IMPACTS OF AN ALTERED WATER AND SALINITY REGIME ON THE CONDITION OF WETLANDS IN THE UPPER SOUTH EAST OF SOUTH AUSTRALIA

By

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ABSTRACT

The Upper South East (USE) region of South Australia covers over 1M ha and is the largest area affected by dryland salinity in South Australia. In 1999, it was estimated that 40% of the region was affected by salinity. To mitigate the threat of flooding and secondary salinisation, an extensive network of drains has recently been constructed. Whilst these drains may have a positive effect on the agricultural land, the impacts they will have on the hundreds of wetlands in the region is as yet, unknown. It is likely that the hydrologic regimes the wetlands are exposed to will be highly modified and the quality of the water that supplies them will be greatly affected by high salinity levels.

This work examined the impact of these landscape scale changes on wetlands in the South East region of South Australia and investigated ways in which water from the drainage system might be used for ecological benefit in wetlands. The aims were to:

- determine whether there have been changes in species composition that can be linked to changes in the salinity and hydrology regimes experienced in the wetlands and to gain a better understanding of the processes and mechanisms that drive the change in species composition and cause salt to accumulate in wetlands via the development of a conceptual model;
- produce curves predicting the probability of occurrence in relation to salinity for species common in wetlands in the South East of South Australia;
- investigate the effects of an increase in salinity with decreasing water depth as a result of evapoconcentration on the growth and survival of three common freshwater macrophytes, and to determine the consequences of longterm exposure to elevated salinity conditions;
- assess the impact of a pulsed discharge of saline drainage water of varying concentrations and durations on key wetland species in an effort to determine how to make best use of the scarce water resources in the region and; and
- assess the combined effects of salinity and hydrology on the seed banks of wetlands that have experienced drought and elevated salinity conditions.

The results of vegetation surveys conducted pre-2000 and post-2000, indicate an overall change in species composition; species requiring fresh conditions are rarer or not recorded and are replaced by species preferring more saline conditions. This change is accompanied by a shift from fresher to saltier conditions and from wetter to drier conditions. Data from groundwater observation bores coupled with flow volumes in the local watercourses supports the process of salt accumulation in wetlands described in the conceptual model.

The curves predicting the probability of occurrence in relation to salinity display a wide range in tolerances across the 15 species for which they were constructed, and highlight the variance due to between wetland differences. These curves, used in combination with knowledge gained from other studies will enable salinity thresholds to be set for many of the common species found in the South East region. Employing these thresholds to drain operation will allow wetlands to be managed in a way that will promote the occurrence of target species.

The study on evapoconcentration effects showed that the percentage of biomass allocated to below ground structures was > 95, > 90, > 75 and > 80% for adult and juvenile *T. procerum*, and for *B. arthrophylla* and *B. medianus* respectively, across all salinity treatments suggesting that long term exposure to elevated salinity conditions results in a large investment in below ground biomass by all species. This study also indicated that the initial lifestage at time of exposure to the salinity regimes had a significant effect on the final dry weights of the *T. procerum* plants. The differences in the dry weights and leaf length and number were greatest between adults and juveniles in the lower salinity treatments (1500 and 6250 μS cm^{-1}), with the adults having much larger weights and measures. At higher salinities (12500 and 18750 μS cm^{-1}), there were no differences. Salinities refer to the salinity of the surface water, not soil salinity.

For the plants tested in the pulse salinity regime experiment, the immediate effect of high salinity environments on non-halophytic plants was not detectable after three to six weeks of exposure, but the short term impact of the pulse did affect the ability of submerged plants to recover.

The seed bank trial showed that the previous drought and salinity conditions experienced by a wetland did affect the seed bank however the water and salinity regime imposed mitigated these impacts. The study provides evidence that extended periods of drought conditions may lead to a seed bank which has a reduced abundance of seeds and repeated exposure to high salinity changes the species composition of the seed bank and reduces the overall diversity.

Our knowledge of wetland plants, habitats, individual wetlands and their pattern in the landscape enables interpretation of how wetland plants have changed and will continue to change in the landscape. The challenge is to use, and build on this knowledge to predict what future wetland landscapes might look like under different management or development scenarios in the USE and to decide what is sustainable.

DECLARATION OF ORIGINALITY

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Abigail May Goodman and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Abigail May Goodman

April 2012

PUBLICATIONS ASSOCIATED WITH THIS THESIS

CHAPTER 6 Pg 68

The response of freshwater plants to salinity pulses

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Aquatic Botany (2010), 93: 59–67

Goodman, A.M. (Candidate)
Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author.
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Supervised development of work, helped in data interpretation and manuscript evaluation.
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CHAPTER 7 Pg 86

The effect of inundation and salinity on the germination of seed banks from wetlands in South Australia

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FOREWARD

This thesis has been prepared as a series of chapters in a format that will be suitable for future publication in scientific journals. To maintain the sense of individual chapters, this has inevitably led to some repetition between chapters.

Chapter 6: The response of freshwater plants to salinity pulses and *Chapter 7: The effect of inundation and salinity on the germination of seed banks from wetlands in South Australia*, have been published in the international journal of Aquatic Botany. In the interest of continuity of the thesis, these chapters have been included as part of the word document. In the publications, salinity was reported in mg L^{-1} but these have been converted to $\mu S\ cm^{-1}$ for inclusion in the main body of the thesis. Copies of these publications have been added as Appendices I and II respectively.

ACKNOWLEDGEMENTS

This research was conducted as part of an Australian Research Council Linkage project titled “Innovative Approach to the Optimal Management of Water Resources and Application to the Upper South East Region of South Australia”, in partnership with the Department for Water (formerly the Department for Water, Land and Biodiversity Conservation). I would like to thank my supervisors Professor Holger Maier and Professor Graeme Dandy for their advice and guidance throughout my PhD and for the pun fun we had during my meetings. Dr Matt Gibbs provided lots of practical advice which I greatly appreciate. To my primary supervisor Assoc. Professor George Ganf, thank you for teaching me to think like a scientist and for always having the time and patience to sit down and talk things through.

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I have really enjoyed being a postgrad and this is largely due to the wonderful people who shared this time with me, particularly Melanie Lancaster, Liz McTaggart, Angela Dunsford, Colin Bailey and Nicole Arbon. Thank you to my supportive family who always accepted “OK” as a response to their enquires about my PhD, helped celebrate the little milestones and spent a Christmas in the ponds. Finally, I would like to thank my fiancée Brad for providing constant understanding, encouragement and perspective and for sacrificing numerous weekends to help set up and monitor pond experiments or to negotiate high voltage electric fences in order to reach dry, thistle infested, fly struck wetlands in 36° heat whilst maintaining his sense of humour.

1 Literature Review

Wetlands worldwide, have only recently been recognised as areas of ecological complexity and conservation importance (Davis, et al. 2006). In many countries, the number of naturally occurring wetlands has decreased drastically and in Australia, they have decreased by more than 50% since European settlement (Smith, et al. 2007). A wetlands is land that is permanently or temporarily under water or waterlogged, with temporary wetlands having surface water or water logging of sufficient frequency and/or duration to affect the biota (Paijmans, et al. 1985). A large variety of wetlands exists in Australia and they range from shallow depressions that are only filled for short periods during wet years to permanent marshes and deep billabongs (Hart, et al. 1990). All wetlands are characterized by the dominant influence of water on their vegetation, wildlife and soils; however the boundaries of many wetlands are not clearly defined because the water level changes annually. They usually contain true aquatic plants and provide important habitats for invertebrates, fish, water birds, reptiles, amphibians and mammals (Hart, et al. 1990).

1.1 Importance of hydrology on the structure of aquatic macrophyte communities

Water is one of the primary factors which structures plant communities within wetland ecosystems (Fennessy, et al. 1994). The water regime is characterised by the changes in water depth, duration, frequency, rate, timing and predictability of inundation and drying phases experienced by an aquatic system (Casanova and Brock 2000, Geoff, et al. 2007, Porter, et al. 2007, Rea and Ganf 1994). Modification of the landscape such as groundwater abstraction, catchment clearing, drainage and discharge (Froend and McComb 1994), and regulation of rivers, has altered natural patterns in water level fluctuation (Leyer 2005, Rea and Ganf 1994). These alterations are a major cause of deteriorating conditions in many Australian aquatic ecosystems (Lloyd, et al. 2004, Sim, et al. 2006), as they have resulted in considerable reduction of both the seasonal flow variability and peak flows.

Flow variables that are emerging as ecologically important are the volume, variability, rates of change, the magnitude and frequency of extreme flows, and their seasonal predictability (Deegan, et al. 2007, USEDS&FMP 1993, Young, et al. 2000). As a result of anthropogenic modification to water regimes, many ‘permanent’ wetlands are now dry during extended periods of low rainfall, and many ‘temporary’ wetlands are permanently inundated (Nielsen, et al. 2003, Smith and Brock 2007) and water levels are held static.

Aquatic macrophytes grow in soil saturated with water or in the water itself (Leck and Brock 2000). The hydrological parameters, including average groundwater level, flooding duration and flooding depth, as well as the rate of water level fluctuation and disturbance frequency and intensity, all influence the response patterns of aquatic macrophytes (Bunn and Arthington 2002, Casanova and Brock 2000, Leyer 2005, Nielsen and Chick 1997). The water regimes of aquatic systems are increasingly seen as the driver in maintaining wetland function and diversity (Bunn and Arthington 2002, Casanova and Brock 2000, Rea and Ganf 1994, Smith and Brock 2007), because hydrology influences germination and seedling recruitment as well as growth of aquatic macrophytes and plays an important role in structuring the composition and zonation of aquatic vegetation (Casanova and Brock 2000, Froend and McComb 1994, Nicol and Ganf 2000, Smith and Brock 2007). Despite growing recognition of the relationships between hydrology and aquatic diversity, ecologists still struggle to predict and quantify biotic responses to altered flow regimes (Bunn and Arthington 2002).

1.2 Secondary Salinisation

Secondary salinisation results from changes in land use and is a problem that is seen globally. It is an increase in salinity at or close to the soil surface resulting from human disturbances of the natural hydrological cycle (Bell 1999, Cocks 2003, Cramer and Hobbs 2002, Eamus, et al. 2006, Halse, et al. 2003, Hart, et al. 1991, Hart, et al. 2003, James, et al. 2003, NLWR 2001). Major land degradation problems across Australia have been attributed to secondary salinisation and these are predicted to become considerably worse over the next 30-50 years (Hart, et al. 2003, NLWR 2001).

