in distribution, differences arise due to geographical influences and the local environment.

In Western Australia there has been little published information on diatom species from severely salt-affected waters. Blinn *et al.* (2004) analysed diatom communities from a range of wetlands in the wheatbelt region of the south-west, finding a number of halotolerant taxa. Similarly, John (1998) carried out an extensive study further south into the composition of streams in the Blackwood catchment and recorded several saline species. However, as yet, no published literature has focussed on the tolerance limits of diatom assemblages from hypersaline systems.

Many water bodies in the wheatbelt are experiencing elevated salt loads and prolonged periods of inundation, with adverse effects on species diversity (Halse *et al.* 2003; Pinder *et al.* 2005). In addition, while some of the wetlands in this area are naturally acidic, many have been subjected to acidification due to the rise of groundwater through acid sulphate soils, discharging into aquatic systems (Blinn *et al.* 2004). The increasing occurrence of acidic hypersaline waters (Halse *et al.* 2003;

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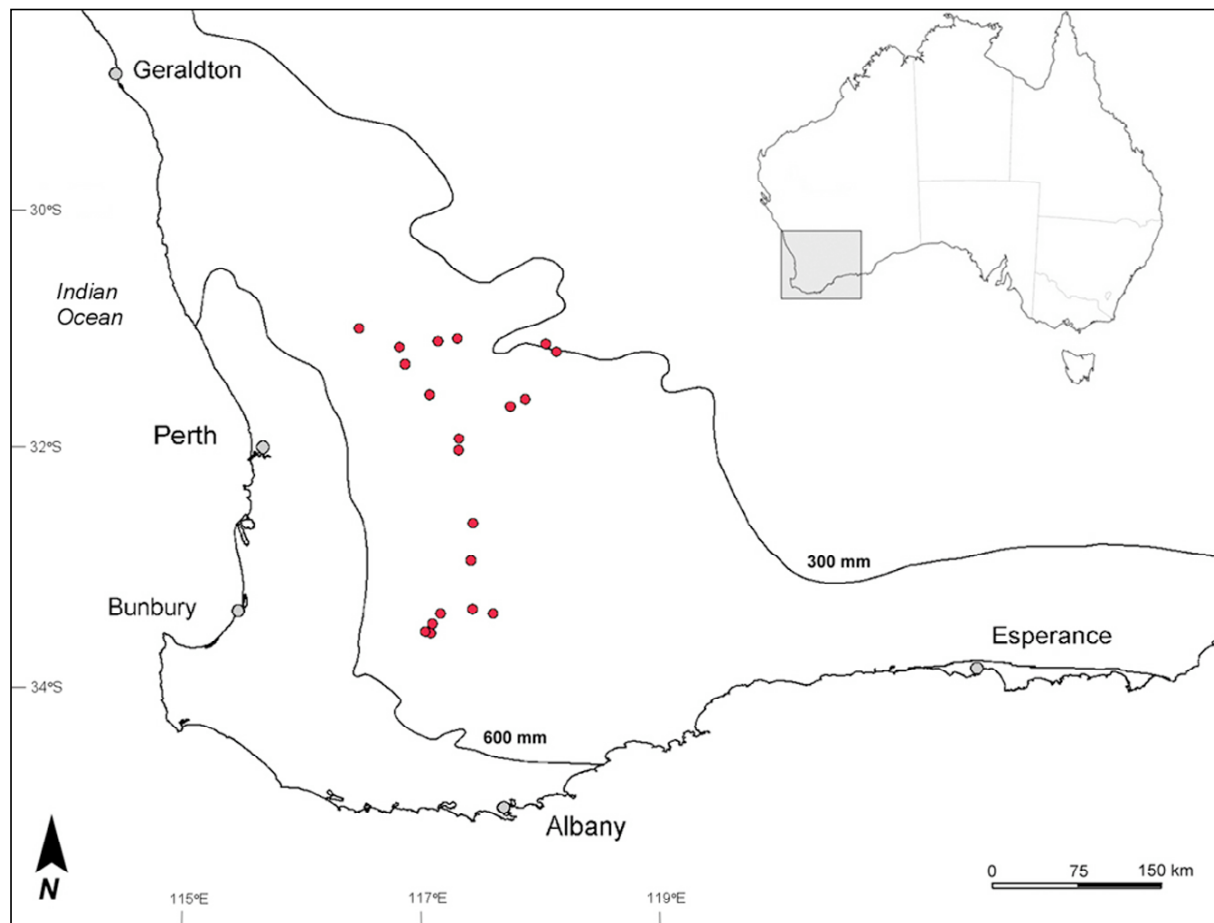
Timms 2005), means that baseline data documenting the response of biota to these extreme conditions is increasingly valuable.

The objective of this study was to explore the diversity of diatoms from hypersaline wetlands in the wheatbelt region, focussing on the influence of salinity and pH. As part of this investigation, regional comparisons were made between hypersaline taxa from eastern Australia and other parts of the world. The research also aimed at determining whether differences in diatom assemblages could be distinguished between 50–100 ppt and above 100 ppt, following Hammer's (1986) suggestion that an extreme hypersalinity category (>100 ppt) may be justified. The data collected from this study contributes to the understanding of species-environment relations in aquatic systems of the wheatbelt.

### **5.3 Materials and Methods**

#### *5.3.1 Study sites*

Study sites consisted of 20 wetlands with salinities greater than or equal to 50 ppt, and were part of the larger dataset used in the previous chapters of the thesis (**Figure 5.1**). Monitoring locations were situated in an area that receives between 300 and 600 mm of annual rainfall, referred to as the wheatbelt (Cale *et al.* 2004). The peak inundation period for most of these sites is spring, following winter rains, which was when sampling was undertaken. Wheatbelt lakes are characterised by their shallow, temporary nature and are generally dominated by sodium chloride, following an ionic spectrum similar to that of seawater (McComb and Lake 1990). They are also subjected to wide seasonal fluctuations, and extremely high salt concentrations, reaching saturation levels with the onset of the drying phase of their hydrological cycle (Williams 1983). Site names and GPS coordinates, as well as the site codes, allocated to each wetland for the purpose of statistical analyses are indicated in **Table 5.1**.



**Figure 5.1:** Location of the 20 hypersaline wheatbelt study sites (indicated by ●) in south-west Western Australia. The 600 and 300 mm annual isohyets approximately define the wheatbelt region.

**Table 5.1:** Site name, code and GPS coordinates of the 20 hypersaline wheatbelt sites.

Code	Site name	GPS coordinates	Code	Site name	GPS coordinates
SW76	Ashwell Rd Lake	33° 31.61' S 117° 16.24' E	SW86	Lake Dowerin	31° 14.63' S 117° 03.30' E
SW77	Baandee Lake	31° 36.04' S 117° 56.59' E	SW87	Lake Koombekine	31° 05.96' S 117° 00.30' E
SW78	Coomelberrup Lake	33° 21.51' S 117° 47.80' E	SW88	Lake Ninan	30° 56.16' S 116° 39.88' E
SW79	Cowcowing Lakes	31° 02.92' S 117° 19.87' E	SW89	Lake Wallambin	31° 01.43' S 117° 29.74' E
SW80	Dumbleyung Lake	33° 19.21' S 117° 37.49' E	SW90	Lake Yealering	32° 35.67' S 117° 37.61' E
SW81	Flagstaff Lake	33° 30.22' S 117° 15.56' E	SW91	Norring Lake	33° 26.83' S 117° 17.16' E
SW82	Hines Hill Lake	31° 32.45' S 118° 04.07' E	SW92	Parkeyerring Lake	33° 21.56' S 117° 21.17' E
SW83	Kevills Lake	31° 52.56' S 117° 30.43' E	SW93	Pink Lake	31° 58.44' S 117° 30.36' E
SW84	Lake Brown	31° 04.30' S 118° 14.51' E	SW94	Queerearrup Lake	33° 30.89' S 117° 13.46' E
SW85	Lake Champion	31° 08.31' S 118° 20.01' E	SW95	Toolibin Lake Inflow	32° 54.58' S 117° 36.57' E

### 5.3.2 Sampling procedure and identification

Study sites were sampled once only between 1999 and 2002, with environmental variables including pH, salinity (ppt), dissolved oxygen ( $\text{mgL}^{-1}$ ), temperature ( $^{\circ}\text{C}$ ) and peripheral vegetation density (allocated score) measured at each location. The JJ periphytometer was used to sample diatom communities (John 1998). This method ensured that diatoms were representative of ambient water quality, in contrast to surface sediment samples, which can be inaccurate due to changing sedimentation rates and environmental conditions (Battarbee *et al.* 2001). The artificial substrates were retrieved after a climax population had been reached (approximately 14 days) and diatom slides were prepared according to John (1983). The sampling protocols adopted for this study have been explained in Chapters 3 and 4.

A minimum of 300 diatom valves were counted from each site and taxonomic identification was carried out using specialised literature (Cholnoky 1966; Archibald 1983; John 1983; Gasse 1986; Ehrlich 1995; John 1998; Snoeijs and Balashova 1998; Fourtanier and Kociolek 1999; John 2000; Witkowski *et al.* 2000; Lange-Bertalot *et al.* 2003). Scanning electron microscopy (SEM) was employed for a number of species that were difficult to resolve under the light microscope. Voucher slides and SEM samples have been deposited at the International Diatom Herbarium, Department of Environmental Biology, Curtin University of Technology in Perth, Western Australia.



### 5.3.3 Scanning electron microscopy (SEM)

SEM was conducted at the Department of Applied Physics, Curtin University, Bentley Campus, following extensive cleaning of samples. Diluted samples were pipetted onto 12 mm diameter coverslips and placed onto a gentle heat to evaporate all traces of water, leaving behind the diatom frustules. The resultant sample was mounted onto electron microscopy stubs using double-sided carbon tape and sealed with carbon paint, both of which are electrically conductive. A sputter coater device was used to coat the sample in a thin, conductive layer of gold, ready for viewing. Photomicrographs were taken using the Philips XL30 SEM to capture images of diatoms. Imaging was performed using the secondary electron detector (SE) over a range of magnifications to gain a complete view of frustule ultrastructure. Images were stored in digital format (tiff) providing a permanent record for future reference. SEM photomicrographs were used for detailed investigation of diatom morphology, enabling identification where possible, of hypersaline species.

### 5.3.4 Analysis of environmental and species data

Ordination of environmental variables was performed using a correlation based principal components analysis (PCA) to determine the main gradients in the data. Salinity was  $\log_{10}$  transformed to reduce skewness, with PCA results presented as a proportion of variance explained over the principal components axes and associated correlation coefficients (McCune and Grace 2002).

The number of species (S) and Shannon diversity (H', Shannon and Weaver 1963) indices were derived for the data. The structure of diatom communities was explored using hierarchical agglomerative classification and non-metric multi-dimensional scaling (NMDS) (Kruskal and Wish 1978), on square-root transformed species abundances. Similarities between sites were calculated with the Bray-Curtis coefficient (Bray and Curtis 1957) and classification was based on the group-average linking algorithm. Analysis of similarities (ANOSIM) (Clarke and Green 1988), was used to determine differences between diatom assemblages and site groupings, with p-values and associated R statistics reported. The BIOENV procedure (Clarke and Ainsworth 1993), was implemented to establish the most influential environmental variables related to diatom composition, based on the weighted Spearman rank correlation coefficient ( $\rho_w$ ). Univariate and multivariate analyses were performed in

PRIMER 5 (Plymouth Marine Laboratory 2002), with a more comprehensive outline of these procedures provided in Chapter 4.

## 5.4 Results

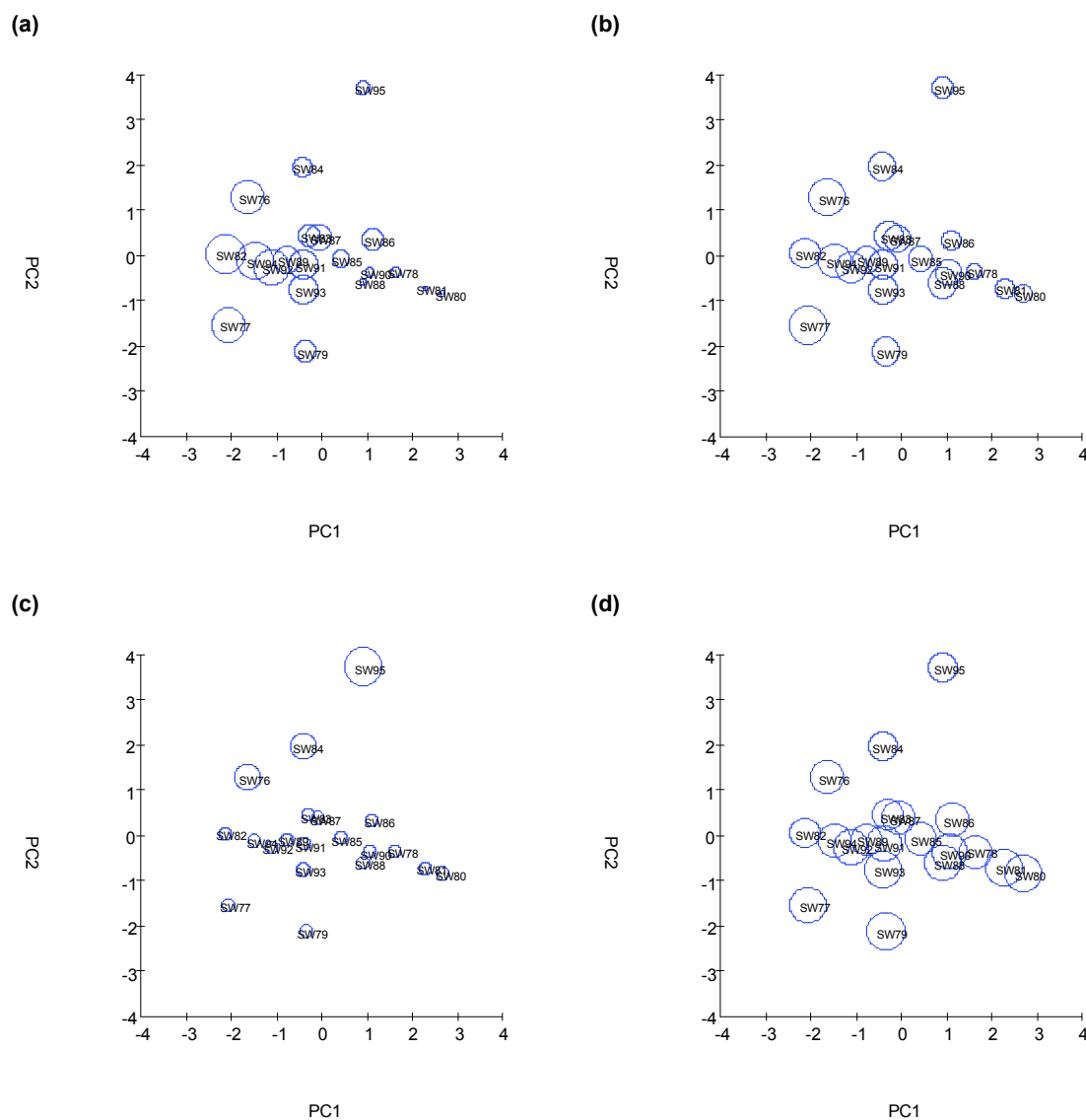
### 5.4.1 Environmental data

The salinity of the wheatbelt sites ranged from 50.20 to 156.80 ppt and pH values varied from 2.90 to 8.64 (**Table 5.2**). Dissolved oxygen ranged from 1.11 to 8.38 mgL<sup>-1</sup>, and temperature from 12.30 to 28.10 °C. Peripheral vegetation scores were 3 or less for all sampling locations, with 1 indicating little or no riparian community (**Table 5.2**).

**Table 5.2:** Environmental variables measured from the 20 hypersaline wheatbelt sites. Arithmetic means are shown for salinity (ppt), dissolved oxygen (mgL<sup>-1</sup>), temperature (°C) and vegetation (score), geometric mean is indicated for pH.