Dryland salinity and salinity due to irrigation are the two ways in which secondary salinisation is caused. Irrigation salinity occurs when water from irrigation moves through the soil, raising the water table and mobilising salt (Eamus, et al. 2006, Halse, et al. 2003). This problem is exacerbated when slightly saline water is used for irrigation. Dryland salinity is caused by the replacement of deep-rooted native vegetation with shallow rooted agricultural crops and pastures, resulting in a reduction in evapotranspiration and an increase in recharge (Bell 1999, Cramer and Hobbs 2002, Eamus, et al. 2006, Hart, et al. 1991, Hart, et al. 2003, NLWR 2001). Both causes result in a rise in the level of regional groundwater tables, bringing salt from low in the soil profile toward the surface and within the root zone of plants (Hart, et al. 1991).

A survey of world secondary salinity suggests that about 76.6 Mha are affected (Ghassemi, et al. 1995). The estimates for Australia indicate that about 2.5 Mha are currently affected by secondary salinity (Robertson, 1996), but hydrological modelling suggests that in the next 50 years, about 17 Mha could be at risk (National Land and Water Resources Audit, 2001). Until recently, a major focus of the effect of dryland salinity has been on its consequences for agriculture where it is predicted that it will have dramatic impacts on productivity and income (Cramer and Hobbs 2002). In southern Australia, secondary salinity also poses one of the most significant threats to ecosystems (Cramer and Hobbs 2005). For aquatic ecosystems, naturally saline systems such as salt lakes and salt marshes have been well described (Sim, et al. 2006, Sim, et al. 2006, Strehlow, et al. 2005, Williams 1998), but salinisation as a consequence of the clearing of native vegetation and irrigation is less well studied (Brock, et al. 2005, Davis, et al. 2003, Nielsen, et al. 2003).

Dryland salinity is difficult to manage because of the lasting nature of its effects on soil and water resources (NLWR 2001). Due to the large spatial and temporal scales over which salinisation occurs, even if our current best land-management practices were fully implemented, salinisation would continue to increase in aquatic ecosystems throughout Australia (Nielsen, et al. 2003). The aquatic systems which comprise rivers, floodplains, riparian zones and wetlands, are the most severely affected by salinisation as they occupy the lowest areas in the landscape where salt accumulates (Brock, et al. 2005, Hart, et al. 2003, James, et al. 2003, NLWR 2001, Walker, et al. 2002). Despite this, few studies have focused on how secondary salinisation affects the health and distribution of native plants, the composition of vegetation assemblages, or interrelated ecosystem processes in freshwater systems (Cramer and Hobbs 2002, Nielsen, et al. 2003, Rea and Ganf 1994). Although the effects of increasing salinisation on aquatic biota have been extensively reviewed, the ecological consequences of salinisation in Australian freshwaters are not well understood (Hart, et al. 1991, James, et al. 2003, Nielsen, et al. 2003). More knowledge about the relationship between flow patterns, salt concentrations and environmental damage is required so that predictions can be made as to the consequences of management actions (Nielsen, et al. 2003).

Salinity is the total concentration of dissolved inorganic ions in water or soil. As measuring the concentration of all ions is time consuming and expensive, salinity is often inferred by measuring the electrical conductivity (EC) of a water or soil sample. EC is standardised to 25°C and is usually expressed in $\mu S\ cm^{-1}$ or $mS\ cm^{-1}$, with $1000\ \mu S\ cm^{-1} = 1\ mS\ cm^{-1}$ (Kefford, et al. 2007). When salinity is measured as the total soluble salts ($mg\ L^{-1}$) the conversion often used is electrical conductivity $\mu S\ cm^{-1}$ = total soluble salts divided by a factor of 0.64 (Hart, et al. 1991). In this work, salinity values refer to the salinity of surface water, not soil salinity except where stated. Salt is a natural component of the Australian landscape that has been deposited from a variety of sources over millions of years (Nielsen, et al. 2003). Prior to the removal of the terrestrial vegetation, salt was carried into wetlands and often concentrated by evaporation. The salt that accumulated in the sediment of wetlands was removed by flushing during the next high-flow event (Nielsen, et al. 2003). Under natural conditions in many wetlands and rivers, periods of low flow and high evaporation, combined with the intrusion of groundwater, causes natural salinity levels to be high for periods of time (Hart, et al. 1991, James, et al. 2003, Nielsen, et al. 2003). Most aquatic systems in Australia are characterised by high temporal variability in dissolved salt concentrations even where there is no salinity problem (Hart, et al. 2003). There is a general pattern of higher salinity at low flows and lower salinity at high flows (Halse, et al. 2003, Hart, et al. 2003, Nielsen, et al. 2003), however, modification of flows through changes in temporal and spatial patterns has altered the flow-salinity patterns (Nielsen, et al. 2003). It is also common for salinity to be higher in flows generated from first rains when salt at or close to the surface dissolves into the surface runoff (Halse, et al. 2003). Secondary salinisation has increased the amount of salt entering aquatic systems (Nielsen, et al. 2003).

1.3 Importance of salinity on the structure of aquatic macrophyte communities

Wetland plant communities comprise the basis of healthy wetland ecosystems and the consequences of plant loss are both many and far-reaching. These include the direct loss of floral diversity, reduced potential for plant population recruitment, reduction in primary production, the subsequent loss of faunal diversity through the loss of habitat and food, decreased aeration of sediments, and increased nutrient levels as a consequence of the decrease in plant uptake (Davis and Froend 1999). Salinisation has been identified as one of the greatest threats of degradation to freshwater ecosystems in Australia (James, et al. 2003, Nielsen, et al. 2003) and the plant communities have been recognized as possibly the most sensitive component of the wetland ecosystem to increases in salinity (Hart, et al. 1991). Plant communities at wetland edges typically exhibit strong zonation along water depth gradients (Geoff, et al. 2007), although and it is likely that zonation in response to salinity regime also occurs (Halse, et al. 2004).

There are a multitude of potential impacts of salinity on aquatic systems, including direct toxic effects, changed chemical processes and loss of habitat in the water, riparian zones and adjacent flood plains (James, et al. 2003). Ecological impacts of salinity are influenced by a range of factors including the sensitivity of a species to salinity (including sublethal and lethal effects), rates of salinity increase, length of exposure and the lifestage at which a species is exposed to salinity (Cocks 2003). In terms of their response to salinity, plants can be broadly divided into two groups; halophytes, which are species that are salt tolerant, and non-halophytes, which are species that achieve best growth in freshwaters (Hart, et al. 1991). Halophytes are the native flora of saline soils, which survive completing their whole life cycle in such environments. For non-halophytes, including freshwater aquatic macrophytes, elevated salinity can result in reduced growth or death (Greenway and Munns 1980, Hart, et al. 1991, James, et al. 2003).

1.3.1 Physiological and Growth Response

Plants and animals have adapted to a wide range of aquatic environments, and have developed a range of physiological mechanisms and adaptations to maintain the necessary balance of water and dissolved ions in cells and tissues (Hart, et al. 1991). Salinity, as it relates to plants, is the occurrence of a high concentration of soluble salts in the soil or solution in which plants grow, the most important ions being sodium, potassium and chloride (Flowers and Yeo 1986). High salinity can result in reduced growth or death in plants due to toxic effects caused by an excess of ions, or ‘water deficiency’ due to difficulties in extracting water from the surrounding medium (Hart, et al. 1991, Munns and Tester 2008).

The physiological processes that confer salinity tolerance in halophytes are well understood and have been extensively reviewed (Flowers, et al. 1977, Flowers and Yeo 1986, Greenway and Munns 1980, Munns and Termaat 1986). The ability of a species to maintain (regulate) the optimal internal osmotic concentration against external gradients determines the salinity tolerance of the species (Hart, et al. 1991). There are two main types of mechanisms for salt tolerance: those minimising the entry of salt into the plant, and those minimising the concentration of salt in the cytoplasm (Munns 2002). To minimise the entry of salt to the plant, some halophytes control the concentration of sodium and chloride in the tissue through selective processes such as ion exclusion at the roots and by keeping salt away from meristems, particularly in the shoot, and from leaves that are actively expanding and photosynthesizing (Ashraf 2004, Hester, et al. 2001). Once salt is in the plant tissue, it can be tolerated by anatomical adaptations and intracellular partitioning. Anatomical adaptations resulting in salt glands or bladders (modified epidermal cells) can enable the excretion of sodium and chloride from the plant (Hester, et al. 2001, Munns and Tester 2008). Intracellular partitioning enables the storage of high internal ionic concentrations of salt in the vacuole, leaving the cell cytoplasm with a relatively low ionic concentration (Flowers, et al. 1977, Greenway and Munns 1980, Munns and Tester 2008).

In glycophytes, photosynthesis may be reduced in response to salinity due to a decrease in transpiration as a result of stomatal closure and leaf decline (Mensforth 1996). Osmotic balance is essential for plants growing in saline media and when this is not achieved by glycophytes, it results in loss of turgidity, cell dehydration and ultimately, the death of cells (Ashraf 2004). Toxic effects originate because the high external salt concentration induces greater diffusion of ions into root cells and results in elevated ionic concentrations in the cytoplasm and this can reduce plant performance (Hart, et al. 1991, Lessani and Marschner 1978). Although there has been considerable work conducted on the sensitivity of agricultural crops to salinity increases, there is little data on the effect of salinisation on native vegetation (Hart, et al. 1991).

Excess salts in the environment results in a decrease in growth of many plants (Munns and Termaat 1986). Morphological characteristics may be associated with salinity tolerance and since a primary effect of salt stress is a water deficit, morphometric variables such as leaf size, shape, and number that influence transpirational water loss, may be associated with salt tolerance within a species. The earliest response of a non-halophyte exposed to salinity is that its leaves grow more slowly. Root growth is almost always less affected than shoot growth, so the root:shoot ratio increases (Munns and Termaat 1986). When plants are exposed to salinity in laboratory experiments, there is a rapid and temporary drop in growth rate followed by a gradual recovery to a new reduced rate of growth (Munns 2002).

1.3.2 Morphological and community salinity effects

Past reviews have provided a great deal of evidence for the decline of aquatic biodiversity in response to salinisation (Hart, et al. 1991, James, et al. 2003, Nielsen, et al. 2003, Williams 1999). Increased salinity is a stress that has been shown to reduce diversity in terrestrial systems (Briggs and Taws 2003, Hobbs, et al. 2003) and freshwater aquatic systems (Brock, et al. 2005, James, et al. 2003). Salinity effects occur at various stages in the life history of a plant (Hart, et al. 1991, James, et al. 2003, Nielsen, et al. 2003), however studies on the effects of salinity have generally focused on impacts on adult life stages, which are potentially the most tolerant life stages (Nielsen, et al. 2003). Many taxa are able to survive at elevated salt concentrations, however reproduction, recruitment and growth of juveniles may be substantially reduced and this has profound consequences on subsequent generations (Hart, et al. 2003, Nielsen, et al. 2003).