Site code	pH	Salinity	Dissolved oxygen	Temperature	Vegetation
SW76	8.05	79.20	7.00	28.10	2
SW77	4.35	122.90	7.49	28.10	1
SW78	5.74	87.20	2.37	12.30	1
SW79	2.90	156.80	4.80	20.80	1
SW80	8.64	154.10	1.14	13.10	1
SW81	7.78	130.00	1.11	14.30	1
SW82	3.65	50.50	8.38	21.90	1
SW83	7.22	59.40	4.47	21.50	1
SW84	6.55	50.20	4.36	21.00	2
SW85	6.21	73.70	3.71	17.90	1
SW86	8.56	77.90	4.60	14.10	1
SW87	8.57	71.40	5.60	20.10	1
SW88	7.54	109.40	1.54	22.10	1
SW89	5.37	66.30	5.90	20.40	1
SW90	7.26	96.20	2.11	19.00	1
SW91	8.45	95.60	6.40	22.00	1
SW92	8.03	92.80	7.80	23.60	1
SW93	7.79	121.10	6.40	22.40	1
SW94	8.30	87.20	8.30	25.20	1
SW95	8.48	51.30	3.37	15.70	3
<b>Mean</b>	<b>6.70</b>	<b>91.66</b>	<b>4.84</b>	<b>20.18</b>	<b>1</b>

Principal components analysis of the hypersaline wetlands (**Figure 5.2a-d**) determined that 68.30 % of the total variation was explained in the first two dimensions, with 37.50 % over PC axis 1 and 30.80 % in PC axis 2. Dissolved oxygen and temperature were negatively correlated with axis 1 (correlation coefficients of -0.68 and -0.64 respectively, **Figure 5.2a-b**), where as vegetation and salinity showed a strong association with axis 2 (correlation coefficients of 0.70 and -0.63 respectively, **Figure 5.2c-d**).

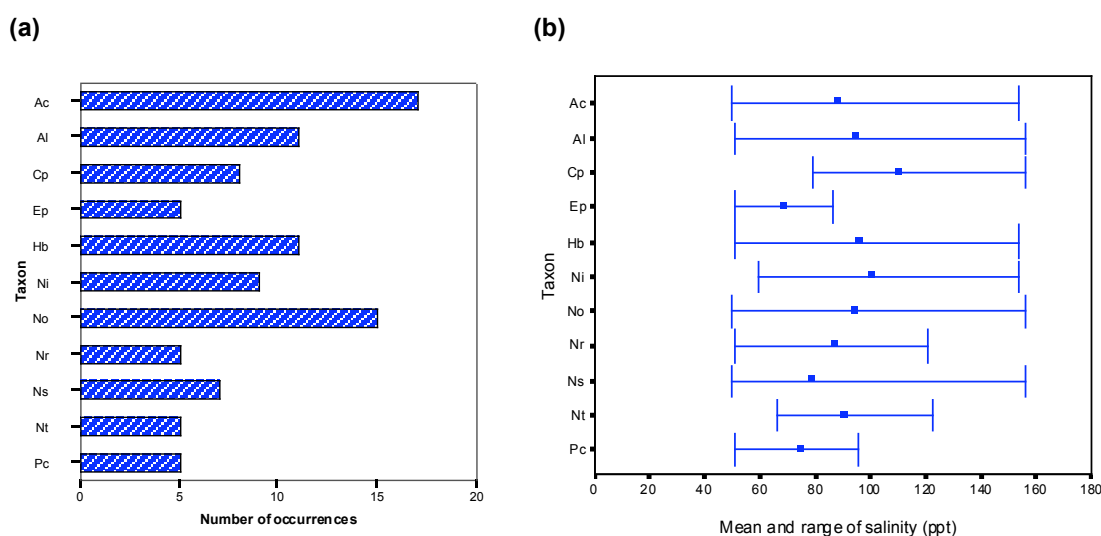


**Figure 5.2:** 2-dimensional PCA ordination of environmental variables from the 20 hypersaline wheatbelt sites. PCA plots are shown with circles overlaid proportional in diameter to (a) dissolved oxygen ( $\text{mgL}^{-1}$ ), (b) temperature ( $^{\circ}\text{C}$ ), (c) vegetation and (d) salinity concentration (ppt,  $\log_{10}$  transformed). Only those variables with correlation coefficients of greater than  $\pm 0.5$  have been displayed, in order of correlation to the PC axes.

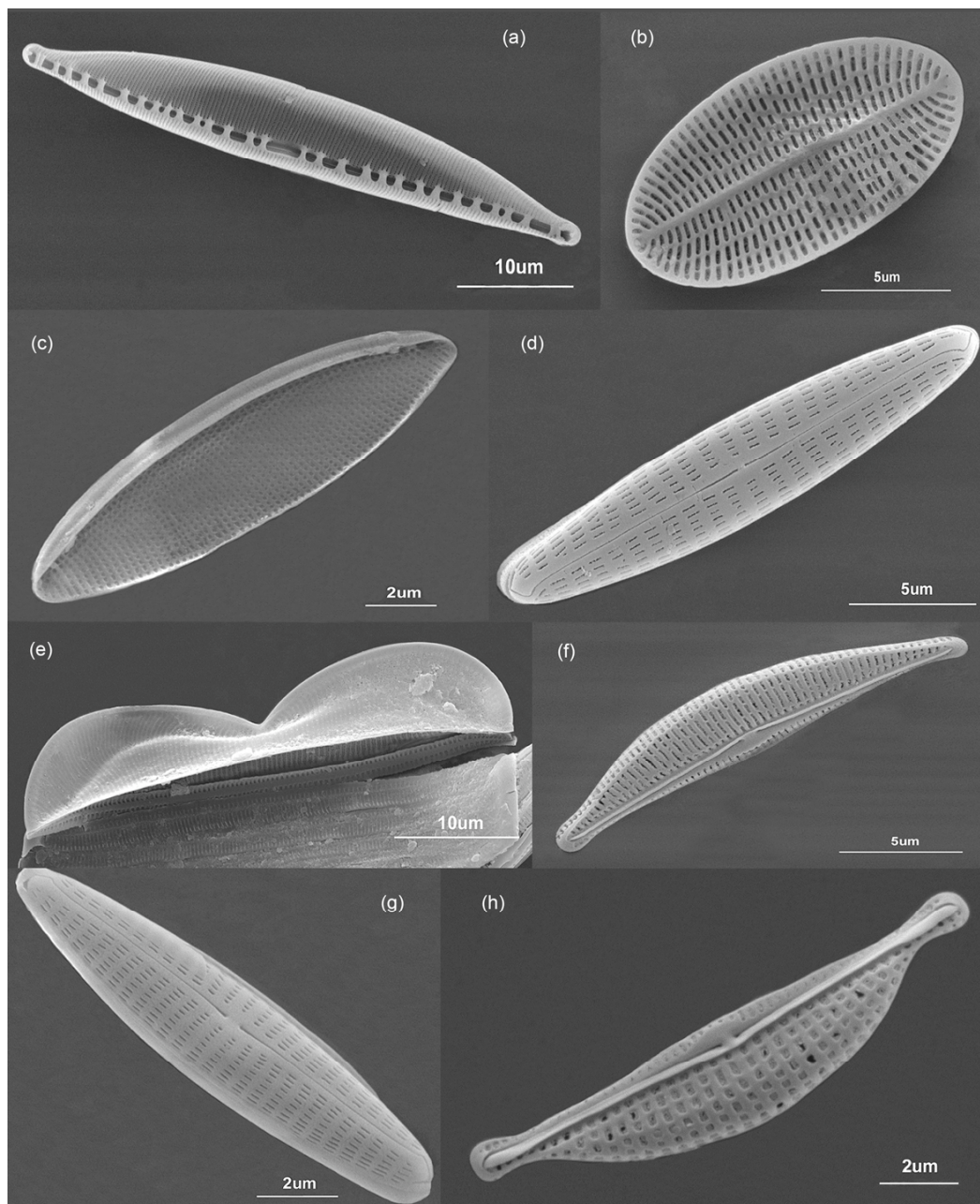
### 5.4.2 Diatom community structure

A total of 47 diatom taxa were identified from the 20 lakes, dominated by species from the *Navicula* and *Amphora* genera. Presence/absence data for diatoms that recorded a maximum abundance of greater than 1 % is provided in **Table 5.3**. *Amphora coffeaeformis* and *Nitzschia ovalis* were the most frequently encountered diatoms (occurring in 17 and 15 sites respectively) and also displayed a wide salinity tolerance (**Figure 5.3a-b**). In comparison, *Nitzschia* sp. aff. *rostellata* and *Proschkinia* sp. aff. *complanata* were less common (both occurring in 5 sites), as well as being observed over a much narrower salinity range (**Figure 5.3a-b**). Scanning electron microscopy was used to photograph some hypersaline species, with selected images presented in **Figure 5.4a-h**.

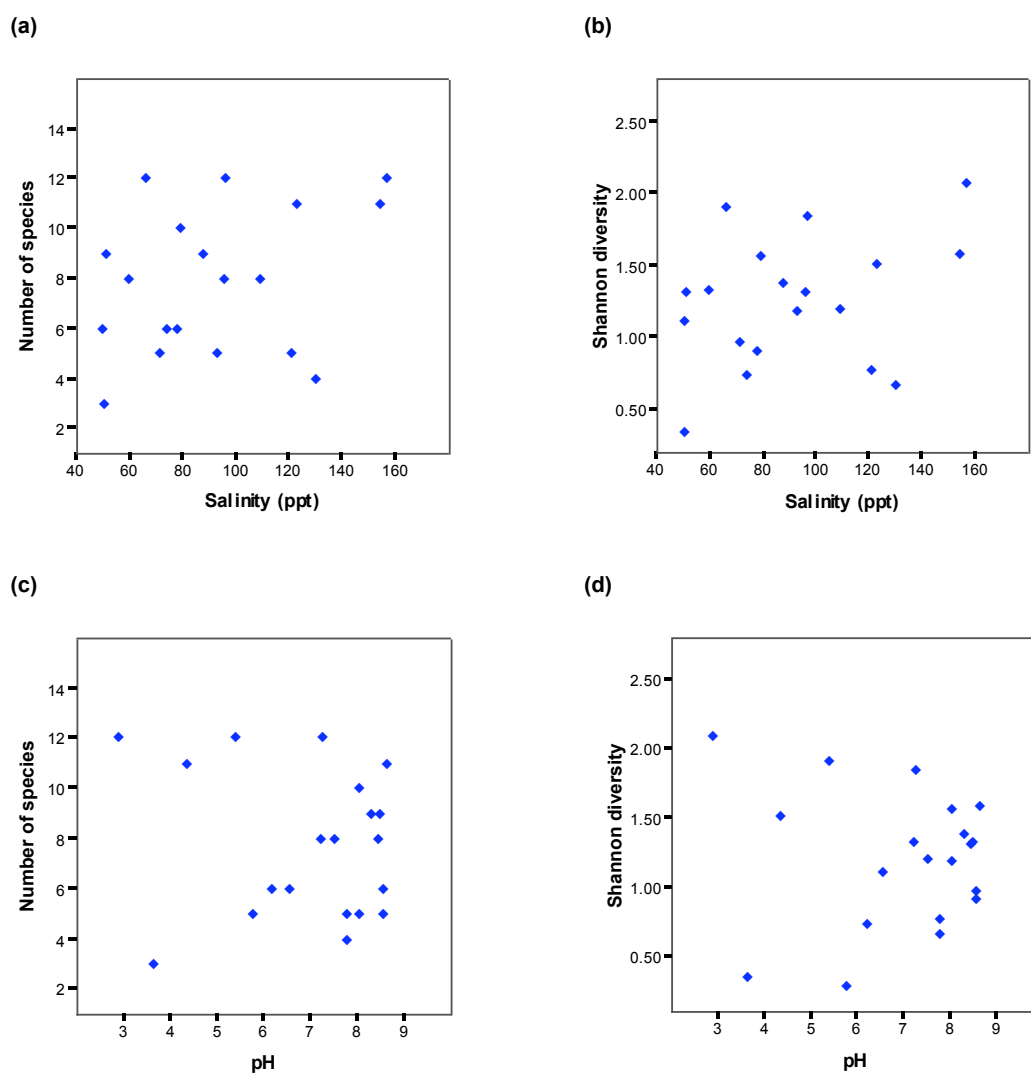
There was no apparent relationship between the number of species and salinity range (**Figure 5.5a-b**), with taxa numbers being highly variable (ranging from three to 12). A similar trend was observed with salinity and Shannon diversity. However, these two indices were related to pH and displayed a weak positive association, where the maximum number of diatom species occurred when pH was close to neutral (**Figure 5.5c-d**).



**Figure 5.3:** (a) Diatom species that occurred in five or more wheatbelt sites and (b) average salinity and associated range in which taxa occurred. Ac-*Amphora coffeaeformis*, Al-*Amphora* sp. aff. *luciae*, Cp-*Cocconeis placentula*, Ep-*Entomoneis* sp. aff. *paludosa*, Hb-*Hantzschia* sp. aff. *baltica*, Ni-*Navicula* sp. aff. *incertata*, No-*Nitzschia ovalis*, Nr-*Nitzschia* sp. aff. *rostellata*, Ns-*Navicula* sp. aff. *salinicola*, Nt-*Navicula tenelloides*, Pc-*Proschkinia* sp. aff. *complanata*.



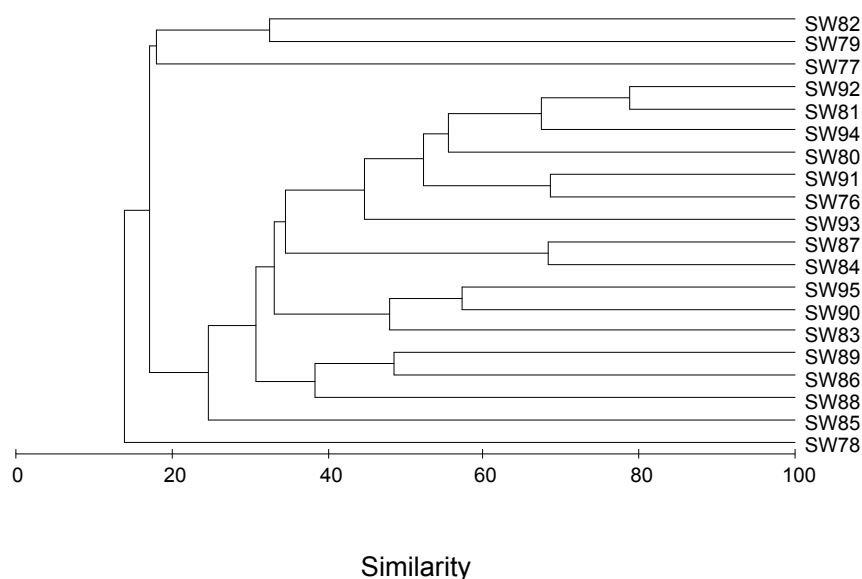
**Figure 5.4:** Scanning electron microscopy (SEM) images of common hypersaline diatom taxa. (a) *Hantzschia* sp. aff. *baltica*, (b) *Cocconeis* *placentula*, (c) *Nitzschia* *ovalis*, (d) *Navicula* sp. aff. *incertata*, (e) *Entomoneis* sp. aff. *paludosa*, (f) *Amphora* *coffeaeformis*, (g) *Navicula* sp. aff. *salinicola* and (h) *Amphora* sp. aff. *luciae*.



**Figure 5.5:** Number of species and Shannon diversity of hypersaline wheatbelt sites in relation to salinity concentration (a-b) and pH (c-d). The y-axis scale is different for each of the indices presented.

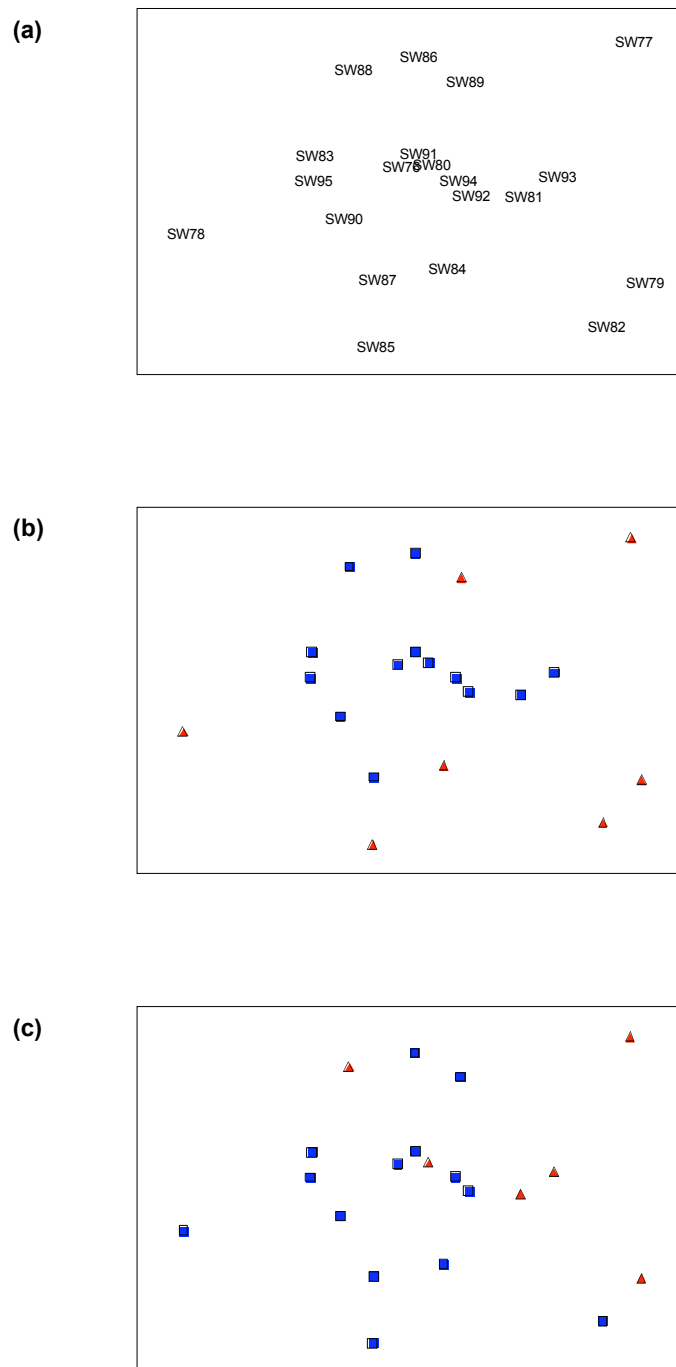
### 5.4.3 Multivariate analysis of diatom data

Hierarchical clustering was performed using Bray-Curtis similarities (**Figure 5.6**) to group sites according to diatom community structure. The resulting dendrogram showed that site SW78 was an outlier, with a similarity of only 15 %. In comparison, sites such as SW86 and SW89 were located within the main dendrogram cluster and had a similarity of close to 50 %. SW81 and SW92 were the two most comparable lakes, with a similarity of almost 80 % based on species composition.



**Figure 5.6:** Dendrogram for the hierarchical clustering of the 20 hypersaline wheatbelt sites using group-average linking of Bray-Curtis similarities, calculated on square-root transformed diatom abundance data.

The NMDS ordination of diatom data followed patterns from the hierarchical classification, and generated an acceptable stress level of 0.18 (**Figure 5.7a**). The main groupings on the NMDS plot were most related to pH, with sites that had a pH of greater than 7 clustering together (**Figure 5.7b**). ANOSIM supported these results showing a significant difference ( $p < 0.01$ ) between the two groups, however the R statistic indicated the level of segregation was intermediate (0.45). Salinity categories including sites between 50 and 100 ppt and sites greater than 100 ppt were also represented on the NMDS (**Figure 5.7c**). Some degree of overlap was apparent, confirmed by ANOSIM testing, which revealed there was no significant difference ( $p > 0.05$ ) derived from diatom assemblages.



**Figure 5.7:** NMDS ordination of the 20 hypersaline wheatbelt sites based on square-root transformed diatom abundance data. (a) NMDS plot showing site distribution using site codes. (b): NMDS plot with pH categories superimposed, where  $\blacktriangle$  are sites with pH less than 7 and  $\blacksquare$  are sites with pH greater than 7 and (c): NMDS plot with salinity groupings superimposed, where  $\blacksquare$  indicates sites between 50 to 100 ppt and  $\blacktriangle$  represents sites greater than 100 ppt.



The results of the BIOENV analyses determined that pH best explained the patterns in the diatom data, with the highest correlation value of  $\rho_w=0.54$  (**Table 5.4**). Subsequent combinations of environmental variables had weaker correlation coefficients ( $\rho_w$  values of approximately 0.40) and included pH with various arrangements of salinity and temperature.

**Table 5.4:** Summary of BIOENV results, with variable combinations that provide the highest Spearman rank correlation coefficients ( $\rho_w$ ) between environmental ( $\log_{10}$  salinity) and square-root diatom data. Bold type indicates overall optimum, only those combinations with a correlation coefficient greater than 0.40 have been shown.

Number of variables	Best variable combination	Correlation ( $\rho_w$ )
<b>1</b>	<b>pH</b>	<b>0.54</b>
2	pH, salinity	0.43
3	pH, salinity, temperature	0.41
2	pH, temperature	0.40

## 5.5 Discussion

### 5.5.1 Environmental variables

A number of the environmental variables measured in the study were found to be important in the PCA and can be attributed to the highly unpredictable nature of hypersaline wetlands in the wheatbelt. Such widely fluctuating conditions are dependent on factors including rainfall and the proportion of catchment cleared, which alters the hydraulic gradient and surface flows of these aquatic systems (Schofield *et al.* 1988; McComb and Lake 1990; Strehlow *et al.* 2005). For example, groundwater intersects the lake bed at Parkeyerring Lake (SW92) resulting in surface water being exposed to successive years of evapo-concentration and salt accumulation (Kay *et al.* 2001). Coomelberrup Lake (SW78) receives saline water from drainage channels located on surrounding agricultural land, further concentrating salts in the water column (Cale *et al.* 2004). Closed lake systems in particular are more susceptible to increased salinity, attaining saturation levels over long, dry summers (Williams 1983; Nielsen *et al.* 2003). Rising water temperatures are also associated with an increase in the concentration of dissolved salts (Boulton and Brock 1999) and the solubility of oxygen decreases significantly at elevated salinities (Borowitzka 1981). The degradation of riparian plant communities is associated with salinisation (Cramer and Hobbs 2002), with Dumblebung Lake

(SW80) and Lake Ninan (SW88) having experienced the death of fringing vegetation caused by salinity and waterlogging (Halse *et al.* 1993; Blinn *et al.* 2004). This has an adverse impact on all groups aquatic biota including diatoms.

### 5.5.2 Species composition

Diatom species numbers ranged from three to 12, following previous studies in the wheatbelt by Blinn *et al.* (2004), which reported less than 20 taxa in hypersaline waters. The majority of species also displayed a broad salinity tolerance, a finding observed with other groups of biota (Williams 1998). Of the 47 taxa recorded in total, only 11 were dominant, with *Amphora coffeaeformis* and *Nitzschia ovalis* being the two most widespread species, followed by *Navicula* sp. aff. *incertata* and *N.* sp. aff. *salinicola*. These taxa have the potential to be used as indicators of hypersalinity for wetlands in the south-west. The majority of halotolerant diatoms from the wheatbelt have also been recognised from other regions. Gasse (1986) identified *Amphora coffeaeformis* and *Navicula* sp. aff. *salinicola* from high salinities in water bodies from East Africa. Similarly, Compère (1994) found *A. coffeaeformis* and *Nitzschia ovalis* were common in wetlands with increased salt concentrations in northern Egypt. In south-east Australia *Amphora coffeaeformis*, *Entomoneis paludosa* and *Navicula incertata* were frequently recorded taxa at elevated salinities (Gell and Gasse 1990; Gell *et al.* 2002). Research from south Western Australia (Blinn *et al.* 2004) determined that *Amphora coffeaeformis*, *Nitzschia ovalis* and *Navicula tenelloides* were dominant hypersaline species, all of which were observed in this study. Specifically, *A. coffeaeformis* is regarded as one of the most salt-tolerant diatoms from inland areas of Western Australia (John 1998).

The morphological variability of a number of commonly encountered species from genera including *Amphora*, *Entomoneis*, *Hantzschia* and *Navicula* was difficult to establish in many wheatbelt lakes. Taxonomic discrepancies may result from the confusion associated with the original descriptions of many small, finely silicified diatoms that occur in saline waters. This is probably related to the availability of silica, which is considered to be an essential nutrient for diatom growth and can be a limiting factor as salinity increases (Cooper 1999). The concept of *N. salinicola* for example is ill-defined, and there are some problems in relation to the identity of type specimens (Archibald 1983). Gasse (1986) found the dimensions of this species to

vary significantly, referring to *N. sp. aff. salinicola* in the nomenclature. Uncertainty also exists for *Amphora luciae*, with Cholnoky's (1960) original definition and drawings not in agreement (Archibald 1983). The type material for this taxon has also been noted as confusing by Snoeijs and Balashova (1998). Other diatoms widely found were *Hantzschia sp. aff. baltica* and *Entomoneis sp. aff. paludosa*, which mostly conformed to the description given in the taxonomic literature except for slight variations in morphology. These aspects require further examination to determine the true status of saline species from the area, with implications for comparisons of species environment relations across regions.

### 5.5.3 Analysis of community structure in relation to salinity and pH

The relationship between salinity and the number of species/Shannon diversity was poor and confirmed by ANOSIM, which showed no significant difference between community structure above and below 100 ppt. As most taxa were consistently observed over this range, Hammer's (1986) suggestion of an extreme hypersalinity category (>100 ppt) is not supported based on these results, and the current classification system appears to explain distribution patterns (freshwater <3 ppt, hyposaline 3-20 ppt, mesosaline 20-50 ppt and hypersaline  $\geq 50$  ppt). Although, it should be noted that the dataset was limited in this case and may be contributing to a lack of statistical power in the analysis. Taking into account water bodies that cover the entire spectrum of salinity from fresh to hypersaline waters, a strong inverse relationship generally exists with diversity (Blinn 1993; Clavero *et al.* 2000; Potapova and Charles 2003). However, when the focus is solely on hypersaline lakes, only salt tolerant assemblages are present and other variables such as pH may play an important role influencing species composition (Williams 1998).

BIOENV results indicated pH was the most important factor in relation to community structure, although the correlation coefficient was not particularly high. This was also shown by the weak positive relationship based on species numbers and diversity. The pH of a water body can be influenced by the geology of the catchment (McComb and Lake 1990), with naturally acidic/saline wetlands common in some inland areas of the wheatbelt, having formed by local hydrogeochemical processes (Mann 1983). Acidification can also be generated from the continued application of fertilisers to crops (Archer 2001; Fitzpatrick 2002), or the installation of deep

drainage channels (Halse *et al.* 2003). The latter process causes the oxidation of acid sulphate soils, with subsequent run-off into surrounding water bodies (Bell and Mann 2004). For example, Lake Champion (SW85) is associated with acidic groundwater (Kay *et al.* 2001), and a pH of 6.21 was recorded in this study. This was within the range that Cale *et al.* (2004) recorded (2.68-6.50), finding that conditions persisted for most of the sampling period. Baandee Lake (SW77) has been previously identified as acidic (pH=6.30) by Blinn *et al.* (2004), however a much lower value (pH=4.35) was attained on this monitoring occasion. A distinct lack of published literature is available for the comparison of sites including Cowcowing (SW79) and Hines Hill (SW82) lakes where the pH was less than 4, with this research providing baseline data for future investigations.

Although pH was shown to be a significant variable, halotolerant diatoms continued to dominate the majority of acidic hypersaline sites, however overall differences in species composition were observed. *Navicula* sp. aff. *arvensis* and *Luticola mutica* were both recorded from Cowcowing Lakes (SW79), and though not abundant, reflected the water quality (pH=2.90). Blinn *et al.* (2004) identified *Navicula arvensis* from wetlands in the wheatbelt with a pH range of between 3.5-7, and noted the presence of *Luticola mutica* in waters with pH as low as 6. The latter has also been reported from acidic lakes (pH range of 4.5-5.3) in the north-eastern United States (Camburn and Charles 2000). The extreme environment of wetlands affected by acidification and salt loading suggests that few taxa would be capable of tolerating these conditions (Blinn *et al.* 2004). However, the number of species ranged from very low (3) to relatively high (12) in wheatbelt lakes with pH less than 7. Taxa numbers greater than 10 were unexpected and may be attributed to the one-off sampling regime, or changes in water chemistry over the sample period. Inundation levels related to the hydroperiod of wheatbelt systems can also influence the diversity of these water bodies. Therefore, it is recommended that any future studies include more sites and increase the number of samples at each location, to determine if species assemblages persist. It is also important to acknowledge that there may be other variables responsible for influencing diatom community structure not taken into account, including factors such as ionic composition (constituent ions), nutrients and heavy metals (Peterson *et al.* 1997; Reed 1998; Gell *et al.* 2002; Potapova and Charles 2003; Blinn *et al.* 2004).

## 5.6 Conclusion

The results from this study have shown that for salinity concentrations between 50 and 150 ppt, diatom taxa displayed broad tolerance ranges, with approximately 10 species dominating in these conditions. For example, *Amphora coffeaeformis*, *Nitzschia ovalis*, *Navicula* sp. aff. *incertata* and *N.* sp. aff. *salinicola* may be considered potential indicators of hypersalinity for wetland systems in the wheatbelt region. Statistical analysis also determined that there was no discernable difference between diatom assemblages above 50 ppt, with the suggestion of an extreme hypersalinity category unwarranted based on this dataset. The importance of low pH was also highlighted, with acidification arising as the next major problem for salinised lakes in the area. Further research is required to determine the variables that control diatom assemblages in acidic hypersaline wetlands from the south-west, with implications for the diversity of all groups of aquatic biota.

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## Chapter 6: A Diatom-Based Transfer Function for Lakes and Streams Severely Impacted by Secondary Salinity in the South-West of Western Australia

### 6.1 Abstract

The association of diatom assemblages to salinity was further investigated through the development of a diatom-based transfer function on lakes and streams from the south-west. The relationship between environmental variables and species composition was explored using canonical correspondence analysis (CCA) and partial CCA. Salinity was shown to account for a significant and independent amount of variation in the diatom data, enabling an inference model to be developed on the final dataset, consisting of 89 sites and 150 diatom taxa. The most successful model was derived using tolerance-downweighted weighted averaging. Summary statistics showed that the transfer function performed very well with a high coefficient of determination and low prediction errors that remained high after the cross-validation method of jackknifing ( $r^2_{\text{apparent}}=0.97$  and  $r^2_{\text{jackknifed}}=0.89$ ). The optima of freshwater diatoms was similar to those reported elsewhere, although optima values for hypersaline species tended to be higher, reflecting the euryhalinity of taxa in this range. This suggests that salinity can be accurately predicted using diatom relative abundances, and the model can be applied to paleolimnological reconstructions. However, the transfer function also provides the basis for use in future biomonitoring studies, to evaluate the success of remediation measures implemented to secondary salinised systems.

### 6.2 Introduction

Salinity is an important water resource management issue for the south-west of Western Australia, as well as being a significant factor that influences diatom community structure, established in previous chapters. This part of the thesis aimed to extend this theme by developing a predictive model (transfer function) based on diatom assemblages and their relationship to increasing salt loads in the region.

Diatom-based transfer functions have been applied to a range of environmental variables for aquatic systems around the world. For example, Battarbee *et al.* (1997) created a predictive model for the acidification of surface waters in central Norway.

Similarly Enache and Prairie (2002) derived transfer functions for pH, total phosphorus and dissolved organic carbon for lakes in western Quebec, Canada. Tibby (2004) also developed a transfer function for total phosphorus in south-eastern Australian water storages and Reid (2005) was able to construct models for a number of variables including pH and chlorophyll *a* across the North and South Islands of New Zealand. In western North America, a 219 lake dataset was used to assess the reliability of salinity inference models and provide a sensitivity analysis of associated diatom taxa (Wilson *et al.* 1996).