Although freshwater aquatic plants are not tolerant of increasing salinity, there is a general acceptance that freshwater ecosystems undergo little ecological stress when subjected to salinities up to 1500 $\mu S\ cm^{-1}$ (Nielsen, et al. 2003). At salinities above 1500 $\mu S\ cm^{-1}$, reduced growth rates and reduced development of roots and leaves of aquatic plants are observed (James, et al. 2003, Nielsen, et al. 2003). Species richness and abundance has been observed to decline with increasing salinity (Brock, et al. 2005, Hart, et al. 1991, Porter, et al. 2007). A field based study by Brock (1981) found that in wetlands in which salinity levels approach 6250 $\mu S\ cm^{-1}$, normally widespread freshwater aquatic macrophytes such as *Myriophyllum propinqua*, *Triglochin* *procerum, Crassula helmsii* and *Isoete muelleri* were no longer found. Several subsequent studies using these and other species have confirmed this finding (James and Hart 1993, Smith, et al. 2009, Warwick and Bailey 1997, Warwick and Bailey 1998). In a recent field study, Smith, et al.(2009) found that a considerable loss of freshwater macrophyte species may occur with a relatively small increase in salinity and reported a predicted probability of occurrence of less than 0.1 for many species at conductivities greater than 1000 µS cm^{-1}.

Sub-lethal effects such as reduced growth rate and vigour have been recorded at salinities between 1500-6250 µS cm^{-1}. The common aquatic macrophytes *Myriophyllum crispatum, Eleocharis acuta, Stekenia tricarinatus* and *Triglochin procerum* all displayed sublethal effects including reduced length and shoot length when grown at salinities greater than 1500 µS cm^{-1} (James and Hart 1993). The shoot and root biomass of *Cladium jamaicense* and *Eleocharis cellulosa* and the height of *Typha domingensis* were reduced by exposure to up to 6250 µS cm^{-1} (Macek and Rejmánková 2007). Salinities of up to 13000 µS cm^{-1} have been shown to reduce growth rates (by reducing leaf number, leaf area culm number and height) in *Bolboschoenus medianus*, although increased nutrient loads mitigated this response (Morris and Ganf 2001). Salinity not only reduced the recovery of *Vallisneria australis* after its release from a period of drying that dessicated aboveground organs, but prohibited recovery when the soil dried out (Salter, et al. 2008). When exposed to conditions of increasing salinity from 3000 µS cm^{-1} up to 8500 µS cm^{-1}, significant reductions in the density, species richness and diversity were recorded (James, et al. 2009). The salt sensitivity database (Morris, et al. 2009) includes a comprehensive review of the studies on the effects of salinity on aquatic macrophytes.

1.3.3 Seed bank response

Wetland sediments contain a reservoir of dormant seeds and vegetative propagules of aquatic plants and eggs of aquatic invertebrates collectively termed the ‘seed bank’ (Nielsen, et al. 2003, Skinner, et al. 2001). In Australian wetlands that are subject to wide environmental fluctuations, plant communities depend on seed or vegetative propagules for regeneration (Brock, et al. 1994).The seed bank provides an important mechanism for the persistence of species in ephemeral wetlands, providing a mechanism for regeneration of plant communities after natural or artificial disturbances such as drought, prolonged inundation or grazing (Brock, et al. 1994, Nicol, et al. 2007). In wetlands, fluctuating water levels create opportunities for recruitment of new individuals from seed banks, and drawdown periods often favour the establishment of species adapted for life in shallow water (Cherry and Gough 2006). There has been much work done on the germination of wetland plants in relation to water availability and hydrology (Casanova and Brock 2000, Leck and Brock 2000, Nicol and Ganf 2000, Nicol, et al. 2003). Most salinity and water regime research examining biodiversity impacts has reported effects on the adult life stages, which generally are more tolerant of disturbances such as drying and salinity than juvenile or reproductive stages (Brock, et al. 2005). Trials investigating the effects of salinity on the emergence of plants from wetland sediments have been undertaken (Nielsen, et al. 2003, Nielsen, et al. 2007).

The emergence of aquatic plants from the seed bank is reduced when exposed to constant levels of salinity above 1500 $\mu S\ cm^{-1}$, and when exposed to salinities of 7350 $\mu S\ cm^{-1}$, very few aquatic plants are capable of emerging and surviving (Brock, et al. 2005, Nielsen, et al. 2003, Nielsen, et al. 2007, Nielsen, et al. 2008). The combined effect of salinity and water regime had a marked influence on both the species richness and abundance of aquatic plants germinating from sediments from a range of wetlands. Salinity had a particularly strong effect in reducing germination from sediments in damp conditions when compared to the flooded conditions (Brock, et al. 2005). Pulses of high salinity (7800 $\mu S\ cm^{-1}$) for a short duration followed by a return to freshwater conditions did not impact on the emergence of aquatic plants from wetland sediment (Nielsen, et al. 2007).

Some species are able to geminate at higher salinities and a study has shown that *Phragmites australis*, *Juncus acutus*, and *Juncus kraussii* were able to germinate in salinities up to or in excess of 29000 $\mu S\ cm^{-1}$ (Greenwood and MacFarlane 2006). The upper salinity limits for germination for the halophyte *Ruppia polycarpa* is between 59000-73500 $\mu S\ cm^{-1}$ (Sim, et al. 2006). Regardless of the adult tolerance, the seedlings that emerge from the seed bank may be more sensitive to increased salinity than adult stages (Brock, et al. 2005) and a greater juvenile sensitivity obviously has important management implications (Brock, et al. 2005, Nielsen, et al. 2003). Managing a system using adult thresholds will lead to an inevitable decline in populations over the long-term as recruitment is reduced (James, et al. 2003, Nielsen, et al. 2003). Linking salinity levels directly to mortality or recruitment potential of aquatic biota is not sufficient to predict the outcome of increasing salinity on freshwater systems (Nielsen, et al. 2003). Information on sub lethal effects of increasing salinity on germination, growth or development of aquatic plants is required, however knowledge of this is limited (Nielsen, et al. 2003).

The challenge for saline water management is to understand the morphological, physiological and life-history characteristics that provide some capacity for tolerance, acclimatisation or avoidance of elevated salinity levels and impart a level of resilience to the biota of freshwater communities (James, et al. 2003). Clearly, in situations where dryland wetlands (wetlands in drier regions which are characterised by extreme hydrologic variability), are modified by human impacts, the diversity and abundance of propagules are likely to be affected, and thereby could indicate the wetland’s ecological health (Skinner, et al. 2001).

2 Introduction

2.1 Study Area – Upper South East of South Australia

The Upper South East (USE) of South Australia covers an area of 680 000 ha and is bounded by the towns of Kingston SE, Naracoorte, Keith, Salt Creek and the Coast (Coorong) (Figure 2-1). It has a Mediterranean climate of cool wet winters in which frosts are commonly experienced, and hot dry summers where daily maximum temperatures can top 40°C. The average rainfall ranges from 340 mm at Keith in the north to 420 mm in Naracoorte in the south and is considered to have a low to moderate variability in comparison to other parts of South Australia. Over the last 10 years rainfall has been lower than the longer-term averages, however there is no suggestion that the current dry period is the result of climate change in the region as it is consistent with the longer-term rainfall distribution pattern (DFW 2010). Drought conditions were experienced over much of south eastern Australia, including the SE of South Australia from 2005 to the end of 2010, with 2006 the worst year (ABS 2010). The average annual Potential Evapotranspiration (PET) is 1300 mm. The region supports agricultural activities, vineyards and forestry plantations.

Over the last 400 000 years, multiple marine incursions have shaped the USE. It is a low relief landscape consisting of a series of ranges (stranded dunes) that are 20-50 m wide and run parallel to the coast, separated by flats that are 2-10 km wide. There is a lack of defined surface watercourses, but where they exist, such as Mosquito, Naracoorte and Morambro Creeks, their catchments originate in western Victoria. The overall gradient of the land towards the coast (west) is 1:1600, and less than 1:5000 to the north (Croft, et al. 1999). Historically, water would flow westward over the flats and towards the coast, until it reached a dune range which directed it northwards through a wide corridor of natural wetlands via a ‘fill and spill’ process. The native vegetation, the open, low-gradient inter-dunal flats and the lack of a defined water course all contributed to slowing the progression of the water (Campbell 1993). There is no natural outlet for surface water except in very wet years when water could flow as far north-west to the coast at Salt Creek and the Coorong. In most years it would terminate in swamps in the north. This flow pattern resulted in extensive swamps, marshes and wetlands that were prevalent at the time of European settlement. (Croft, et al. 1999).

Intensive land clearance began in 1949 and was followed by the construction of privately owned surface water drains to drain the flooded and water logged flats and make them suitable for agriculture. As a result, only 13% of the native vegetation in the USE remains (Bulman, et al. 1993). This remaining native vegetation is not evenly distributed, but is concentrated in areas less suited to agriculture, either on deep sands (at the top of the dunes), saline soils, sheet limestone (Croft, et al. 1999) or wetland areas.

2.2 Salinity in the USE

Salt deposits which are a legacy of the region's marine past, have resulted in saline soils and groundwater. Groundwater is found in two major aquifer systems: a deeper confined limestone aquifer that is thought to be recharged in the lower south east and western Victoria and is associated with freshwater; and an upper unconfined aquifer that is recharged locally and is commonly saline due to the marine salts (Paydar, et al. 2009). The groundwater salinities in the unconfined aquifer vary from less than 2200 $\mu S\ cm^{-1}$ in the southern part of the study area to in excess of 18000 $\mu S\ cm^{-1}$ in the north, with some areas having a salinity exceeding that of seawater (54000 $\mu S\ cm^{-1}$) (USEDS&FMP 1993). Consequently, the region has had a long history of salinisation. When the groundwater is within 2 m of the soil surface it can be brought to the surface via capillary action. At the surface, the water is evaporated and salt that was dissolved in it is left behind. Where this is a naturally occurring process it is called primary salinisation, and this can be seen at Bunbury Conservation Park in the north-east corner of the of the USE. However if the groundwater is brought closer to the soil surface as a result of human activities such land clearance, it is termed secondary salinisation, and dryland salinity is an example of this. The dryland salinity problems in the USE have been caused by the intensive clearing of native vegetation and its replacement with pastures that are less efficient users of water. Overall this has resulted in a reduction in evapo-transpiration and an increase in recharge. This has caused a gradual rise in the regional groundwater level in the order of 0.5–1 m every 10 years (Armstrong and Stadter 1992, USEDS&FMP 1993).