The use of models for assessing salinity concentration is considered to be a valuable statistical tool, generating calibration or training sets that can be employed for quantitative paleolimnological reconstructions (Stevenson and Pan 1999; Battarbee *et al.* 2001; Racca *et al.* 2004). This was demonstrated with the development of a transfer function for ionic composition, which included almost 300 modern diatom samples from African lakes (Gasse *et al.* 1995). In comparison, over 50 samples were used to establish a diatom-based transfer function for conductivity in central Mexican lakes (Davies *et al.* 2002) and a salinity model was produced for 57 lakes in California (Bloom *et al.* 2003). Similarly, a conductivity transfer function was derived from 40 lakes with modern diatoms samples in eastern Tibet, China (Yang *et al.* 2003).

The problem of secondary salinity in eastern Australia has also led to the development of a predictive model that incorporated 62 wetlands from Western Victoria, and determined the optima and tolerances of common diatom taxa (Gell 1997; 1998). However, currently there are no diatom-based transfer functions available for salt-affected lakes and streams in the south-west of Western Australia. Understanding the fluctuations in salinity concentration of water bodies in this area is essential, given the considerable increases that are expected to occur in the next 15-20 years (Short and McConnell 2001; Mayer *et al.* 2004; McFarlane *et al.* 2004). Wetlands located further inland are especially important as they characterise the semi-arid nature of the region, which has led to the formation of a unique array of aquatic systems with intrinsic conservation value (Lane and McComb 1988; Halse 2004).

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The objective of the investigation was to establish a diatom-based transfer function for lakes and streams affected by secondary salinisation in Western Australia. The south-west sites represent a modern dataset from which the response of diatom taxa to salinity can be quantitatively measured. The inference model has the potential to provide insights for paleolimnological studies, or to be integrated into future biomonitoring programs.

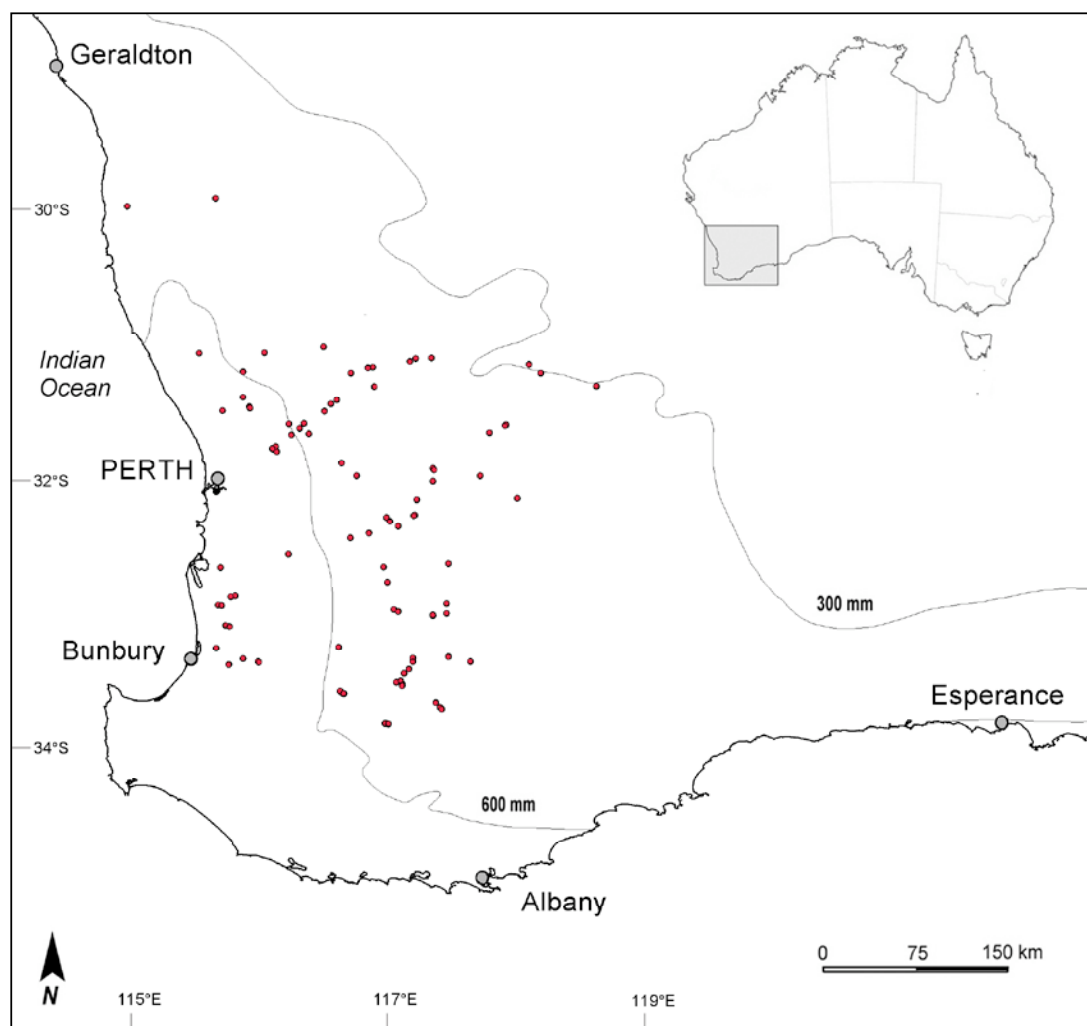
### **6.3 Materials and Methods**

#### *6.3.1 Study sites and collection procedure*

Study sites consisted of the 95 lakes and streams described in previous chapters (**Figure 6.1**). Sites were sampled once only, integrating both flowing and standing waters due to the temporary nature of water bodies in the area. Measurement of environmental variables was conducted at each site and diatom community structure was sampled using an artificial substrate collector; the JJ periphytometer (John 1998). This method ensured uniformity over the sites and that species assemblages were associated with ambient water quality. These techniques were presented in greater detail in Chapters 3 and 4 of the thesis.

#### *6.3.2 Sample preparation and enumeration*

Samples were prepared using the nitric acid digestion procedure (John 1983), after which enumeration and identification of diatoms was carried out with specialised taxonomic literature. The extended methodology for these techniques has been provided in Chapter 4. The resulting dataset was then converted into percentage abundances for statistical analyses.



**Figure 6.1:** The location of the 95 study sites (indicated by ●) in the south-west region of Western Australia. The 600 and 300 mm annual isohyets approximately define the wheatbelt area.

### 6.3.3 Data analysis

Detrended correspondence analysis (DCA), detrending by segments, was used to investigate patterns in diatom community structure and to confirm that the dataset showed a unimodal response. As the gradient length of the ordination axis exceeded 4, canonical correspondence analysis (CCA) was deemed to be an appropriate statistical technique for the data (Leps and Smilauer 2003). Both analyses were carried out with diatom abundances square-root transformed to reduce skewness.

The relationship between sites, environmental variables and diatom assemblages was explored using CCA with biplot scaling. Salinity (ppt) was  $\log_{10}$  transformed and downweighting of rare taxa was employed. Monte Carlo tests were run with 999 random permutations to test the significance of canonical axes and to assess the relative importance of environmental variables. A series of partial CCAs were implemented to partition the total explained variation in the diatom data (Bocard *et al.* 1992). This technique determined the unique contribution of salinity and the interaction effects that occurred with other environmental variables. All ordination procedures were performed in CANOCO Version 4.5.3 (ter Braak and Smilauer 2004), with partitioning results providing the statistical basis for the development of the transfer function (Battarbee *et al.* 1997).

The statistical software package C2 Version 1.4 Beta (Juggins 2003), was used to derive a diatom-based transfer function for salinity using classical and inverse deshrinking techniques. Simple weighted averaging (WA) and tolerance-downweighted WA transfer functions were developed based on the relative abundance of diatom taxa. Values for tolerance-downweighted WA have been included as they provide an extension of the simple weighted averaging method, which incorporates information on species tolerances and ranges (ter Braak 1987). The subsequent WA regression coefficients calculated from this procedure provided an estimate of species optima and tolerances limits to salinity concentration.

Model performance has been reported throughout the chapter in terms of the coefficient of determination ( $r^2$ ), the root-mean squared of the error (RMSE), and the root-mean squared error of prediction (RMSEP) between observed and inferred values. The  $r^2$  statistic measures the predictive ability of the model and assesses the

strength of the relationship between observed and inferred values, where as the RMSE and RMSEP indicates the prediction errors in the dataset. The degree of maximum bias (Max bias) calculates the tendency to over or under-estimate values along the salinity gradient (Birks 1995; Gasse *et al.* 1995). Both apparent and jackknifed (leave-one-out) values have been presented, allowing for comparison with other published transfer functions. Jackknifing is a cross-validation method involving sample resubstitution, providing a less biased measure and giving a better indication of the true predictive ability of the model (Dixon 2001).

The original dataset of 217 species and 95 sites was reduced to 150 taxa and 89 sites to increase the accuracy of the transfer function. Outliers were those sites that had a difference between the observed and jackknifed-inferred salinity value of greater than one-quarter of the total range of the variable (Gasse *et al.* 1995). These included sites SW44, SW49, SW79, SW80, SW82 and SW90. Diatom taxa with a maximum abundance of less than 1 % were also removed from the final model in order to lessen the influence of rare taxa.

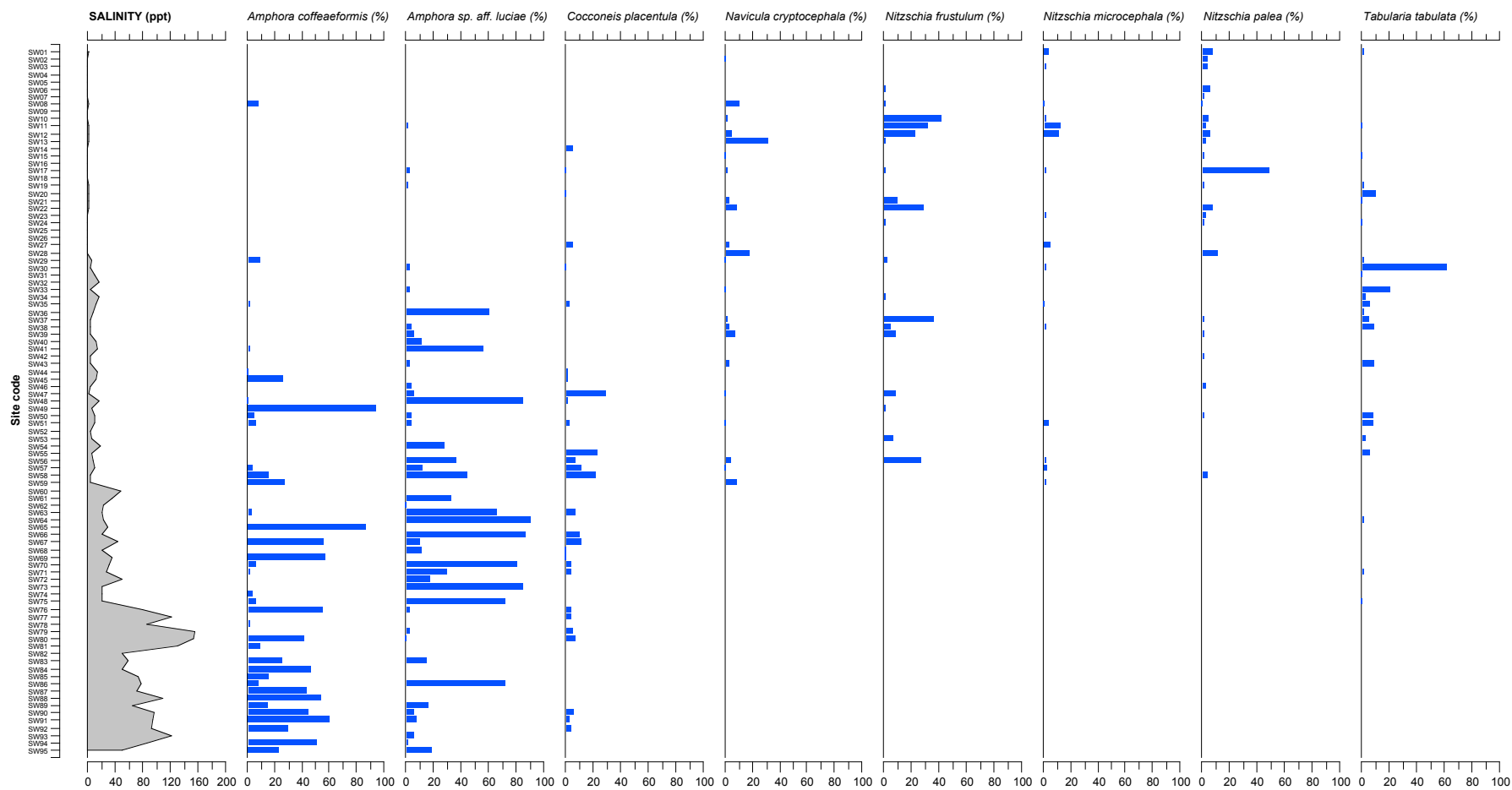
The comparison of model statistics including the optima and tolerance limits of diatom taxa has been based on salinity (ppt) throughout the chapter. As all comparison values quoted in the discussion were below 35 ppt, the optima and tolerance values from other regional studies that were measured in  $\text{gL}^{-1}$ , were also converted to ppt. The difference between the two measurements at these concentrations is negligible ( $<1$ ).

## 6.4 Results

### 6.4.1 Diatom relative abundance

A total of 217 diatom species were identified from the 95 lakes and streams. The abundance of the most commonly occurring species (present in  $\geq 25$  sites) is presented in **Figure 6.2**. *Amphora coffeaeformis* and *Amphora* sp. aff. *luciae* were the most dominant taxa recorded from the south-west water bodies and demonstrated consistently high abundances (in excess of 50 %) in the majority of saline sites ( $>20$  ppt). Species such as *Nitzschia frustulum* and *Nitzschia palea* were regularly found in the freshwater sites ( $<3$  ppt), and had comparatively lower abundance levels (less than 50 %).





**Figure 6.2:** Relative abundance of diatom taxa occurring in 25 or greater sites in relation to salinity concentration in the south-west region.

### 6.4.2 Ordination results

CCA (canonical correspondence analysis) was used to investigate the relationship between sites, environmental variables and diatom assemblages. Axis 1 ( $\lambda_1=0.71$ ) and 2 ( $\lambda_2=0.31$ ) explained 9.60 % of the cumulative variance in the data (**Table 6.1**). The low percentage variance explained is typical of complex datasets that contain many zeroes and a large number of samples and species (Dixit *et al.* 1993). However, the statistical validity of the ordination axes is considered to be of greater importance than the amount of variance explained and can be evaluated using a permutation test (Dixit *et al.* 1993; ter Braak and Smilauer 2002). Both canonical axes were shown to be significant based on Monte Carlo testing ( $p<0.01$ , 999 random permutations). In addition, DCA generated similar eigenvalues ( $\lambda_1=0.81$ ,  $\lambda_2=0.40$ ) and species variance results (11.40 % over 2 axes), further supporting the CCA ordination (**Table 6.1**).

**Table 6.1:** Summary of CCA ordination ( $\log_{10}$  salinity and square-root species data with downweighting of rare taxa) and DCA results (square-root species data with downweighting of rare taxa) for the 95 south-west lakes and streams.