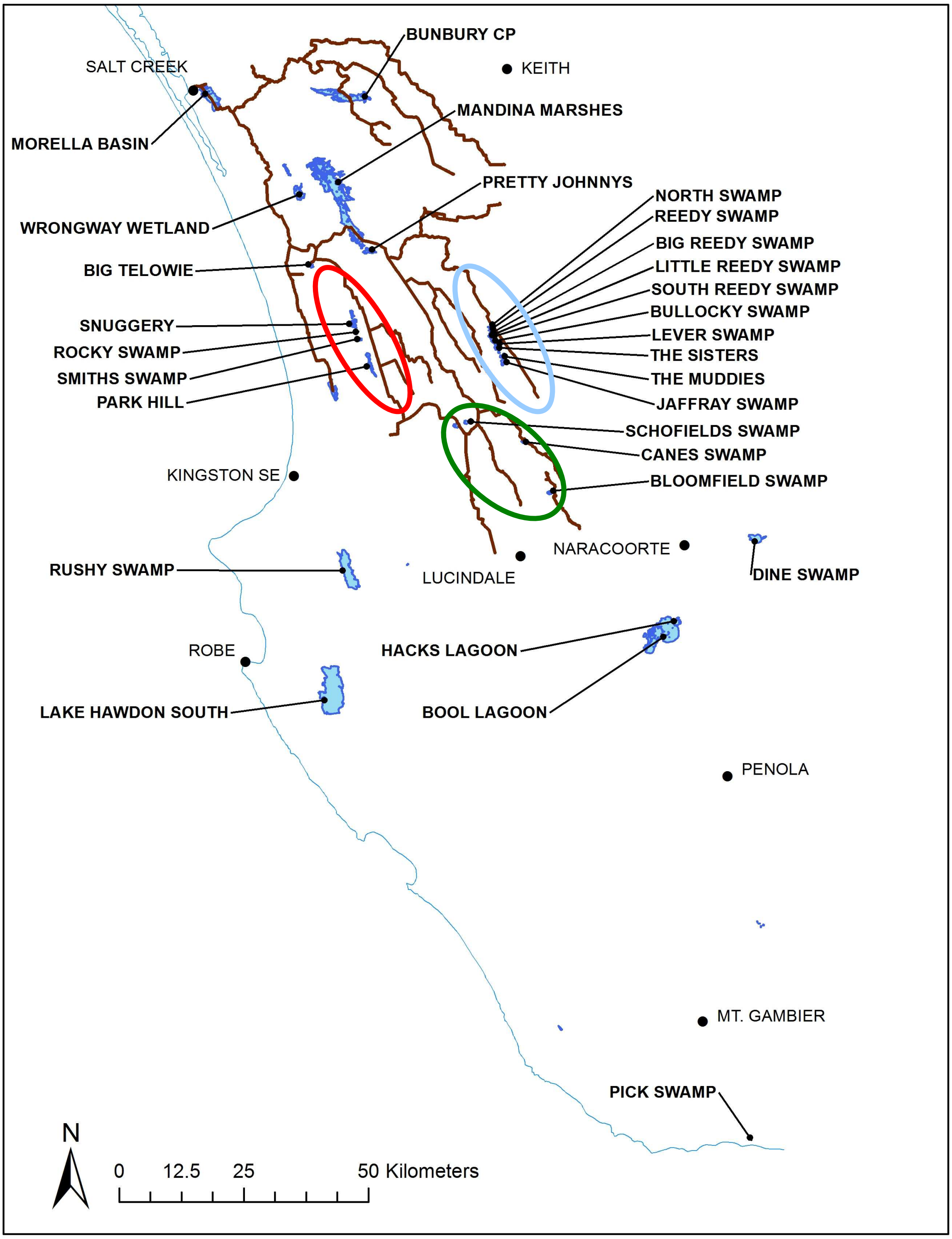


Figure 2-1: Map of the South East region showing: the USEDS&FMP deep drains (burgundy), the West Avenue complex (red); the Marcollat complex (light blue); the Bakers Range complex (green); and the wetlands sampled in the November 2007, November 2009 and January 2010.

2.3 Upper South East Dryland Salinity & Flood Management Program (USEDS&FMP)

The development of extensive new areas of dryland salinity was noted in the 1980's and in 1999 it was estimated that 40% of the USE region (250 000 ha excluding wetlands) was salinity affected, with a further 175 000 ha at risk of degradation (USEDS&FMP 1999). Many of the highly productive flats had rapidly deteriorated. Extended periods of inundation were destroying pastures and increasing the rate of land salinisation, and changes in water regime and quality were causing significant negative changes in the wetlands and remnant native vegetation. In response to community concerns over increasing areas of salinised land and flooding in the USE, the Upper South East Dryland Salinity and Flood Management Program (USEDS&FMP) was developed. The Program outlined an integrated approach to combat rising water tables, while taking into account environmental, economic and social concerns in the region (USEDS&FMP 1999).

The Program has sought to satisfy a number of objectives, including: protecting agricultural and environmental lands from dryland salinity; mitigating widespread and prolonged flooding; providing environmental flows to protect and enhance wetland and watercourse ecological values; and protecting and enhancing the ecological values of remnant natural areas (terrestrial and wetland) through management agreements with private landholders (DFW 2010). A major component was a new regional network of groundwater and surface water drains to control groundwater level and associated soil salinisation. Modelling indicated that a direct impact of groundwater drains would be a lowering of the water table in areas adjacent to the drains, thereby over- coming water logging problems and reducing the evaporative concentration of salt in the soil profile and at the land surface (Armstrong and Stadter 1992).

2.4 Restoring Environmental Flows to the Upper South East Wetlands (REFLOWS)

The Lower South East (LSE) region has had a long history of drainage; by 1966 large scale drainage (of surface water) had been completed in the LSE, funded by the State Government. These drains successfully removed the excess surface water and directed it away from its natural north-west path, sending it out to sea at Robe and Kingston

SE (Taffs 2001). As a consequence of the redirection of water, wetlands and watercourses in the USE suffered from reduced surface water flooding and have declined in health and extent (DFW 2010). As part of the wetland restoration component of the USEDS&FMP, the feasibility of restoring surface water flows from the LSE to the USE was investigated and resulted in a project known as Restoring Environmental Flows to the Upper South East Wetlands (REFLOWS). REFLOWS seeks to partially restore historical surface water flows from key source water catchments in the LSE (via two large constructed flood-ways) to deliver more reliable and substantial environmental flow volumes to key wetland systems in the USE (Paydar, et al. 2009).

2.5 The Drainage System

Construction of the USEDS&FMP commenced in 1995 and was completed in 2011. It consists of a 615 km network of interconnected drains and watercourses carrying: fresh surface water runoff; saline water emanating from the groundwater system; or a mixture of both (Stace 2005). Drains primarily used to control groundwater levels and generally referred to as ‘deep drains’, have been excavated to a depth of greater than 2 m where they intercept saline groundwater. Drains used primarily to collect surface water runoff, thus reducing surface inundation are generally referred to a ‘shallow drains’ and have been excavated to less than 2 m and do not intercept groundwater (Stace 2005). In most cases, the alignment of the drains is parallel to the wetland complexes, on the western side of the flats (Figure 2-1). There are over 100 weirs and regulators within the drainage system to facilitate the appropriate transfer of water through the network. There are numerous telemetered data collection stations both within the drainage network and at selected wetland sites. They record a combination of hydrological and water quality data including water depth, discharge, salinity, pH, dissolved oxygen, rainfall, soil moisture and turbidity. The data from these sites enable managers to consider water quality and quantity when deciding the best way to operate the regulators and weirs so that the optimum reduction in salinity, flood mitigation and environmental outcomes can be achieved.

An adaptive management system for the whole region that is designed to manage the movement of water through the drains, watercourses, wetlands, swamps and regulatory structures has been adopted and is reviewed and updated regularly

(SEWCDB 2011). Whilst these drains may have a positive effect on the agricultural land, the impacts they will have on the hundreds of wetlands in the region is as yet, unknown. It is likely that the new hydrologic regimes the wetlands will be exposed to will be highly modified and the quality of the water that supplies them will be greatly affected by high salinity levels.

2.6 Wetland Management

It is estimated that prior to European settlement, 45% of the landscape in the South East of South Australia was subject to inundation either permanently or seasonally. Currently less than 6% of wetlands in the South East remain, albeit in an altered hydrological state with less than 10% of the remaining wetland areas considered to be intact (Harding 2007). All remaining areas of undisturbed wetland and native vegetation have high conservation value due to the extensive clearance, drainage and agricultural development of the region (USEDS&FMP 1993). The wetlands of the South East provide important breeding, feeding and drought refuge habitats for fauna. Their high conservation value stems from their relation to other wetland habitats in south-eastern Australia, as the wetlands in the South East are more reliable than many of the wetlands further north in the Murray-Darling Basin (Jensen 1993). Due to the recent drought in southern Australia (with 2006 being the driest year), many wetlands in the region did not receive water for five years or more. As an example: the Ramsar listed wetlands of Bool and Hacks Lagoons were continuously wet from 1985-1993; they contained surface water each year from 1996-2000; but since then they have only received water three times up until 2010 when they were filled by summer rains and floods (DEH 2006). Part of the USEDS&FMP was a coordinated wetland management program which aims to restore natural surface flow regimes to many wetlands. The program will assist to protect wetlands remaining in this area from the damaging effects of rising saline groundwater (Evans and Brindal).

Prior to European settlement, surface water ran westward, off the interdunal flats and into swamps and wetland. However since the construction of the drainage network, the water running off the flats is collected by the drains. In some cases, ‘smart’ drains have been constructed that allow the surface water from the flats to pass over the groundwater drain and into wetlands. Where this does not occur there are regulators in the drains that enable water from the drains to be directed into the wetlands. The water in the drains often has a higher salinity than the surface water, as it contains saline groundwater. The regulators can be operated so that when the salinity of the water in the drains is high, the regulator in the drain is left open and the water bypasses the wetland and continues northward in the drain. However, if the water is of an appropriate quality for use in the wetland, the regulator in the drain can be closed and the water directed out of the drain and into the wetland complexes as an environmental flow.

The USEDS&FMP state that “the series of weirs protect unique wetlands in the area by preventing the flow of saline water into the wetlands, but allowing good quality water to enter and maintain the health of the ecosystem”. In order to achieve this, it is recognised that threshold levels that reflect the salt tolerance of the aquatic macrophytes in the wetlands is required, however, little is known about how to manage the timing, concentration, frequency and duration of releases of salt water to minimise impact on the aquatic biota of wetlands (Nielsen, et al. 2007). The body of work presented here addresses some of these knowledge gaps.

2.7 Aims

Using a combination of pond and laboratory experiments and field surveys in wetlands in the South East of South Australia, this work attempts to: advance the understanding of salt tolerance of common freshwater aquatic macrophyte species and assess the impact of the interaction of salinity and water regime in different combinations on selected macrophyte species and on germination from the seed bank of representative wetlands.