Analysis	Axes	1	2	3	4	Total
<i>CCA</i>						
Eigenvalues		0.71	0.31	0.15	0.12	
Species-environment correlations		0.95	0.81	0.69	0.74	
Cumulative % variance: species data		6.70	9.60	11.00	12.20	
Cumulative % variance: species-environment		51.10	73.30	84.00	92.50	
Sum all eigenvalues						<b>10.639</b>
Sum all canonical eigenvalues						<b>1.397</b>
<i>DCA</i>						
Eigenvalues		0.81	0.40	0.30	0.28	
Lengths of gradient		7.34	3.40	3.35	3.39	
Cumulative % variance: species data		7.60	11.40	14.20	16.90	
Sum all eigenvalues						<b>10.639</b>

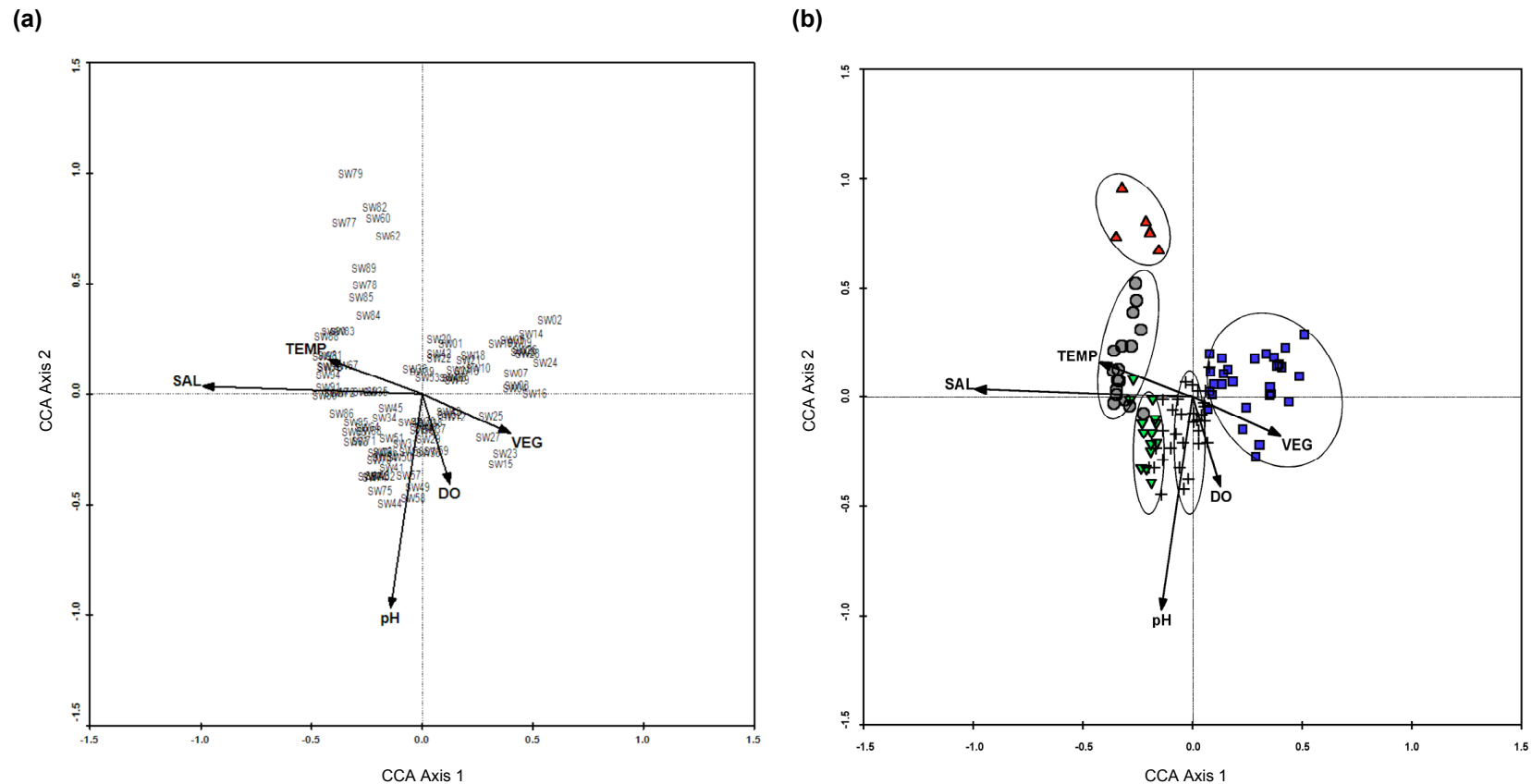
The first two axes accounted for almost 75 % of the cumulative variance of species-environment relation (**Table 6.1**). CCA determined that salinity and pH mainly influenced diatom community structure, indicated by the length of their vectors on the biplot (**Figure 6.3a**). Axis 1 was highly correlated to salinity with an intraset correlation of -0.94. This was demonstrated by a clear gradient separating sites into the different salinity categories, ranging from freshwater (<3 ppt) and hyposaline (3-20 ppt), through to mesosaline (20-50 ppt) and hypersaline ( $\geq 50$  ppt), based on

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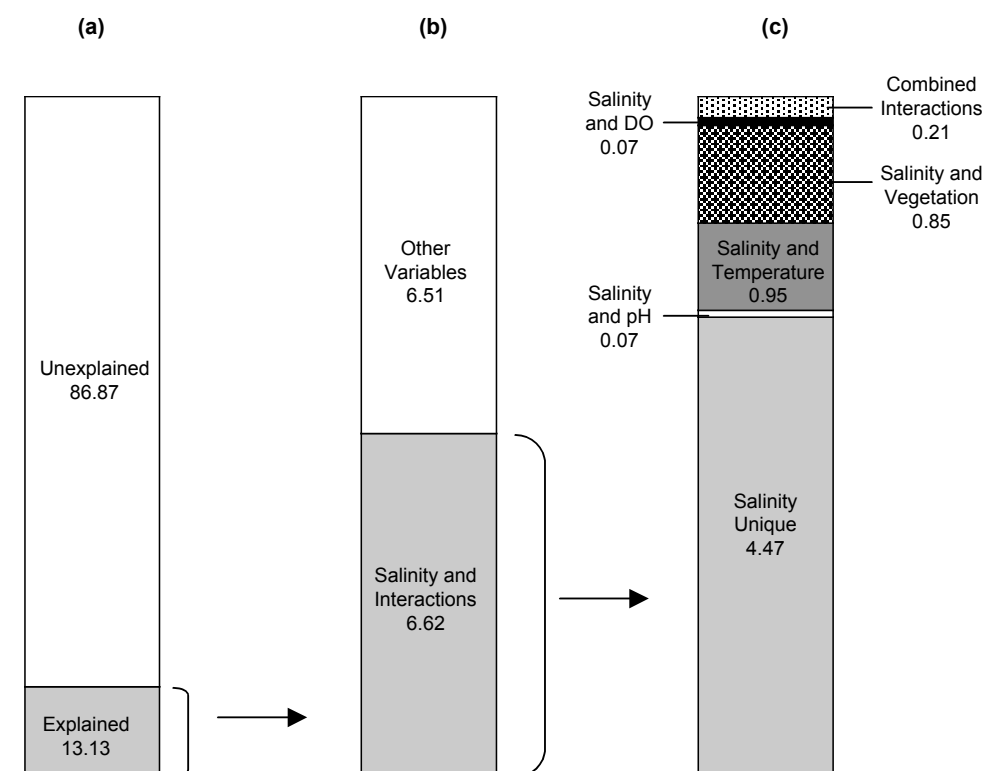
species composition (**Figure 6.3b**). Axis 2 was strongly related to pH, having an  $r$ -value of -0.78. This was illustrated by a small group of outliers on the biplot (**Figure 6.3b**), representing both acidic ( $\text{pH} < 5$ ) and saline ( $> 20$  ppt) water bodies, which had distinct diatom assemblages associated with these conditions.

Overall the five environmental variables used in the ordination analyses accounted for 13.13 % of the total explained variance. **Figure 6.4a-c** summarises the results of the partial CCAs into which this total explained variance is partitioned. It shows the contribution of salinity compared to the other environmental variables as well as the unique amount of variance explained by salinity and its interactions with other variables.

From the 13.13 % (**Figure 6.4a**) of total explained variance salinity accounted for 6.62 %, with pH, dissolved oxygen, temperature and vegetation only contributing to 6.51 % collectively (**Figure 6.4b**). Examining the partitioning of variance for salinity (**Figure 6.4c**), the results indicated that there was minimal interaction between salinity and pH (0.07 %), the two most highly correlated variables in the CCA. Monte Carlo testing (999 random permutations) also showed that both of these variables were highly significant ( $p < 0.01$ ) in the analyses. However, salinity accounted for the largest proportion of unique diatom variance (4.46 %), meaning that it represented an independent amount of variation in the diatom data, enabling the development of a statistically significant transfer function.



**Figure 6.3:** CCA biplot showing (a) sites and environmental variables and (b) site groupings in relation to salinity and pH gradients. The ordination is based on the analysis of 95 lakes and streams and the environmental variables of salinity (SAL,  $\log_{10}$  transformed), pH, temperature (TEMP), dissolved oxygen (DO) and vegetation (VEG), with square-root transformed diatom data and downweighting of rare taxa. Axis 1 was strongly related to salinity, where (■) are freshwater <3 ppt sites, (+) are hyposaline 3-20 ppt sites, (▼) are mesosaline 20-50 ppt sites and (●) are hypersaline  $\geq 50$  ppt sites. Axis 2 was correlated to pH where (▲) are both acidic (pH<5) and saline sites (>20 ppt).



**Figure 6.4:** Summary of the results of partitioning the total variance in the diatom data into (a) proportion of explained and unexplained variance, (b) proportion explained by salinity including interaction effects and that explained by other environmental variables and (c) proportion of unique variance explained by salinity and interaction with each variable. Results are based on 217 taxa from 95 sites, with downweighting of rare taxa.

#### 6.4.3 Salinity transfer function

The strong relationship between salinity and diatom assemblages from the southwest study sites was used to derive a salinity transfer function. The summary statistics for both simple weighted averaging (WA) and tolerance-downweighted WA (Tol WA) methods and classical (cla) and inverse (inv) deshrinking techniques have been presented in **Table 6.2**. These values represent the reduced dataset of 89 sites and 150 diatom taxa, based on  $\log_{10}$  transformed salinity.

The coefficients of determination for simple WA and tolerance-downweighted WA were high ( $r^2=0.95$  and  $0.97$  respectively), indicating the transfer function performed very well. Jackknifed  $r^2$  values were lower in all cases ( $r^2_{\text{jackknifed}}=0.88-0.89$ ) and prediction errors were higher ( $\text{RMSEP}_{\text{jackknifed}}=0.21-0.22$ ) than the corresponding

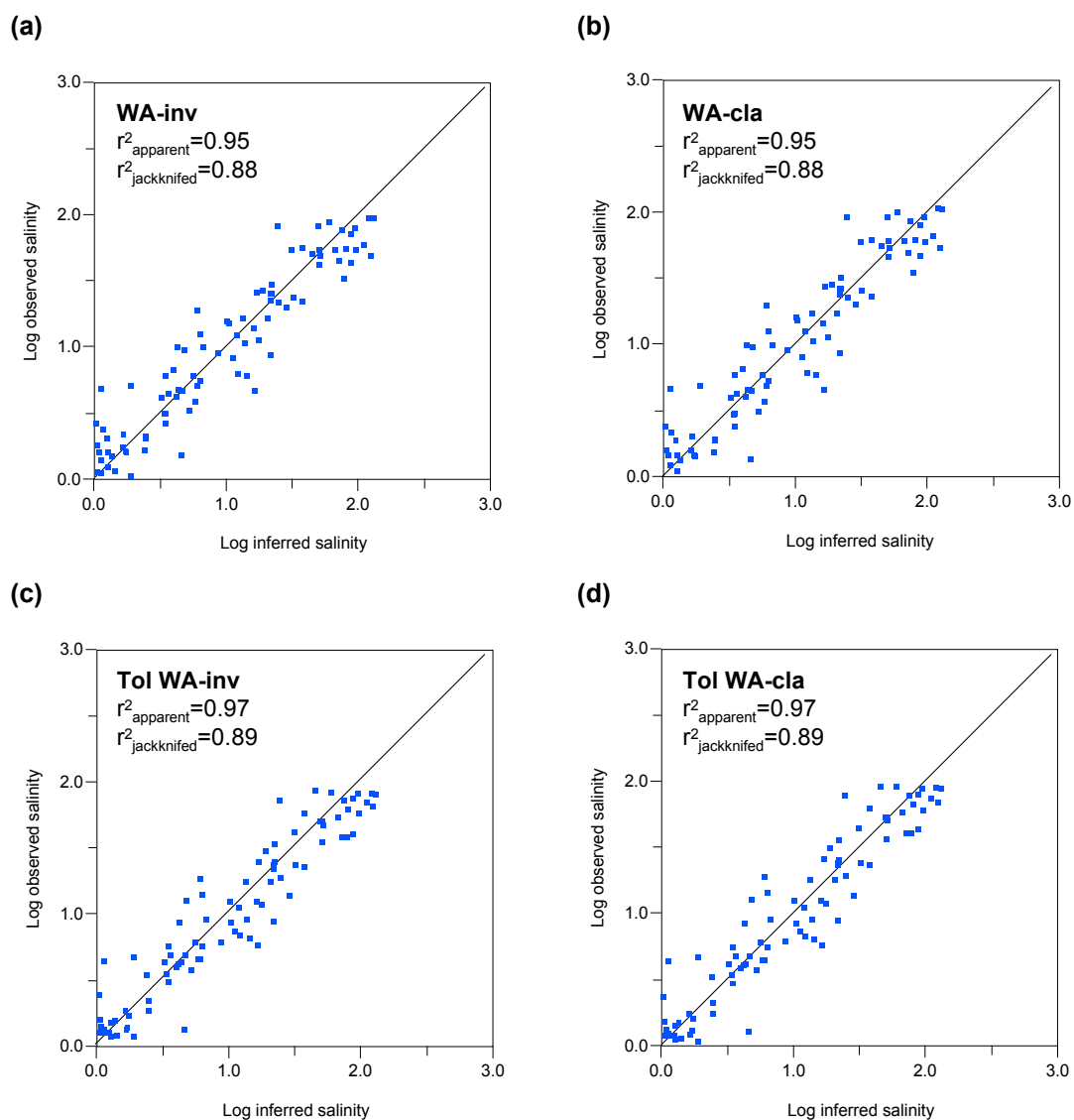
apparent measures. However the strength of the relationship between observed and inferred salinity remained consistently high after cross-validation in the form of jackknifing was conducted on the data.

Tolerance-downweighted WA estimations performed slightly better (**Table 6.2**) than simple WA for both inverse and classical deshrinking ( $r^2_{\text{apparent}}=0.97$ ,  $r^2_{\text{jackknifed}}=0.89$ ). Tolerance-downweighted WA also had lower prediction errors ( $\text{RMSE}_{\text{apparent}}=0.11-0.12$ ,  $\text{RMSEP}_{\text{jackknifed}}=0.21$ ) and maximum bias values ( $\text{Max bias}_{\text{apparent}}=0.06-0.08$ ,  $\text{Max bias}_{\text{jackknifed}}=0.17-0.20$ ), compared to simple WA methods. The various weighted averaging procedures have been presented as a series of scatterplots in **Figure 6.5a-d**, which clearly show the strong linear relationship between observed and inferred salinity values.