2.7.1 Aim 1: Evidence of recent salinity change on the flora of South East wetlands

The changes to the hydrology and salinity regimes of the wetlands in the South East and the potential threats these pose to the flora have been discussed. There is little evidence however to show that these changes have as yet, had an impact on the wetland ecosystems. The first aim of this study (Chapter 3) was to determine whether there have been changes in species composition that can be linked to changes in the salinity and hydrology regime experienced in the wetlands. To gain a better understanding of the processes and mechanisms that drive the change in species composition and cause salt to accumulate in wetlands, a conceptual model that describes how changes in groundwater, surface water and rainfall influence wetland salinity and water availability was developed. The causal factors described in the conceptual model were tested by assessing the evidence for salt accumulation in wetlands by comparing the salinity of water in wetlands to that of the surrounding groundwater, taking into account the reduced flows in the years post-2000.

2.7.2 Aim 2: Predicting probability of occurrence of wetland plants

There is general acceptance that sublethal effects can be detected in freshwater aquatic macrophytes grown in salinities greater than 1500 $\mu S\ cm^{-1}$ and that at salinities greater than 6250 $\mu S\ cm^{-1}$, freshwater macrophytes are no longer found. The operation of the USEDS&FMP requires managers to make decisions about when and where drainage water can be diverted into wetlands based on the salinity of the water. Currently these decisions are being made using the salinity tolerances mentioned above as a guideline. An important aim of wetland management is to protect adequately the most sensitive plant species within the wetland (James and Hart 1993), however at present there is limited information on the salinity tolerance of the individual species found in wetlands. Smith, et al.(2007) produced curves relating species occupancy at a site to the salinity of that site for species found in the Wimmera region of Western Victoria. The second aim (Chapter 4) employs a similar approach and uses field data collected across the salinity range found in the South East wetlands to produce curves predicting the probability of occurrence in relation to salinity for species common in wetlands in the South East of South Australia.

2.7.3 Aim 3: Effects of evapoconcentration

The conceptual model that was developed as part of Aim 1 explains the process whereby as the wetland dries and water evaporates, salt accumulates in the wetland through the process of evapo-concentration. Following on from this, the third aim (Chapter 5) was; to explore the effects of an increase in salinity with decreasing water depth as a result of evapoconcentration on the growth and survival of three common freshwater macrophytes: *Triglochin procerum, Baumea arthrophylla* and *Bolboschoenus medianus,* and to determine the consequences of longterm exposure to elevated salinity conditions. It has been stated that while the adults of many taxa are able to survive at elevated salt concentrations, the growth of juveniles may be substantially reduced (Hart, et al. 2003, Nielsen, et al. 2003). Therefore, as part of this study the salt sensitivity of adult and juvenile *T. procerum* was also investigated.

2.7.4 Aim 4: Effects of a pulsed salinity regime

As part of the USEDS&FMP, weirs and regulators were constructed along deep groundwater drains to enable water to be transferred from the drainage system and into wetland complexes. Depending on its salinity, the drainage water has the potential to be of ecological benefit to the wetlands. Due to the way in which the drains are operated, it is likely that the wetlands will receive water diverted from the drains in a pulse. However James, et al. (2003) caution that a pulsed release of saline water into freshwater systems should be avoided as it is likely to cause higher mortality and loss of biodiversity in a system than does a slow build-up to the same level. When the effect of a pulse application of salinity on the germination from a seed bank was studied, it was found that pulses of high salinity for a short duration followed by a return to freshwater conditions did not impact on the emergence of aquatic plants from wetland sediment (Nielsen, et al. 2007). Aim 4 (Chapter 6), was to assess the impact of a pulsed discharge of saline drainage water of varying concentrations and durations on key wetland species in an effort to determine how to make best use of the scarce water resources in the region.

2.7.5 Aim 5: Effects of inundation and salinity on the germination from the seed bank

The results from Aim 1 clearly demonstrate a change in the composition in the flora of wetlands in the South East. These changes have been attributed to the impacts of altered salinity and hydrology regimes. The seed bank provides an important mechanism for the persistence of species in ephemeral wetlands (Brock, et al. 1994, Nicol, et al. 2007), and emergence from the seed bank depends on environmental conditions, past and present (Brock, et al. 2005). Aim 5 (Chapter 7) was to assess the combined effects of salinity and hydrology on the seed banks of wetlands that have experienced drought and elevated salinity conditions.

3 Evidence of salt accumulation in wetlands and change in species composition

3.1 Introduction

Macrophytes contribute greatly to the structural diversity of wetland environments providing important refuge area for insect larvae and small fish (Reid and Brooks 2000), and are an important food source for water fowl. It is because of their high ecological importance and their capacity to register long-term change in ecosystem structure and function that macrophytes are used as indicators of wetland condition. The South East of South Australia is prone to secondary salinisation as the landscape has not yet reached a hydrological balance and salt is moved laterally around the landscape and up and down the soil profile. This is likely to have significant ecological impacts. An extensive biological survey of the Western Australian Wheatbelt showed that 15 assemblages of non-saline and saline wetlands were at risk from secondary salinisation. In this region, habitats that once were occupied by *Baumea articulata*, *B. arthrophylla, B. rubiginosa*, as well as species belonging to the genera *Utricularia* and *Triglochin* have been replaced by more salt tolerant species such as *Lepilaena preissii*, *Sarcocornia quinquifolia* and *Triglochin mucronata,* a species similar to *T. striatum* (Lyons, et al. 2004). There is a high possibility that similar changes in the aquatic biota may occur in the South East of South Australia if the wetlands become drier and more saline. This could threaten the long term survival and distribution of the freshwater macrophytes which currently comprise the wetland flora.

Groundwater is the dominant source of water for agriculture and industry in the South East region of South Australia (Paydar, et al. 2009). There are two major aquifer systems in the region: the shallow unconfined and the deeper confined aquifers, however, a majority of the groundwater use is sourced from the unconfined aquifer (DFW 2010). The water balance constructed for the entire South East shows that the groundwater resource in this region is over-allocated based on a mass balance, and over the last 10 years (2000 to 2009), rainfall has been lower than longer-term averages, with a noticeable decline in groundwater tables compared to the previous three decades (DFW 2010). Many wetlands in the South East of South Australia are fed by both surface water and groundwater and are classified as groundwater dependent ecosystems (SKM 2010). Therefore, the management of the upper unconfined aquifer is of particular importance, as it interacts with the groundwater dependent ecosystems; recent work has concluded that of the 6% of wetlands that remain in the region, less than 10 % of these (only 0.6% of the original wetland area) remain in good ecological condition, and that 77% of remnant wetlands are highly likely to be groundwater dependant (DFW 2010).

Winter precipitation is a critical factor in establishing the duration a wetland is inundated, and summer evaporation is critical in defining the capillary rise of groundwater to the surface in dried wetlands and the consequent deposition of salt (Mensforth 1996). The depth from ground surface to the unconfined aquifer water level oscillates throughout the year depending on the season (DFW 2010) but is less than 2 m over much of the region (Paydar, et al. 2009). Due to their groundwater dependence, changes to the depth to the unconfined aquifer are likely to affect the water available to wetlands (SKM 2010). Depending on climatic factors such as El Niño events resulting in below average rainfall and La Niña events resulting in above average rainfall (BOM 2011), the volume of water contributed to the groundwater or surface water will vary from year to year. There is clear evidence that groundwater dependent ecosystems are at risk in the South East as a result of falling groundwater levels, particularly in areas dominated by blue gum and pine plantations (DFW 2010). Therefore it is likely that the wetlands will receive less water in the future.

Wetlands occupy the lowest areas in the landscape and therefore are: the site of water runoff collection and retention; more likely to have large groundwater interactions; and the area where salt accumulates (Brock, et al. 2005, Hart, et al. 2003, James, et al. 2003, NLWR 2001, Walker, et al. 2002). Although the effects of increasing salinisation on aquatic systems are reviewed extensively, the ecological consequences of salinisation in Australian freshwaters are not well understood (Hart, et al. 1991, James, et al. 2003, Nielsen, et al. 2003), nor the mechanisms by which salt accumulates in wetlands. The landscape scale effects of dryland salinity in the South East region have been well documented (Cann, et al. 1992, NLWR 2001, USEDS&FMP 1993, Walker and Mensforth 1996), but given the changes that have been documented in the Western Australian Wheatbelt, there is a need for early identification of changes in the species composition at the wetland scale in the South

East. It is also necessary to determine if these changes are related to changes in the salinity and hydrology regimes experienced by these wetlands.

By analysing plant datasets collected in the mid 1990s, corresponding to average rainfall, and plant datasets collected in the mid to late 2000s capturing data from the below average rainfall years, the first aim of this study was to determine whether there have been changes in species composition that can be linked to changes in the salinity and hydrology regime experienced in the wetlands. The second aim was to gain a better understanding of the processes and mechanisms that drive the change in species composition and cause salt to accumulate in wetlands. A conceptual model that describes how changes in groundwater, surface water and rainfall influence wetland salinity and water availability was developed. The causal factors described in the conceptual model were tested by assessing the evidence for salt accumulation in wetlands by comparing the salinity of water in wetlands to that of the surrounding groundwater, taking into account the reduced flows post-2000.

3.2 Materials and Methods

3.2.1 Species Composition

Wetlands from four complexes in the South East region were surveyed pre-2000 and post-2000 (Table 3-1). Wetland complexes rather than individual wetlands were compared as this enabled greater confidence that all species representative of the area were recorded. Species lists from studies conducted between 1994 and 1999 were used to determine the presence/absence of species in the four wetland complexes pre-2000 (Table 3-1). Unfortunately no environmental variables were documented during these studies. To determine the presence/absence of species post-2000, wetlands in each of the wetland complexes were surveyed between November and December 2009 (Figure 2-1). In each wetland, twenty metre transects with dimensions 20m×0.5m were used to survey the macrophyte community. The transect locations in each wetland were selected to ensure that all the species present at each wetland were recorded. In each transect, the presence of individual macrophyte species was identified and recorded. The number of transects varied between wetlands to allow spatial variation in the macrophyte community to be incorporated. Where available, species lists from the South Australian Wetland Inventory Database (SAWID) (DWLBC 2010) were also used. Using the PC-Ord 5.0 software (McCune and

Mefford 1999), an NMS ordination relating the pre-2000 and post-2000 surveys of each wetland complex to the species found was produced.

Table 3-1: Summary of survey data for pre-2000 and post-2000 comparison.