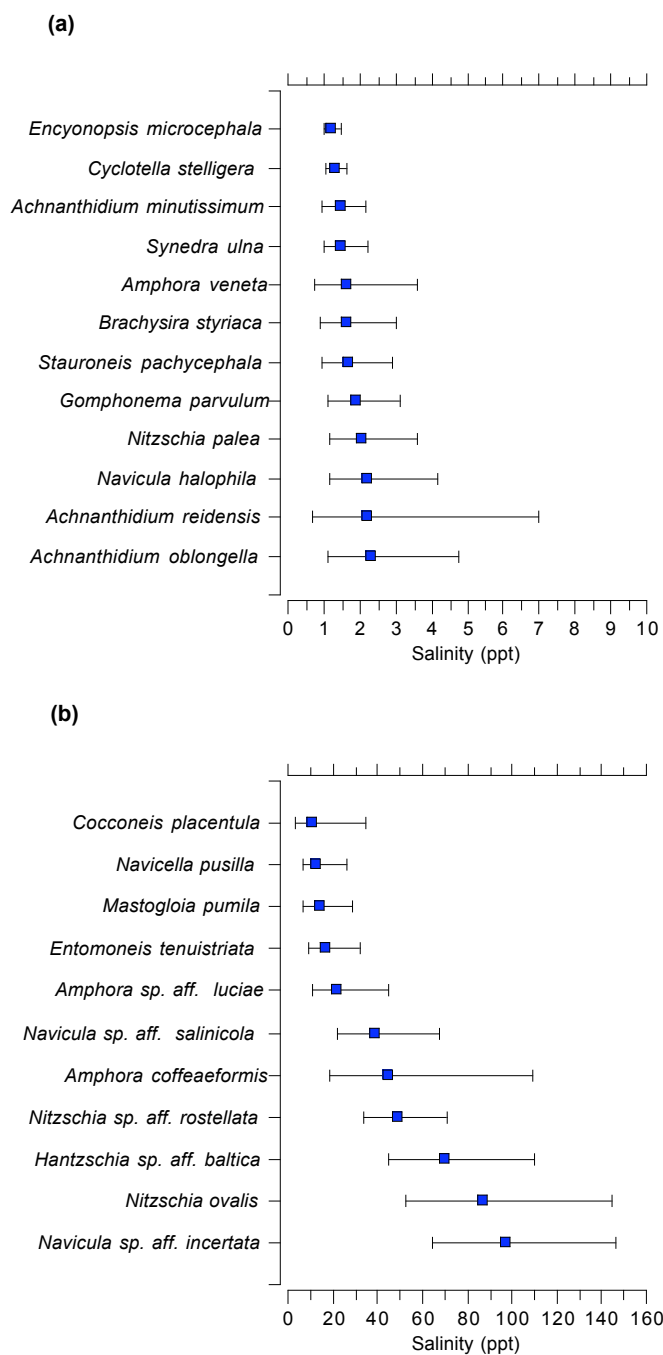
**Table 6.2:** Summary statistics for the performance of simple weighted averaging (WA) and tolerance-downweighted WA (Tol WA) transfer function for salinity ( $\log_{10}$  transformed ppt). Inverse (inv) and classical (cla) deshrinking methods have been reported for apparent and jackknifed values. Bold type indicates the best performing procedure.

Summary Statistics	WA-inv	WA-cla	Tol WA-inv	Tol WA-cla
<i>Apparent</i>				
$r^2$	0.95	0.95	0.97	<b>0.97</b>
RMSE	0.15	0.15	0.11	<b>0.12</b>
Max Bias	0.09	0.07	0.08	<b>0.06</b>
<i>Jackknifed</i>				
$r^2$	0.88	0.88	0.89	<b>0.89</b>
RMSEP	0.22	0.22	0.21	<b>0.21</b>
Max Bias	0.21	0.16	0.20	<b>0.17</b>

The salinity optima and upper and lower tolerance limits of the 150 taxa used to construct the model are displayed in **Appendix 6.1**. A high Hill's N2 (Hill 1973) value indicates a greater number of effective occurrences in the dataset and that the salinity estimates for these taxa are more reliable. Overall the majority of diatoms (approximately 100) had optima below 10 ppt, with less than 20 species displaying and optima of above 50 ppt. The optima and tolerance range for some of the most commonly occurring freshwater and saline taxa have been presented in **Figure 6.6a-b**.



**Figure 6.5:** Relationship between measured (observed) and diatom-inferred salinity ( $\log_{10}$  ppt) based on apparent values for (a) simple WA inverse deshrinking, (b) simple WA classical deshrinking, (c) tolerance-downweighted WA inverse deshrinking and (d) tolerance-downweighted WA classical deshrinking methods. Both  $r^2$  apparent and jackknifed measures have been indicated.



**Figure 6.6:** Backtransformed estimated salinity optima and upper and lower tolerance limits of diatom taxa (in order of increasing optima), with a maximum abundance of greater than 1 % and occurring in 10 or more sites from the modern dataset of 95 lakes and streams in the south-west. (a) Represents freshwater species with optima of less than 3 ppt and (b) shows saline diatom species with optima greater than 10 ppt.



Species that were abundant in the freshwater sites (Hill's  $N2 > 3$ ) and had the lowest salinity optima included *Encyonopsis microcephala* (1.21 ppt), *Achnantheidium minutissimum* (1.46 ppt) and *Gomphonema parvulum* (1.89 ppt). These taxa also showed the narrowest tolerance ranges of between 0.5 and 2 ppt. Diatoms with the broadest tolerance ranges (50-100 ppt) tended to display much higher salinity optima. These included species such as *Navicula* sp. aff. *incertata*, *Hantzshia* sp. aff. *baltica* and *Nitzschia ovalis*, which all had optima of greater than 50 ppt (Hill's  $N2 > 3$ ).

## 6.5 Discussion

### 6.5.1 Transfer function performance

The ordination procedure determined that salinity accounted for an independent and significant amount of the total variation in the diatom data, allowing for the development of the transfer function. The inference model performed very well and showed a highly significant relationship between modern diatom assemblages from the south-west and salinity. The coefficient of determination ( $r^2$ ) was high and apparent error values low (RMSE), with jackknifed prediction errors increasing marginally, highlighting the importance of using a cross-validation method on the dataset (Birks 1995; Battarbee *et al.* 2001). However jackknifed  $r^2$  values remained consistently high using both simple and tolerance-downweighted weighted averaging (WA), indicating the transfer function can be used to accurately predict salinity, taking into account prediction errors (Davies *et al.* 2002).

The method of tolerance-downweighted WA was shown to perform better than simple WA techniques and is recommended for use in the south-west. Classical deshrinking was only slightly better than inverse deshrinking, with the choice of procedure dependent on the required prediction values (Gasse *et al.* 1995). If greater accuracy is needed at the upper or lower end of the salinity spectrum, then classical deshrinking is preferred. However, if the focus is on inferring mid-range salinity concentrations, then inverse deshrinking should be used (Birks 1995; Gasse *et al.* 1995).

The transfer function derived for the south-west was comparable to those from other regions of the world, with performance being similar to previously published models

( $r^2_{\text{apparent}}=0.97$  and  $r^2_{\text{jackknifed}}=0.89$ ). For example, the conductivity transfer function for African lakes had an  $r^2_{\text{apparent}}=0.92$  and  $r^2_{\text{jackknifed}}=0.80$ , using tolerance-downweighted WA with both classical and inverse deshrinking (Gasse *et al.* 1995). A salinity inference model from British Columbia, in Canada generated an  $r^2$  value of 0.92, also created with tolerance-downweighted WA methods and based on seasonal water chemistry measurements (Wilson *et al.* 1994). An electrical conductivity model developed for Spanish salt lakes, showed an  $r^2_{\text{apparent}}=0.91$  based on simple WA, however cross-validation led to a much lower  $r^2_{\text{jackknifed}}=0.57$  (Reed 1998). Weighted averaging techniques with inverse deshrinking was also applied to a salinity transfer function from the northern Great Plains of North America, to produce a high quality model ( $r^2_{\text{apparent}}=0.91$ ) (Fritz *et al.* 1991). In Australia a transfer function by Gell (1997) produced an  $r^2$  statistic of 0.87 and a jackknifed value of 0.79 using tolerance-downweighted WA for lakes in Western Victoria. By comparing the south-west model with those developed from these areas it is possible to determine the level of consistency between datasets and the ecological optima derived for common diatom taxa (Davies *et al.* 2002).

### 6.5.2 Comparison of taxa optima

In general, the estimated salinity optima of diatom species inhabiting freshwater and low salinity concentrations were agreeable with other regions. Hill's N2 (Hill 1973), a measure of the number of the effective occurrences of each taxon, tended to be higher for these species, indicating they were well represented in the dataset and that their estimated optima is likely to be more reliable (Cumming and Smol 1993). Species dominating freshwaters included *Achnanthydium minutissimum*, with optima of 1.5 ppt in the south-west of Western Australia, compared to 0.6 ppt in the Canadian dataset (Wilson *et al.* 1994), and 1.4 ppt in the south-east of Australia (Gell 1997). Another common freshwater taxon was *Synedra ulna*, which had optima of 1.5 ppt (south-west), 1.6 ppt (northern Great Plains) (Fritz *et al.* 1993) and 2.1 ppt (south-east Australia). *Navicula cryptocephala* displayed higher optima of 3.0 ppt in the south-west, 2.7 ppt in the northern Great Plains and 3.1 ppt in south-east Australia. A species that has been shown to withstand low to moderate salinity is *Nitzschia frustulum*, which had optima of 3.9 ppt and 4.0 ppt in the south-west and south-east of Australia, much lower than optima values derived from the Spanish (Reed 1998) and Canadian training sets (9.4 and 16.8 ppt respectively).

The estimated optima of saline species in the south-west tended to be higher than those from other datasets. For example, the optima for *Amphora coffeaeformis* was 44.9 ppt in the south-west, 26.9 ppt in south-east Australia and 7.8 ppt in Africa (Gasse *et al.* 1995). This was also the case for *Navicula incertata*, 97.0 ppt (south-west), compared to 34.7 ppt (south-east Australia) and 21.1 ppt (northern Great Plains). However, for the commonly encountered *Navicula salinicola* optima estimates showed some degree of disparity, 38.8 ppt in the south-west, 63.1 ppt in south-east Australia, 20.1 ppt in Africa and 22.3 ppt in Spain. Another species with a cosmopolitan distribution is *Cocconeis placentula*, well-documented as having highly variable optima. The south-west study generated an optima similar to south-east Australia (10.8 ppt and 12.0 ppt respectively), compared to much lower optima derived for Africa (0.3 ppt), the northern Great Plains (2.3 ppt) and Canada (0.2 ppt). The salinity optima of a number of species common to hypersaline sites may be overestimated in the south-west due to the lower number of sample sites compared to those in the freshwater and low salinity ranges. However, in general taxa with higher salinity optima also displayed a broad tolerance, reflecting the euryhalinity of diatoms at the upper end of the salt spectrum, with the majority of these species able to withstand fluctuating conditions (Gasse *et al.* 1987).

A number of saline diatom taxa were frequently encountered in the south-west, and were absent from similar water bodies around the world. These included species such as *Amphora* sp. aff. *luciae* (optima=22.1 ppt), *Hantzschia* sp. aff. *baltica* (optima=70.1 ppt) and *Nitzschia ovalis* (optima=86.9 ppt). These taxa all displayed high Hill's N2 values (>5) and can therefore be considered influential within the training set (Cumming and Smol 1993; Reed 1998). This indicates that although many saline species may be ubiquitous in their distribution (Clavero *et al.* 2000), the south-west appears to have a unique array of diatom assemblages inhabiting inland salt lakes. Many of the taxa also require further examination to determine if they are species that have been previously documented elsewhere or if they are endemic, having evolved as a result of the isolated and internal nature of wetlands in Western Australia (De Deckker 1983; Halse *et al.* 2003).

### 6.5.3 Discrepancies between datasets

Compared to other datasets, the south-west transfer function contained a relatively large number of sites (95 reduced to 89) that incorporated all parts of the salinity gradient, ranging from freshwater to hypersaline (0.04–156.80 ppt). The British Columbia (Canada) dataset consisted of 65 lakes, later combined to form a dataset of 111 samples (Wilson *et al.* 1994) and then further expanded to 219 lakes (Wilson *et al.* 1996), spanning a large gradient of ionic concentrations, with a subsequent strong correlation between measured and diatom inferred salinity (Cumming and Smol 1993). The African lakes dataset is well-recognised as one of the largest with 282 modern diatom samples, created by merging a number of regional subsets, and increasing the strength of the relationship between diatoms and hydrochemical variables (Gasse *et al.* 1995). The larger training sets tend to incorporate a more representative range of salinities, leading to the development of estimated optima for diatom taxa that should generally be agreeable for the different geographical zones (Birks 1995; Battarbee *et al.* 2001).

In contrast, discrepancies between species optima may arise as a result of small sample size or through the use of a dataset that is biased towards the upper or lower end of the salinity scale. For example, the relatively small dataset (53 samples) from Central Mexico is acknowledged as having a bias in the low and medium conductivity ranges (Davies *et al.* 2002), whereas the Spanish (74 samples reduced to 70) (Reed 1998) and northern Great Plains (66 lakes reduced to 53) (Fritz *et al.* 1993) training sets contained limited freshwater sites and were therefore biased towards the upper end of the salinity gradient. This has implications in that the full ecological range of a species may not yet have been established or encountered for areas in which these transfer functions have been constructed. Other explanations for differences in species optima may be related to local environmental conditions and water chemistry, which can limit species distribution (Davies *et al.* 2002).

A number of studies have highlighted the problem of using integrated sediment samples, especially in shallow systems, which can often contain diatoms that have accumulated over months or seasons and are then associated with a single water chemistry measurement (Fritz *et al.* 1991; Fritz *et al.* 1993; Gasse *et al.* 1997). Diatoms identified from these samples may also be partially broken or dissolved and

are therefore not truly indicative of present species composition (Battarbee *et al.* 2001). This study has attempted to address these concerns through the use of an artificial substrate collector – the JJ periphytometer. This method of sampling allows for the colonisation of assemblages that can be directly related to ambient environmental conditions (John and Hellereen 1998), which subsequently aims to increase the accuracy of the transfer function (Gasse *et al.* 1997). However, future research could include a larger number of samples to encompass the spatial and temporal variability of diatom growth at a particular site, as well as to adequately cover the full range of taxa that may occur (Battarbee *et al.* 2001). This is an important consideration for temporary water bodies situated further inland in the south-west of Western Australia, which are mostly shallow and particularly sensitive to changes in the hydrocycle (Williams 1983; Timms 2005).

#### 6.5.4 Potential applications

The salinity transfer function developed for the south-west now has the potential to provide insights for studies on the reconstruction of lake salinities through core analysis of diatom assemblages. Gell (1998) used the transfer function derived from south-east Australia to infer past lake water salinity in western Victoria, Australia. Similarly Gasse (2002) reconstructed the history of conductivity changes in western Africa based on the statistical model for this region. Fritz *et al.* (1991) also evaluated the use of the salinity transfer function for the northern Great Plains, by testing the model on a lake in North Dakota. Generally, there is adequate agreement between diatom inferences and the observed sediment record, however discrepancies can arise and are usually attributable to localised anthropogenic activities (Davies *et al.* 2002). This problem is especially relevant to the south-west as many wetlands have been subjected to intensive agricultural impacts in the form of acidic and saline drainage (Davis 2004; Halse 2004), a process which adversely affects diatom community structure. The extremely fluctuating environments of water bodies in this region also influence the productivity of diatom taxa, and may affect the accuracy of the transfer function in paleolimnological reconstructions (Gasse *et al.* 1995).

The use of the model for predicting future trends in salinity requires further investigation, including testing the transfer function against sediment records from appropriate aquatic systems. Understanding past lake history is an essential

component to effective restoration and management efforts (Battarbee *et al.* 2001). By gaining insight into the response of wetlands to disturbance regimes such as salinisation, the model can be applied in a predictive manner to determine future changes (Anderson 1995). For example, the use of high resolution cores for monitoring recent trends (decadal variability) is an important and relatively unexplored area of research and coupled with long-term sediment records can provide an indication of lake recovery time, following rehabilitation (Anderson 1993; Battarbee 2000). It is considered to be a more efficient management option to diagnose the early stages of secondary salinity, rather than implement costly large-scale remediation programs in the south-west of Western Australia. In this way, the transfer function would be most effective when used in conjunction with water chemistry measurements for long-term monitoring strategies of salt-affected lakes and streams.

The diatom-based salinity transfer function for the south-west was shown to be statistically robust, comparable to other models and provides a calibration dataset for a new geographical region. The model is an important statistical tool that can now be merged with training sets from south-east Australia, or different parts of the world in order to enhance strength and predictive ability. This would also increase the accuracy of estimated salinity optima and tolerance ranges of diatom species by improving the representation of individual taxa (Gasse *et al.* 1995; Reed 1998). Another factor that may potentially improve statistical rigor is the incorporation of a greater number of environmental variables, such as ionic composition or nutrient concentration (Davies *et al.* 2002). The relationship of these variables to salinity needs to be explored as they may have an important role in wetlands located in the agricultural area of the south-west. This would allow for the development of transfer functions for other factors, similar to those that have been established elsewhere.