Watercourse	Time Period	Report or Survey Used	Survey Year
Bool and Hacks Lagoons	Pre-2000	Brownlow (1997)	1996
Bool and Hacks Lagoons	Post-2000	Survey work in Nov 2009 for this study	2009
West Avenue Watercourse	Pre-2000	Stewart, et al.(2002)	1999
West Avenue Watercourse	Post-2000	DWLBC (2010) and survey work in November 2009 for this study	2004 - 2009
Bakers Range Watercourse	Pre-2000	Stewart (1996)	1996
Bakers Range Watercourse	Post-2000	DWLBC (2010) and survey work in November 2009 for this study	2006 - 2009
Marcollat Watercourse	Pre-2000	Dowling (1997)	1994
Marcollat Watercourse	Post-2000	Survey work in November 2009 for this study	2009

3.2.2 Development of Conceptual Model

To explain the factors driving the change in species composition over the pre-2000 and post-2000 period, the processes and mechanisms that occurred in the wetland complexes need to be better understood. A conceptual model was developed to aid with this. This was done by combining personal experience, knowledge and observation with results and thoughts from the published literature.

3.2.3 Evidence to support the Conceptual Model - Discharge at gauging stations

To confirm that surface water availability was reduced in the years post-2000, the discharge in ML/day from January 1990 to December 2009 was plotted for gauging stations at Mosquito Creek upstream from Bool and Hacks Lagoon, and at the Callendale Regulator where water can be directed down Bakers Range watercourse (Figure 2-1). Records for the Marcollat watercourse gauging station only started in Jan 1997 (DFW 2011). A gauging station with a long enough record was not available for the West Avenue watercourse.

3.2.4 Evidence to support the Conceptual Model - Surface and groundwater salinities

Salinity data was collected to enable the comparison of surface and groundwater salinities. In November 2009, 18 wetlands representing the salinity gradient from south to north in the South East region were sampled and seven wetlands still holding water were re-sampled in January 2010 (Figure 2-1). At each wetland, where there was sufficient water depth, conductivity readings were taken at approximately 10 cm above the soil surface using a TPS WP-81 Conductivity meter. Surface water conductivities were recorded at each transect, correct at a standard temperature of 25°C.

The online Obswell database provides information on the network of observation bores for South Australia including water level and salinity data. Due to a lack of information on groundwater-surface water interactions, it was not possible to demonstrate a connection between the unconfined aquifers in which the bores are located and the wetlands which they neighbour. Therefore the bores closest to the wetlands sampled were identified and the conductivity readings recorded at these bores in the period 1st August 2009 to 31st October 2009 were used (DWLBC 2009). The differences between the conductivities recorded in the wetlands and those recorded in the groundwater are considered in the context of the conceptual model.

3.3 Results

3.3.1 Comparing Species Composition

The 2-D ordination (Figure 3-1) illustrates the pre- and post- 2000 change in the floristic composition of four wetland watercourses/complexes in the South East (stress=6.4). The pre-2000 survey (open triangles) position Bool and Hacks lagoons, West Avenue and Bakers Range watercourses in the bottom right hand corner of the ordination. However, the post-2000 surveys (closed triangles) are shifted up and slightly to the left. Similarly, the post-2000 surveys for the Marcollat watercourse were shifted to the left and higher in the ordination. Overall, there was a general movement of the surveys from the bottom of the ordination toward the top, and from the right of the ordination toward the left. The blue dots situated in the centre of the triangles representing the wetland complexes are species which are faithful to only that wetland complex (Figure 3-1). *Azolla filiculoides, Carex apressa, Centaurium* *spicatum, Isolepis platycarpa, Lemna trisulca, Lepilaena australis, Melaleuca squarrosa, Myriophyllum propinqum, Schoenoplectus validus, Spirodela sp, Utricularia sp.* and *Wolffia angustata* were found in the pre-2000 survey at no other location but Bool and Hacks lagoons (shown in dark blue). In the post-2000 surveys, none of these species were recorded at Bool or Hacks lagoons and instead, *Lemna minor*, which was previously absent from any of the wetlands, was recorded. In the West Avenue watercourse (shown in red), *Agrostis sp., Dianella brevicaulis, Juncus caespiticius* and *Trifolium campestre* were recorded in the pre-2000 survey, but in the post-2000 survey these species were no longer present at these wetlands but *Ruppia tuberosa* was recorded. In the Bakers Range watercourse (shown in green), *Centaurium tenuiflorum, Cotula vulgaris, Hydrocotyle sp, Muehlenbeckia sp*. and *Potamogeton australiensis* were recorded in the pre-2000 surveys, but in the post-2000 surveys there were no records of these species but *Trifolium repens* was present. In the Marcollat watercourse, (shown in light blue) in the pre-2000 survey, *Juncus procerus, Suadea australis, Wilsonia humilis* and *Wahlenbergia luteola* were recorded, but in the post-2000 surveys these species were not recorded although *Cyperus gymnocaulos* was.

The labelled blue dots symbolize species that are not just representative of one wetland complex but may be present at two or more, at one or both time periods. For example, *Lepilaena cylindrocarpa, Lepilaena patentifolia* and *Lepilaena pressii* are three species that were not recorded in any wetlands in the pre-2000 surveys but were recorded at Bool and Hacks lagoons, West Avenue and Marcollat watercourses in the post-2000 surveys.

No environmental variables were collected in the pre-2000 surveys corresponding to the presence/absence data. Therefore, to ascertain what the axes of the ordination represent, the ecology of the species was examined. The species recorded in the surveys, their life forms and the habitat they are associated with are listed in Table 3-2. Many of the species on the right-hand side of the ordination, such as *Azolla filiculoides, Myriophyllum propinqum, Utricularia sp* and *Lemna trisulca,* are species requiring standing water to thrive (Cunningham, et al. 1981, Sainty and Jacobs 2003). Species positioned on the left-hand side of the ordination are those better suited to waterlogged or drained conditions including *Cyperus gymnocaulos, Suaeda australis, Wilsonia backhousei* and *Wilsonia humilis* (Cunningham, et al. 1981, National

Herbarium of NSW 2011). This indicates a gradient on the horizontal axis (Axis 1) from wetter conditions on the right to drier conditions on the left. Glycophyte species, such as *Azolla filiculoides, Myriophyllum propinqum, Utricularia sp* and *Wolffia angustata* are known to be highly salt sensitive (Brock 1981) and were positioned low in the ordination space, whereas halophytic species, such as *Lepilaena cylindrocarpa, Lepilaena patentifolia, Lepilaena pressii* and *Ruppia tuberosa* (Brock 1981, National Herbarium of NSW 2011) were positioned high in the ordination space. This indicates a salinity gradient on the vertical axis of the ordination (Axis 2) from fresher to saltier conditions. The overall movement of the surveys from the pre-2000 to post-2000 floristic composition was from the bottom of the ordination space toward the top, and from the right to the left- hand side. This corresponds to fresher wetter conditions in the pre-2000 period and increased salinity and drier conditions in the post-2000 period. Several species recorded in the pre-2000 surveys require both fresh and wet conditions and it is these species: *Azolla filiculoides, Myriophyllum propinqum, Utricularia sp*, *Lemna trisulca* and *Wolffia angustata* that were not recorded in the 2009 survey. The absence of adult plants raises questions about the condition of the seed bank and whether or not it is intact.

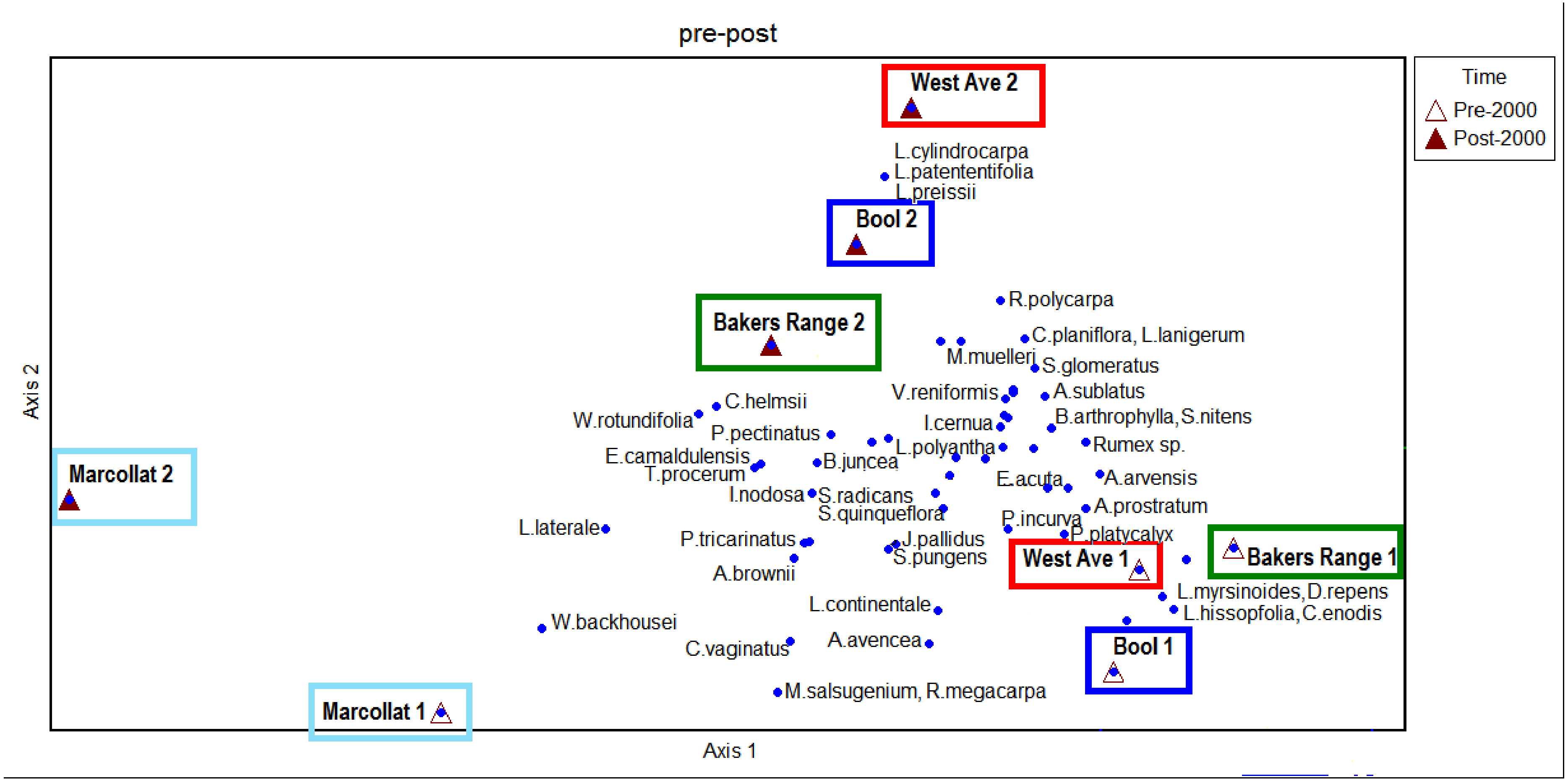


Figure 3-1: NMS Ordination showing the positions of four wetland watercourses/complexes in the South East: Bakers Range watercourse; Bool and Hacks lagoons; Marcollat watercourse; and West Avenue watercourse, in relation to their floristic compositions pre-2000 (open triangles) and post 2000 (closed triangles).

Table 3-2: Species recorded in pre- and post-2000 surveys

Species	**Habitat**	**Life Form**	**Lifespan**
Acacia longifolia var. sophorae	Terrestrial	Tree	Perennial
Acacia melanoxylon	Terrestrial	Tree	Perennial
Acaena novae-zelandiae	Floodplain	Groundcover	Perennial
Agrostis avenacea	Terrestrial	Grass	Perennial
Anagallis arvensis	Floodplain	Herb	Annual
Angianthus preissianus	Floodplain	Groundcover	Annual
Apium prostratum	Floodplain	Herb	Perennial
Apodasmia brownii	Floodplain	Sedge	Perennial
Atriplex prostrata	Floodplain	Herb	Annual
Aster subulatus	Floodplain	Herb	Annual
Azolla filiculoides	Aquatic	Free floating	Annual
Baumea arthrophylla	Aquatic	Emergent sedge	Perennial
Baumea articulata	Aquatic	Emergent sedge	Perennial
Baumea juncea	Aquatic	Emergent sedge	Perennial
Bolboshoenus caldwellii	Aquatic	Emergent sedge	Perennial
Bromus hordeaceus	Terrestrial	Grass	Annual
Bulbine bulbosa	Floodplain	Herb	Perennial
Carduus tenuiflorus	Terrestrial	Herb	Annual
Carex apressa	Aquatic	Emergent sedge	Perennial
Centaurium erythraea	Floodplain	Herb	Annual
Centaurium spicatum	Floodplain	Herb	Annual
Centaurium tenuiflorum	Floodplain	Herb	Annual
Centrolepis polygyna	Floodplain	Herb	Annual
Chenopodium glaucum	Floodplain	Groundcover	Annual
Chorizandra enodis	Aquatic	Sedge	Perennial
Cotula australis	Floodplain	Herb	Annual
Cotula coronopifolia	Floodplain	Herb	Annual
Crassula helmsii	Aquatic	Submerged	Annual
Cuscuta planiflora	Floodplain	Parasitic	Annual
Cyperus gymnocaulos	Aquatic	Emergent sedge	Perennial
Cyperus vaginatus	Aquatic	Emergent sedge	Perennial
Dianella brevicaulis	Terrestrial	Herb	Perennial
Dianella revolute	Terrestrial	Herb	Perennial
Dichondra repens	Floodplain	Groundcover	Annual
Distichlis distichophylla	Floodplain	Grass	Perennial
Eleocharis acuta	Aquatic	Emergent sedge	Perennial
Epilobium billardierianum sp. billardierianum	Floodplain	Herb	Perennial
Eucalyptus camaldulensis	Terrestrial	Tree	Perennial
Eucalyptus fasciculosa	Terrestrial	Tree	Perennial
Gahnia filum	Aquatic	Emergent sedge	Perennial
Gahnia trifida	Aquatic	Emergent sedge	Perennial
Hydrocotyle laxiflora	Floodplain	Herb	Perennial
Hydrocotyle muscosa	Floodplain	Herb	Perennial
Isolepis cernua	Aquatic	Emergent sedge	Perennial
Isolepis fluitans	Aquatic	Emergent sedge	Perennial
Isolepis nodosa	Aquatic	Emergent sedge	Perennial
Isolepis platycarpa	Aquatic	Emergent sedge	Perennial
Juncus bufonius	Floodplain	Rush	Perennial
Juncus caespiticius	Floodplain	Rush	Perennial

(Table 3-2 cont.)

Species	**Habitat**	**Life Form**	**Lifespan**
Juncus krausii	Floodplain	Emergent rush	Perennial
Juncus procerus	Floodplain	Rush	Perennial
Junus pallidus	Floodplain	Emergent rush	Perennial
Lemna minor	Aquatic	Free floating	Annual
Lemna trisulca	Aquatic	Free floating	Annual
Lepidosperma laterale	Floodplain	Emergent sedge	Perennial
Lepidosperma sp.	Floodplain	Emergent sedge	Perennial
Lepilaena australis	Aquatic	Submerged	Annual
Lepilaena cylindrocarpa	Aquatic	Submerged	Annual
Lepilaena patentifolia	Aquatic	Submerged	Annual
Lepilaena preissii	Aquatic	Submerged	Annual
Leptospermum continentale	Terrestrial	Tree	Perennial
Leptospermum lanigerum	Terrestrial	Tree	Perennial
Leptospermum myrsinoides	Terrestrial	Tree	Perennial
Lilaeopsis polyantha	Floodplain	Herb	Annual
Lobelia alata	Floodplain	Herb	Annual
Lythrum hissopifolia	Floodplain	Herb	Annual
Melilotus indica	Floodplain	Herb	Annual
Melaleuca brevifolia	Terrestrial	Tree	Annual
Melaleuca halmatuorum	Terrestrial	Tree	Annual
Melaleuca squarrosa	Terrestrial	Tree	Annual
Mimulus repens	Floodplain	Herb	Annual
Montia sp.	Floodplain	Herb	Annual
Muehlenbeckia sp.	Floodplain	Climbing Shrub	Perennial
Myriophyllum muelleri	Aquatic	Submerged	Annual
Myriophyllum propinqum	Aquatic	Submerged	Annual
Myriophyllum salsugenium	Aquatic	Submerged	Annual
Myriophyllum simulans	Aquatic	Submerged	Annual
Myriophyllum verrucosum	Aquatic	Submerged	Annual
Parapholis incurve	Floodplain	Herb	Annual
Phragmites australis	Aquatic	Emergent	Perennial
Potamogeton pectinatus	Aquatic	Submerged	Annual
Potamogeton tricarinatus	Aquatic	Submerged	Annual
Potamogeton australiensis	Aquatic	Submerged	Annual
Pratia platycalyx	Floodplain	Herb	Annual
Psuedo-gnaphalium luteo-album	Floodplain	Herb	Annual
Pteridium esculentum	Floodplain	Fern	Perennial
Ranunculus sp.	Aquatic	Emergent	Annual
Rumex sp.	Floodplain	Herb	Annual
Ruppia megacarpa	Aquatic	Submerged	Annual
Ruppia polycarpa.	Aquatic	Submerged	Annual
Ruppia tuberose	Aquatic	Submerged	Annual
Samolus repens	Floodplain	Herb	Annual
Sarcocornia quinqueflora	Floodplain	Herb	Annual
Schoenoplectus pungens	Aquatic	Emergent sedge	Perennial
Schoenoplectus vallidus	Aquatic	Emergent sedge	Perennial
Schoenus nitens	Floodplain	Sedge	Perennial

(Table 3-2 cont.)

Species	Habitat	Life Form	Lifespan
Selliera radicans	Floodplain	Herb	Annual
Senecio glomeratus	Floodplain	Herb	Annual
Sonchus sp.	Floodplain	Herb	Annual
Spirodela sp.	Aquatic	Free floating	Annual
Sporobolus virginicus	Floodplain	Grass	Annual
Stipa sp.	Floodplain	Grass	Annual
Trifolium campestre	Floodplain	Herb	Annual
Trifolium repens	Floodplain	Herb	Annual
Triglochin procerum	Aquatic	Emergent	Perennial
Triglochin striatum	Aquatic	Emergent	Perennial
Typha domingensis	Aquatic	Emergent	Perennial
Urtica incise	Floodplain	Herb	Perennial
Utricularia sp.	Aquatic	Submerged	Perennial
Villarsia renifomis	Aquatic	Emergent	Perennial
Wahlenbergia luteola	Terrestrial	Herb	Perennial
Wilsonia backhousei	Floodplain	Herb	Perennial
Wilsonia humilis	Floodplain	Herb	Perennial
Wilsonia rotundifolia	Floodplain	Herb	Perennial
Wolffia angustata	Aquatic	Free floating	Annual

3.3.2 The Conceptual Model

The quality, rate of flow and depth to the unconfined aquifer is highly variable across the South East region and this is a reflection of the topography, soil type and recharge rates (MacKenzie and Stadter 1992). Depth to the groundwater varies both seasonally and geographically; the shallow groundwater levels occur in the lower lying areas, such as the interdunal flats, and seasonal (MacKenzie and Stadter 1992) fluctuations can range from 0.05–1.5 m (Mensforth 1996). The unconfined aquifer is recharged locally, mainly by diffuse rainfall on the flats and dunal ranges (when rainfall exceeds potential evapotranspiration), however contributions from surface water discharge into sinkholes and drainage wells, and wetlands and swamps are also important (Paydar, et al. 2009). The magnitude of the recharge varies according to the nature of the soil, the depth of the water table and the nature of the vegetation and variations in land management practices (MacKenzie and Stadter 1992, USEDS&FMP 1993). Over winter, as a result of the recharge the depth from the soil surface to the unconfined aquifer decreases. As the groundwater rises, it intercepts the base of groundwater dependent wetlands. When the soil moisture reaches capacity and with continued rainfall, surface water is generated. These two sources of water; unconfined aquifer water (W_{UAW}) and surface water (W_{SW}) (SEWCDB 2004), both move into wetland basins, causing the wetlands to fill (Figure 3-2a).

In the South East region, groundwater salinity varies between less than 500 µS cm^{-1} in the south and greater than 50000 µS cm^{-1} in the north of the region (DWLBC 2009, MacKenzie and Stadter 1992, Mensforth 1996, Paydar, et al. 2009). Hence, high conductivity aquifer water that enters the wetland also brings salt. In contrast, the surface water is fresh with lower conductivities, frequently <1000 µS cm^{-1}. When filling, the resulting salt content of the wetland water (W_w) at any particular moment in time will be a product of the mixing of the W_{SW} and W_{UAW} plus any as described by Equation 1.

Equation 1

$$C(W_W) = \frac{C(W_{UAW}) \cdot V(W_{UAW}) + C(W_{SW}) \cdot V(W_{SW})}{V(W_{UAW}) + V(W_{SW})}$$

where: C is salt concentration; and

V is volume.