## 6.6 Conclusion

The transfer function developed for the south-west of Western Australia performed very well in comparison to other models from different geographic regions. The estimated salinity optima of common taxa were most similar to those identified from wetlands in south-east Australia. Generally, the optima of freshwater diatoms was agreeable with those elsewhere, although optima values for some hypersaline species

tended to be higher than previously documented, reflecting the euryhalinity of taxa inhabiting this range. The inference model could be further refined through the inclusion of a greater number of hypersaline sites, or by merging datasets to increase predictive ability. The next step in the research is to explore the model further in relation to paleolimnological reconstructions or to investigate the use of the transfer function as an evaluation technique for lakes and streams impacted by secondary salinisation.

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**Appendix 6.1:** List of diatom taxa and associated optima and tolerance limits in the south-west dataset with greater than 1 % abundance. The total number of occurrences (N), effective number of occurrences (Hill's N2), maximum percent abundance for salinity (Max), optima ( $\log_{10}$  and backtransformed abundance weighted means) and upper and lower tolerance limits (calculated from abundance-weighted standard deviations) have been presented for each species. Upper tolerance limits that exceeded the highest measured salinity concentration of 156.80 ppt have been replaced with this value. Salinity units are ppt.

Taxon	Authority	N	Max	Hill's N2	Opt ( $\log_{10}$ )	Opt
<i>Achnanthydium</i> sp. A		2	47.57	1.03	1.79	60.98
<i>Achnanthydium binodis</i>	Kützing	8	5.45	4.26	0.21	1.62
<i>Achnanthydium hungaricum</i>	Grunow	2	6.41	1.75	0.11	1.28
<i>Achnanthydium minutissimum</i>	(Kützing) Czarnecki	24	36.28	10.50	0.16	1.46
<i>Achnanthydium oblongella</i>	Oestrup	13	17.63	4.83	0.37	2.32
<i>Achnanthydium ploenensis</i>	Hustedt	8	1.26	6.13	0.81	6.48
<i>Achnanthydium reidensis</i>	Foged	14	73.79	2.61	0.35	2.22
<i>Achnanthydium</i> sp. aff. <i>hungaricum</i>	Grunow	2	4.99	1.87	0.03	1.08
<i>Amphora acutiuscula</i>	Kützing	5	4.53	3.70	0.33	2.12
<i>Amphora australiensis</i>	John	5	1.24	4.53	0.93	8.57
<i>Amphora coffeaeformis</i>	(Agardh) Kützing	38	87.25	17.22	1.65	44.88
<i>Amphora fontinalis</i>	Hustedt	4	14.83	2.24	0.19	1.55
<i>Amphora holsatica</i>	Hustedt	7	18.78	1.99	1.03	10.75
<i>Amphora ovalis</i>	(Kützing) Kützing	2	2.03	1.29	0.17	1.48
<i>Amphora paraveneta</i>	Lange-Bertalot	4	8.33	1.80	1.08	12.05
<i>Amphora</i> sp. A		1	59.11	1.00	2.09	123.90
<i>Amphora</i> sp. aff. <i>coffeaeformis</i>	(Agardh) Kützing	13	26.01	6.49	0.93	8.47
<i>Amphora</i> sp. aff. <i>luciae</i>	Cholnoky	41	90.26	18.59	1.34	22.06
<i>Amphora suburgida</i>	Hustedt	7	16.51	3.11	0.89	7.73
<i>Amphora veneta</i>	Kützing	10	43.99	2.95	0.21	1.63
<i>Bacillaria paxillifer</i>	(O.F. Müller) Hendey	21	77.87	5.54	0.63	4.25
<i>Brachysira styriaca</i>	(Grunow) Ross	13	46.71	4.62	0.22	1.65
<i>Brachysira vitrea</i>	(Grunow) Ross	6	3.54	4.10	0.12	1.31
<i>Caloneis silicula</i> f. <i>intermedia</i>	Hustedt	1	1.61	1.00	0.23	1.68
<i>Caloneis ventricosa</i>	(Ehrenberg) Meister	1	1.32	1.00	1.83	67.30
<i>Chaetoceros muelleri</i>	Hustedt	2	29.64	1.80	1.42	26.06
<i>Cocconeis distans</i>	Gregory	5	4.70	3.11	1.65	44.81
<i>Cocconeis placentula</i>	(Ehrenberg) Hustedt	32	29.87	12.23	1.03	10.78
<i>Cocconeis placentula</i> var. <i>euglypta</i>	(Ehrenberg) Cleve	6	50.16	3.08	1.42	26.23
<i>Ctenophora pulchella</i>	(Ralfs ex Kützing) Williams & Round	8	12.39	2.72	0.46	2.88

**Appendix 6.1 (continued):** List of diatom taxa and associated optima and tolerance limits in the south-west dataset with greater than 1 % abundance. The total number of occurrences (N), effective number of occurrences (Hill's N2), maximum percent abundance for salinity (Max), optima ( $\log_{10}$  and backtransformed abundance weighted means) and upper and lower tolerance limits (calculated from abundance-weighted standard deviations) have been presented for each species. Upper tolerance limits that exceeded the highest measured salinity concentration of 156.80 ppt have been replaced with this value. Salinity units are ppt.

Taxon	Authority	N	Max	Hill's N2	Opt ( $\log_{10}$ )	Opt	Low tol limit	Up tol limit
<i>Cyclotella meneghiniana</i>	Kützing	23	30.75	4.54	0.81	6.45	3.59	11.59
<i>Cyclotella stelligera</i>	Cleve & Grunow	11	60.97	3.23	0.12	1.32	1.08	1.62
<i>Cyclotella striata</i>	(Kützing) Grunow	2	7.76	1.08	1.22	16.71	15.15	18.42
<i>Cylindrotheca closterium</i>	(Ehrenberg) Lewin & Reimann	9	94.23	3.61	1.12	13.29	7.81	22.63
<i>Cymbella turgida</i>	(Gregory) Cleve	1	1.93	1.00	0.07	1.18	0.64	2.16
<i>Diadesmis confervacea</i>	Kützing	1	16.50	1.00	0.06	1.14	0.62	2.09
<i>Diploneis subovalis</i>	Cleve	5	1.81	2.48	0.74	5.49	4.02	7.50
<i>Encyonema delicatulum</i>	(Kützing) Mann	6	38.15	2.11	0.22	1.67	1.13	2.47
<i>Encyonema gracile</i>	Ehrenberg	3	2.82	2.42	0.09	1.22	0.88	1.68
<i>Encyonema minutum</i>	(Hilse ex. Rabenhorst) Mann	7	4.81	2.58	0.07	1.17	1.04	1.32
<i>Encyonopsis microcephala</i>	(Grunow) Krammer	10	56.57	3.38	0.08	1.21	0.98	1.50
<i>Entomoneis paludosa</i>	(W. Smith) Reimer	16	7.69	8.01	0.72	5.20	3.50	7.72
<i>Entomoneis pseudoduplex</i>	Osada & Kobayasi	4	6.95	1.74	1.79	61.14	46.88	79.74
<i>Entomoneis</i> sp. aff. <i>paludosa</i>	(W. Smith) Reimer	5	77.34	1.07	1.87	74.42	63.90	86.67
<i>Entomoneis tenuistriata</i>	John	10	20.30	4.42	1.24	17.19	9.18	32.18
<i>Eunotia bilunaris</i>	(Ehrenberg) Mills	3	31.09	1.08	0.05	1.11	0.88	1.41
<i>Eunotia flexuosa</i>	(Brébisson) Kützing	1	1.27	1.00	0.67	4.65	2.54	8.52
<i>Eunotia pectinalis</i>	(O.F. Müller) Rabenhorst	4	5.09	3.18	0.15	1.40	0.82	2.39
<i>Eunotia pectinalis</i> var. <i>minor</i>	(Kützing) Rabenhorst	3	3.81	1.84	0.09	1.23	1.13	1.35
<i>Fallacia auriculata</i>	(Hustedt) Mann	5	5.44	1.89	0.70	5.05	2.69	9.46
<i>Fallacia tenera</i>	(Hustedt) Mann	12	15.69	3.41	0.66	4.59	2.77	7.62
<i>Fragilaria famelica</i>	(Kützing) Lange-Bertalot	2	50.73	1.41	0.63	4.28	3.96	4.63
<i>Fragilaria vaucheriae</i>	(Kützing) Petersen	7	69.01	4.17	0.30	2.01	1.38	2.91
<i>Fragilariforma virescens</i>	Ralfs	1	9.45	1.00	0.14	1.37	0.75	2.52
<i>Frustulia magaliesmontana</i>	Cholnoky	5	2.82	3.35	0.11	1.29	0.94	1.75
<i>Frustulia vulgaris</i>	(Thwaites) De Toni	3	4.82	1.38	0.07	1.18	1.14	1.22
<i>Gomphonema auritum</i>	Braun	4	6.45	1.73	0.10	1.25	0.74	2.10
<i>Gomphonema parvulum</i>	(Kützing) Kützing	15	72.82	3.76	0.28	1.89	1.13	3.15
<i>Gomphonema undulatum</i>	Hustedt	4	1.25	3.22	0.39	2.48	1.28	4.80
<i>Gyrosigma kutzingii</i>	(Grunow) Cleve	5	4.81	2.75	0.23	1.68	0.82	3.48

**Appendix 6.1 (continued):** List of diatom taxa and associated optima and tolerance limits in the south-west dataset with greater than 1 % abundance. The total number of occurrences (N), effective number of occurrences (Hill's N2), maximum percent abundance for salinity (Max), optima ( $\log_{10}$  and backtransformed abundance weighted means) and upper and lower tolerance limits (calculated from abundance-weighted standard deviations) have been presented for each species. Upper tolerance limits that exceeded the highest measured salinity concentration of 156.80 ppt have been replaced with this value. Salinity units are ppt.

Taxon	Authority	N	Max	Hill's N2	Opt ( $\log_{10}$ )	Opt	Low tol limit	Up tol limit
<i>Gyrosigma spencerii</i>	(W. Smith) Cleve	3	1.24	2.04	0.96	9.03	3.95	20.65
<i>Hantzschia amphioxys</i>	(Ehrenberg) Grunow	4	1.99	2.81	1.47	29.43	7.70	112.44
<i>Hantzschia</i> sp. aff. <i>baltica</i>	Simonsen	12	8.26	7.05	1.85	70.12	44.53	110.41
<i>Hantzschia</i> sp. aff. <i>barckhausenii</i>	Lange-Bertalot & Metzeltin	2	14.96	1.45	1.97	93.20	81.71	106.30
<i>Haslea spicula</i>	(Hickie) Bukhtiyarova	16	4.34	9.19	0.87	7.40	3.54	15.49
<i>Hyalodiscus scoticus</i>	(Kützing) Grunow	1	1.25	1.00	0.06	1.15	0.63	2.10
<i>Hippodonta capitata</i>	(Ehrenberg) Lange-Bertalot	4	11.07	1.69	0.22	1.66	0.94	2.95
<i>Luticola mutica</i>	(Kützing) Mann	1	0.31	1.00	0.39	2.47	1.35	4.53
<i>Martyana martyi</i>	(Héribaud) Round	1	12.85	1.00	2.04	110.40	60.26	156.80
<i>Mastogloia pumila</i>	(Grunow) Cleve	11	40.31	2.12	1.15	14.03	6.83	28.83
<i>Melosira nummuloides</i>	(Dillwyn) Agardh	2	22.51	1.70	0.78	6.04	1.05	34.75
<i>Navicella pusilla</i>	(Grunow ex. A. Schmidt) Krammer	20	43.59	2.46	1.11	12.92	6.42	25.99
<i>Navicula accomoda</i>	Hustedt	1	1.88	1.00	0.39	2.47	1.35	4.53
<i>Navicula cincta</i>	(Ehrenberg) Kützing	14	15.06	2.85	0.97	9.38	3.84	22.88
<i>Navicula cryptocephala</i>	Kützing	32	31.82	9.03	0.48	3.04	1.59	5.81
<i>Navicula cryptocephala</i> var. <i>exilis</i>	(Kützing) Grunow	1	2.25	1.00	1.09	12.33	6.73	22.59
<i>Navicula elegans</i>	W. Smith	2	2.27	1.25	1.70	50.18	2.69	156.80
<i>Navicula halophila</i>	(Grunow) Cleve	12	36.39	6.16	0.34	2.20	1.16	4.18
<i>Navicula notha</i>	Wallace	2	22.19	1.17	0.13	1.34	0.42	4.30
<i>Navicula perminuta</i>	Grunow	17	31.72	7.84	0.88	7.66	4.52	12.99
<i>Navicula radiosa</i>	Kützing	8	5.45	5.40	0.16	1.43	0.84	2.43
<i>Navicula ramosissima</i>	(Agardh) Cleve	1	4.63	1.00	0.64	4.33	2.36	7.93
<i>Navicula recens</i>	Lange-Bertalot	2	2.44	1.96	0.81	6.52	6.05	7.03
<i>Navicula salinarum</i>	Grunow	13	58.54	1.91	0.62	4.16	2.21	7.85
<i>Navicula schroeteri</i>	Meister	1	1.76	1.00	0.11	1.28	0.70	2.34
<i>Navicula</i> sp. A		1	4.47	1.00	2.09	123.90	67.63	156.80
<i>Navicula</i> sp. aff. <i>agrestis</i>	Hustedt	13	32.61	5.44	0.76	5.77	2.13	15.61
<i>Navicula</i> sp. aff. <i>cryptocephala</i>	Kützing	2	35.43	1.05	1.82	66.83	54.70	81.65
<i>Navicula</i> sp. aff. <i>duerrenbergiana</i>	Hustedt	2	9.51	1.83	1.64	43.40	13.88	135.75
<i>Navicula</i> sp. aff. <i>incertata</i>	Lange-Bertalot	10	74.29	3.28	1.99	97.03	64.26	146.50

**Appendix 6.1 (continued):** List of diatom taxa and associated optima and tolerance limits in the south-west dataset with greater than 1 % abundance. The total number of occurrences (N), effective number of occurrences (Hill's N2), maximum percent abundance for salinity (Max), optima ( $\log_{10}$  and backtransformed abundance weighted means) and upper and lower tolerance limits (calculated from abundance-weighted standard deviations) have been presented for each species. Upper tolerance limits that exceeded the highest measured salinity concentration of 156.80 ppt have been replaced with this value. Salinity units are ppt.

Taxon	Authority	N	Max	Hill's N2	Opt ( $\log_{10}$ )	Opt	Low tol limit	Up tol limit
<i>Navicula</i> sp. aff. <i>salinicola</i>	Hustedt	11	60.81	4.41	1.59	38.78	22.08	68.11
<i>Navicula subrhynchocephala</i>	Hustedt	5	2.62	3.83	0.60	3.94	3.08	5.03
<i>Navicula symmetrica</i>	Patrick	1	1.29	1.00	1.08	12.07	6.59	22.11
<i>Navicula tenelloides</i>	Hustedt	6	25.71	3.60	1.96	90.37	64.30	127.01
<i>Navicula tripunctata</i>	(O.F. Müller) Bory	24	19.36	10.14	0.86	7.27	3.86	13.70
<i>Navicula tripunctata</i> var. <i>schizemoides</i>	(Van Heurck) Patrick	1	0.63	1.00	0.61	4.05	2.21	7.42
<i>Navicula viridula</i>	Kützing	8	1.88	4.83	0.46	2.88	1.36	6.09
<i>Nitzschia agnita</i>	Hustedt	4	17.03	1.88	0.65	4.45	3.89	5.09
<i>Nitzschia aurariae</i>	Cholnoky	15	18.86	5.71	0.81	6.49	2.75	15.32
<i>Nitzschia desertorum</i>	Hustedt	3	12.31	2.16	0.49	3.11	2.45	3.95
<i>Nitzschia epithemioides</i>	Grunow	3	2.93	1.97	1.33	21.37	11.81	38.68
<i>Nitzschia fasciculata</i>	Grunow	3	3.16	1.90	0.56	3.63	2.08	6.35
<i>Nitzschia frustulum</i>	(Kützing) Grunow	25	42.58	9.67	0.59	3.88	2.21	6.84
<i>Nitzschia gracilis</i>	Hantzsch	4	4.69	3.66	0.20	1.57	0.93	2.66
<i>Nitzschia hybrida</i>	Grunow	2	6.67	1.27	0.67	4.66	4.48	4.84
<i>Nitzschia linearis</i>	W. Smith	4	11.63	2.42	0.14	1.40	1.14	1.71
<i>Nitzschia microcephala</i>	Grunow	26	13.23	11.24	0.58	3.82	1.97	7.42
<i>Nitzschia obtusa</i>	W. Smith	7	1.29	5.72	0.66	4.57	1.52	13.76
<i>Nitzschia ovalis</i>	Arnett	16	80.86	5.98	1.94	86.90	52.17	144.78
<i>Nitzschia palea</i>	W. Smith	27	49.84	6.65	0.31	2.04	1.17	3.58
<i>Nitzschia paleacea</i>	Grunow	9	17.59	3.71	0.63	4.30	2.61	7.11
<i>Nitzschia paleaeformis</i>	Hustedt	3	12.54	2.11	0.23	1.70	0.61	4.77
<i>Nitzschia pellucida</i>	Grunow	5	5.14	3.13	1.51	32.38	11.35	92.37
<i>Nitzschia prolongata</i>	Hustedt	1	56.19	1.00	1.17	14.63	7.99	26.80
<i>Nitzschia romana</i>	Grunow	6	10.90	1.76	0.09	1.22	0.79	1.89
<i>Nitzschia scalpelliformis</i>	(Grunow) Grunow	5	2.25	2.98	0.44	2.76	0.87	8.73
<i>Nitzschia sigma</i>	(Kützing) W. Smith	16	3.57	8.09	0.92	8.30	3.82	18.03
<i>Nitzschia</i> sp. aff. <i>archibaldii</i>	Lange-Bertalot	9	52.99	5.29	1.17	14.76	7.25	30.05
<i>Nitzschia</i> sp. aff. <i>capitellata</i>	Hustedt	3	10.20	2.61	0.62	4.18	3.79	4.62
<i>Nitzschia</i> sp. aff. <i>fontifuga</i>	Cholnoky	13	8.18	9.42	0.56	3.67	2.13	6.31

**Appendix 6.1 (continued):** List of diatom taxa and associated optima and tolerance limits in the south-west dataset with greater than 1 % abundance. The total number of occurrences (N), effective number of occurrences (Hill's N2), maximum percent abundance for salinity (Max), optima ( $\log_{10}$  and backtransformed abundance weighted means) and upper and lower tolerance limits (calculated from abundance-weighted standard deviations) have been presented for each species. Upper tolerance limits that exceeded the highest measured salinity concentration of 156.80 ppt have been replaced with this value. Salinity units are ppt.

Taxon	Authority	N	Max	Hill's N2	Opt ( $\log_{10}$ )	Opt	Low tol limit	Up tol limit
<i>Nitzschia</i> sp. aff. <i>marginata</i>	Hustedt	2	2.56	1.99	1.74	54.64	15.94	156.80
<i>Nitzschia</i> sp. aff. <i>microcephala</i>	Grunow	1	43.09	1.00	1.69	49.50	27.02	90.69
<i>Nitzschia</i> sp. aff. <i>rostellata</i>	Hustedt	13	69.67	2.78	1.69	48.81	33.49	71.13
<i>Nitzschia</i> sp. aff. <i>vitrea</i> var. <i>salinarum</i>	Grunow	1	1.46	1.00	0.62	4.20	2.29	7.69
<i>Nitzschia subinflata</i>	Hustedt	1	3.45	1.00	0.57	3.68	2.01	6.74
<i>Pinnularia microstauron</i>	(Ehrenberg) Cleve	3	13.54	1.20	1.44	27.69	10.84	70.69
<i>Pinnularia</i> sp. aff. <i>subcapitata</i>	Gregory	1	20.07	1.00	1.69	49.50	27.02	90.69
<i>Navicula elginensis</i>	(Gregory) Grunow	1	2.25	1.00	0.07	1.18	0.64	2.16
<i>Planothidium delicatulum</i>	(Kützing) Round & Bukhtiyarova	7	6.77	4.49	0.64	4.41	2.66	7.33
<i>Planothidium dubium</i>	(Grunow) Round & Bukhtiyarova	4	4.48	2.94	1.59	39.23	9.00	156.80
<i>Planothidium haukianum</i>	(Grunow) Round & Bukhtiyarova	6	7.83	3.70	0.68	4.84	3.70	6.32
<i>Planothidium lanceolatum</i>	(Brébisson) Round & Bukhtiyarova	24	15.91	10.12	0.52	3.28	1.33	8.09
<i>Pleurosigma elongatum</i>	W. Smith	11	8.02	2.68	0.83	6.80	2.06	22.50
<i>Proschkinia</i> sp. aff. <i>complanata</i>	(Grunow) Mann	9	94.32	2.20	1.81	64.31	31.91	129.63
<i>Pseudostaurosira brevistriata</i>	(Grunow) Williams & Round	7	42.58	2.90	0.19	1.56	1.16	2.11
<i>Rhopalodia gibberula</i>	(Ehrenberg) O.F. Müller	6	5.10	4.02	0.82	6.60	4.25	10.24
<i>Rhopalodia gibberula</i> var. <i>timsahensis</i>	(O.F. Müller) Freguelli	1	4.31	1.00	1.02	10.56	5.76	19.35
<i>Rhopalodia musculus</i>	(Kützing) O.F. Müller	4	3.88	3.04	0.94	8.65	3.62	20.62
<i>Stauroneis pachycephala</i>	Cleve	14	3.52	8.08	0.23	1.68	0.97	2.91
<i>Stauroneis contruens</i> var. <i>venter</i>	(Ehrenberg) Hamilton	9	13.06	4.20	0.55	3.53	1.47	8.47
<i>Surirella</i> sp. A		1	5.13	1.00	0.06	1.15	0.63	2.10
<i>Synedra levigata</i> var. <i>hyalina</i>	Grunow	1	7.19	1.00	0.06	1.15	0.63	2.10
<i>Synedra</i> sp. aff. <i>radians</i>	Kützing	2	80.83	1.52	1.23	16.98	7.45	38.71
<i>Synedra ulna</i>	(Nitzsch) Ehrenberg	13	13.44	4.96	0.18	1.51	1.03	2.22
<i>Tabellaria flocculosa</i>	(Rabhenhorst) Kützing	4	9.25	2.22	0.21	1.62	1.22	2.15
<i>Tabularia tabulata</i>	(Agardh) Snoeijs	32	61.86	6.30	0.78	6.01	3.44	10.49
<i>Thalassiosira weissflogii</i>	(Grunow) Fryxell & Hasle	18	19.36	6.77	0.91	8.08	4.79	13.65
<i>Tryblionella apiculata</i>	Gregory	9	4.97	4.37	0.96	9.15	4.46	18.74
<i>Tryblionella hungarica</i>	Grunow	11	2.48	6.46	0.76	5.73	2.55	12.89
<i>Tryblionella punctata</i>	W. Smith	5	0.33	5.00	1.70	50.30	30.20	83.79



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## Chapter 7: General Discussion

### 7.1 Synthesis and Future Research

This study investigated the distribution pattern of diatoms from 95 lakes and streams of varying salinities in the south-west region of Western Australia. An integrated sampling regime provided extensive baseline data on many water bodies ranging from fresh to hypersaline that previously had not been examined. The influence of salinity on diatom diversity was explored, in order to identify potential indicator species and to generate a predictive model, reporting the optima and tolerance limits of important taxa. The results of this research revealed a number of key findings in relation to the objectives set out at the beginning of the thesis.

Analysis of the physico-chemical environment of lakes and streams showed that water quality varied greatly. As expected, aquatic systems further inland had significantly higher salt loads than those located further south, due to lower annual rainfall and catchment clearing practices (Schofield *et al.* 1988; Halse *et al.* 2003; Lymbery *et al.* 2003). Salinity was also significantly higher in standing waters compared to flowing waters and can be attributed to evaporative processes concentrating salts in the system (McComb and Lake 1990; Davis 2004). Hypersaline waters displayed significant trends in water quality, with reduced dissolved oxygen levels and elevated water temperatures, compared to freshwaters. These results were mostly related to salinity concentration and the shallow, temporary nature of water bodies in the region (Williams 1998a; 1998b). The pH of hypersaline sites was also lower, a factor that may be associated with underlying geology or surrounding land use practices (McComb and Lake 1990; Archer 2001). The baseline data collected from this study covers a wide geographic area and has the potential to be incorporated into future management strategies to determine long-term trends for salt-affected systems, with monitoring programs currently in place restricted by the lack of funding arrangements (Environmental Protection Authority 2006). These findings also have important implications in terms of the aquatic biota that inhabit these systems.

The investigation of diatom distribution from lakes and streams in the south-west identified a number of important trends according to environmental conditions. The relationship between water quality variables and diatoms showed that salinity was the key factor influencing species composition. This has also been the case in a number of other studies, where a wide range of salinities has been taken into account (Blinn 1993; Compère 1994; Clavero *et al.* 2000). Over 200 diatom taxa were recorded, with freshwater sites supporting the highest number of species (recording a maximum of 33), in comparison to hypersaline sites, where numbers were limited to less than 15. Possible indicators of freshwaters included *Achnantheidium minutissimum* and *Gomphonema parvulum*, with *Amphora coffeaeformis* and *Nitzschia ovalis* characteristic of more saline waters. These taxa have also been found to inhabit similar ranges in water bodies from Eastern Australia (Gell and Gasse 1990; Blinn and Bailey 2001), Africa (Gasse 1986; Compère 1994) and Canada (Hammer *et al.* 1983; Cumming *et al.* 1995). The information collated on diatom indicator species from water bodies of different salinity ranges in the south-west has the potential to be integrated into future monitoring studies for aquatic systems at risk of salinisation. As salinities in this region are predicted to continue rising (Halse *et al.* 2003; Environmental Protection Authority 2006), further analysis of hypersaline diatom assemblages, specifically from the wheatbelt area was undertaken in this study.

A subset of 20 wheatbelt sites representing salinities in excess of 50 ppt and varying in pH were explored, to examine species-environment relations and taxonomy of potential indicators. Dominant taxa included *Amphora coffeaeformis*, *Nitzschia ovalis*, *Navicula* sp. aff. *incertata* and *N.* sp. aff. *salinicola*, which have previously been observed as characteristic of saline conditions in other regions (Gasse 1986; Gell and Gasse 1990; Compère 1994). The findings showed that these species also displayed wide tolerance limits to high salinity and low pH (with a minimum pH of approximately 3). Subsequent investigation revealed reduced species numbers in the acid saline wetlands, however there were limited publications available for comparison, specifically on the interaction between pH and salinity, as well as potential implications for aquatic biota. Although these types of water bodies are a natural occurrence in some parts of the wheatbelt (Mann 1983), human-induced acid

saline lakes are increasing, as a result of continued fertiliser usage or the installation of deep drainage channels exposing acid-sulphate soils (Archer 2001; Fitzpatrick 2002; Halse *et al.* 2003). It is likely that the combination of acidity and hypersalinity will result in few taxa that are able to tolerate these hostile environments (Blinn *et al.* 2004; Timms 2005), meaning that the collection of baseline data is of great importance. These results also revealed the need to conduct further taxonomic research on salt-tolerant diatoms from this region, to confirm morphological variations and determine the level of endemism that exists in south-west water bodies. The findings in relation to indicator species of varying salinity ranges also led to a detailed investigation to establish the tolerance limits of significant taxa.

The development of a diatom-based transfer function for salinity was undertaken using the calibration dataset of south-west sites. The subsequent data provides an important contribution to the research on diatom ecology in Western Australia, given that this type of model previously has not been accessible for the region. The optima and tolerance limits for freshwater diatom taxa were found to be most similar to parts of eastern Australia, where a transfer function was developed for over 50 wetlands (Gell 1997). In comparison however, diatoms characteristic of saline conditions in the south-west generally showed higher optima, indicating broader tolerance limits to the highly variable conditions in lakes and streams from this area. The south-west dataset has the potential to be merged with those from other geographical regions to increase predictive capacity, or to provide insights for paleolimnological studies. This has been the case with previous transfer functions generated for Eastern Australia (Gell 1998), Canada (Fritz *et al.* 1991) and Africa (Gasse 2002), where past lake water salinities were reconstructed. The south-west inference model has also highlighted potential applications as a future management or monitoring tool, an aspect of the study requiring further research.

## 7.2 Problems Encountered During the Study

A number of difficulties were encountered throughout project, mainly related to field logistics and the temporary nature of water bodies, especially in the wheatbelt. Due to the large scope of the study and broad geographical area, the accessibility and remoteness of many sites posed a unique problem, specifically influencing the

sampling regime, resulting in the exclusion of a number of wetlands. In addition, the wheatbelt area in particular, has been experiencing a marked decrease in winter rainfall substantially reducing stream inflows (Environmental Protection Authority 2006), meaning that many large seasonally inundated lakes remained dry throughout the sampling period. Currently, climatic modelling predictions indicate that under increasing greenhouse conditions, average annual rainfall in the south-west of Western Australia is set to decline by as much as 20 % in the next 25 years, altering the hydroperiod of water bodies in the area (Environmental Protection Authority 2006).

Fluctuating climatic conditions can also influence the flooding regime of aquatic systems, resulting in problems associated with artificial substrate collectors (JJ periphytometers). Sudden freshwater inputs or sediment inflows caused by unseasonably high rainfall events can lead to contamination, with diatoms not being representative of ambient conditions (John 1998). Alternatively, some periphytometers were left exposed due to high evaporation rates and a rapid reduction in water levels over the relatively short colonisation period of 14 days. These issues have been reported in previous studies (John 1998; 2000), however the level of consistency and uniformity provided by the use of artificial substrates was considered more important for this project. Generally however, despite the aforementioned logistical difficulties an adequate number of sites were ultimately compiled in order to achieve the research objectives.

### **7.3 Management and Remediation of Salinity**

The findings of this study, although not directly related to the management of salinity, have raised some important issues with regards to the topic. Generally, it is accepted that the effects of salinisation have the potential to be contained by informed water resource management (Williams 1999). Preferred options for reducing or ameliorating salinity impacts include revegetation or engineering strategies, however the need to develop further control methods and alternative dryland agricultural techniques has also been acknowledged (Schofield *et al.* 1988; George *et al.* 1997; Clarke *et al.* 2002). The success of remediation measures is dependent on understanding the complexity of the problem and implementing the appropriate procedures (Timms 2005). Evaluating the success of monitoring

programs is also essential, and should include the assessment of aquatic biota (Environmental Protection Authority 2006), with organisms such as diatoms being ideal indicators of ecological changes (John 2007).

Present day levels of biodiversity in the wheatbelt can only be maintained if significant numbers of water bodies representing a broad spectrum of environments and associated habitats do not become salinised (Halse *et al.* 2003). There have been few studies conducted in the south-west and particularly in the wheatbelt, allowing for limited comparison and it is likely to be many years before trends relating to salinity and biodiversity become evident (Cale *et al.* 2004). In addition, the use of science in wetland management has increased in recent years, although there is still scope for improving and facilitating the transfer of information, in order to optimise required outcomes (Halse and Massenbauer 2005).

#### **7.4 Conclusion**

In conclusion, this study has contributed to the understanding of diatom distribution patterns in water bodies of varying salinities from the south-west of Western Australia. The findings showed that diatoms were sensitive to different salinity ranges and as a result, a number of potential indicator species have been established. The extensive dataset compiled enabled a statistically robust transfer function to be developed, in a region previously not examined for these purposes, prompting the need for further research. The objectives set out in the initial stages of the study were achieved, with the data collected aiming to be integrated into future biomonitoring programs, as well as to provide useful information for management strategies focussing on secondary salinised systems in the south-west.

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