The main groundwater discharge process is evaporative loss; where groundwater is within 2 m of the soil surface, capillary rise causes water (and solutes) to be carried up to the soil surface where water evaporates and the salt is left behind (MacKenzie and Stadter 1992, Mensforth 1996). This occurs over summer and causes the depth to the unconfined aquifer to increase (DFW 2010, Figures 3 &4). The combination of this, and the effects of evapotranspiration (W_{ET}) results in a decrease in the water level of the wetland (Figure 3-2b). Evapotranspiration removes water from the wetlands but leaves salt behind, resulting in an increase in the salt content of the remaining water. This process is termed evapoconcentration. The overall effect is an increase in the conductivity of the wetland water and MacKenzie and Stadter (1992) report that monitoring indicates that salinity increases significantly during the summer period due to evapoconcentration. As the unconfined aquifer recedes, there is evidence that some salt is lost from the water column, most likely to the sediments (James, et al. 2009).

When the wetland is dry and the depth to the unconfined aquifer has dropped below the base of the wetland, salt which was in the wetland water will remain in the sediment of the wetland, at or near the surface. Through capillary action, moisture from the soil and the unconfined aquifer is brought to the surface where the water evaporates, leaving salt behind (Figure 3-2c). After isolated or small rainfall events, water will move laterally through the soil and into the wetland (as depicted by the dashed arrows in Figure 3-2c) and this water is likely to further contribute salt. As a result, salt is accumulated in the wetland basin.

Water in wetlands will remain fresh as long as the amount of salt exported through seepages is the same as that imported through rainfall or inward seepage of saline groundwater. If the groundwater flow through the wetland is relatively fast, the salinity of the wetlands will reflect that of the surrounding groundwater (Mensforth 1996). Based on this, due to the groundwater dependency of wetlands in the South East, and by applying Equation 1, it would be expected that the salinity of the wetlands would be similar to the salinity of the surrounding unconfined aquifer, or fresher due to fresh surface water inputs.

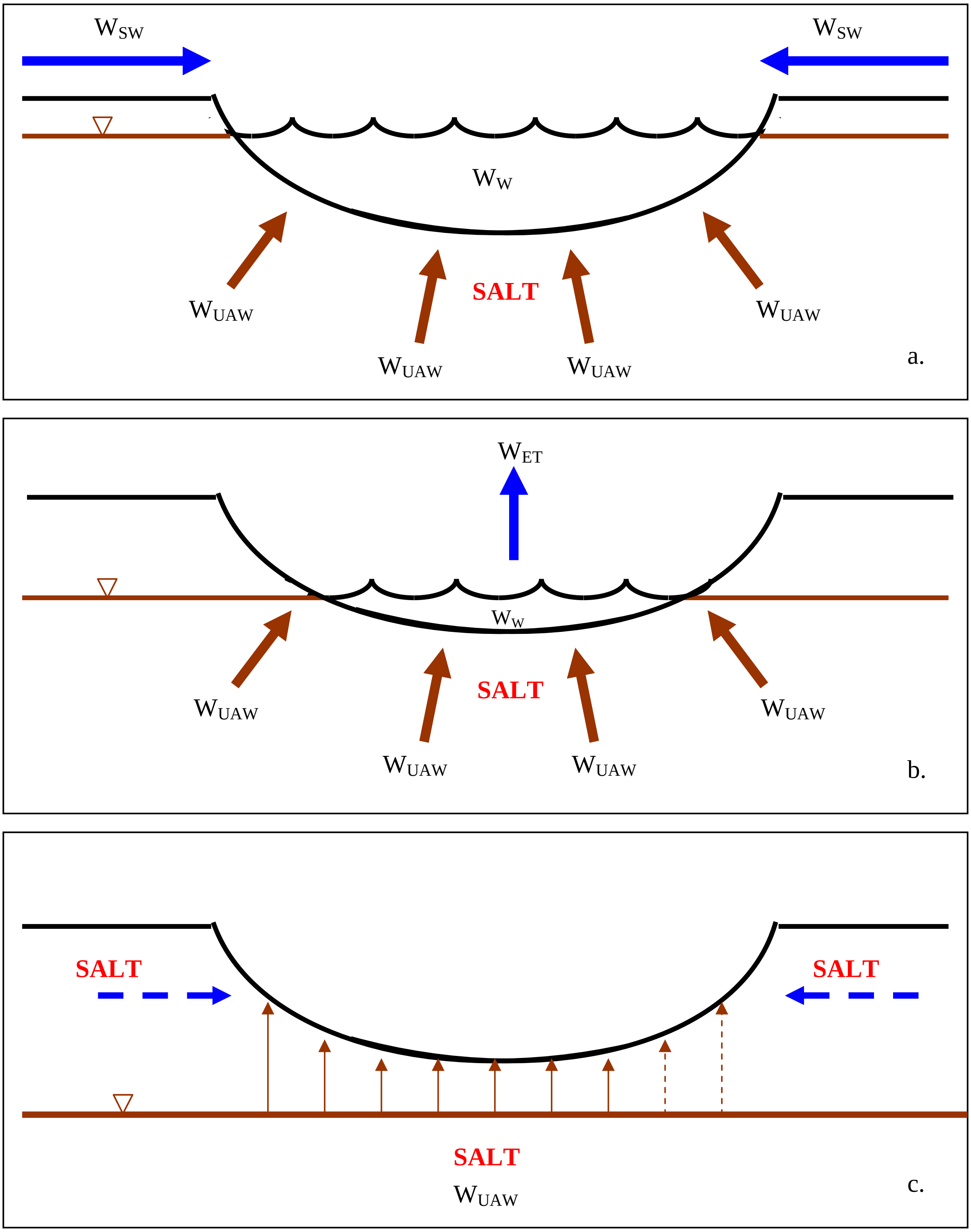


Figure 3-2: Conceptual diagrams illustrating the interaction between the water in the unconfined aquifer and surface water when a wetland is: a) filling; b) drying; and c) completely dry.

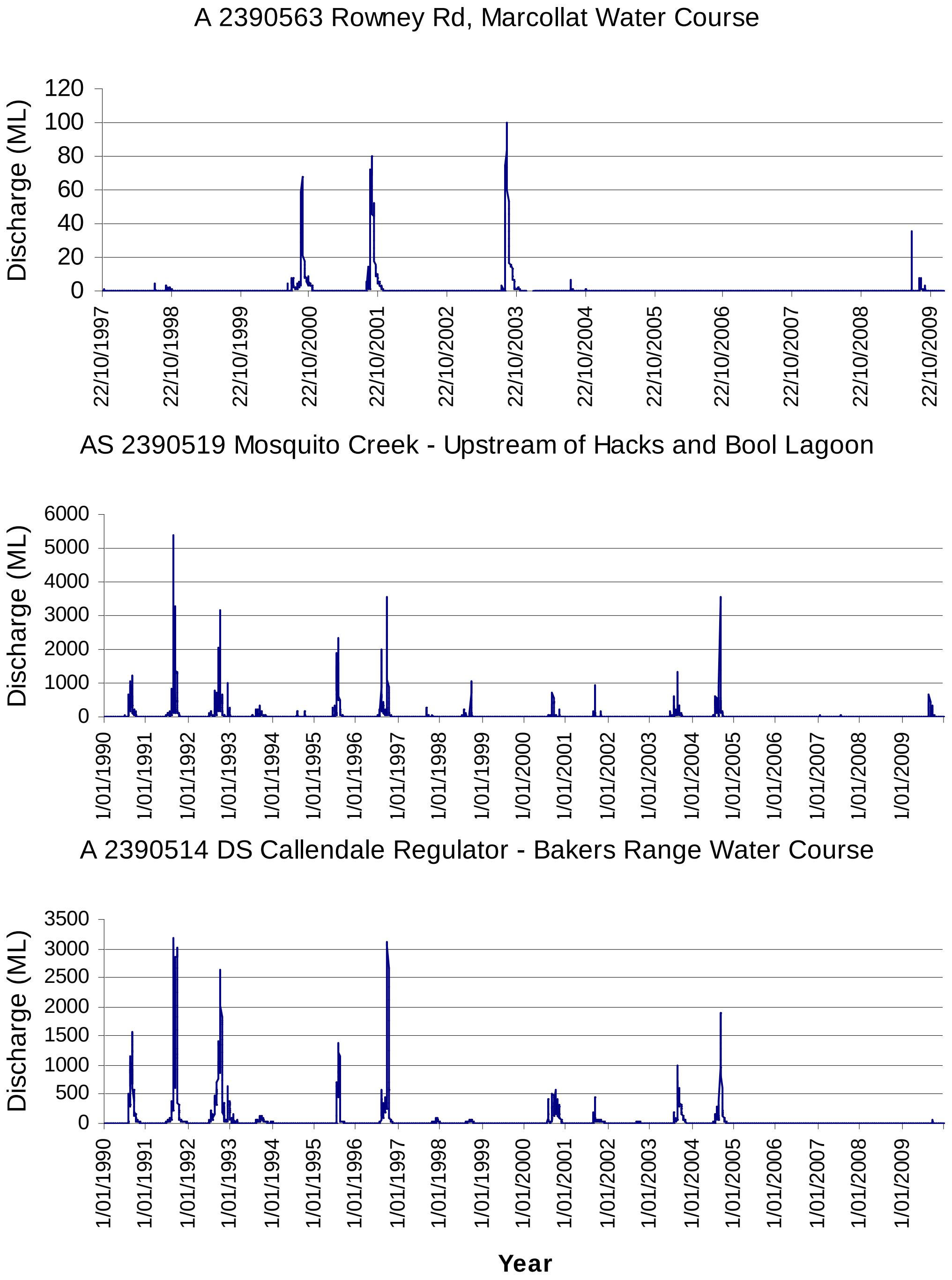
Prior to land use changes, in dry periods salt would have been carried into wetlands and often concentrated by evaporation. However, the salt that accumulated in the sediment of wetlands was removed by flushing during the next high-flow event (Mensforth 1996, Nielsen, et al. 2003). In the Upper South East, where wetlands occur along water courses or part of ‘fill and spill’ chain of wetlands, this is also the case. However, wetlands that are at the end of terminal system or occur as an isolated basin, flushing flows will not occur. Surface water quality changes with season and flow conditions; low flow conditions will result in higher levels of salinity in the surface water as a result of seepage of shallow groundwater and runoff from the upper parts of the catchment (Mensforth 1996). Wetland vegetation relies on the regular flushing of salt from the root zone for continued survival. A change in hydrology that led to the constant presence of a shallow saline water table would reduce the leaching of salt from the root zone and cause a decline in vegetation health (Cramer and Hobbs 2002). Many of the wetlands in the South East are ephemeral and most have experienced extended dry periods. During this time, it is likely that salt has accumulated in the wetland sediments due to the processes described in Figure 3-2. As a consequence of the drought conditions that were present from 2003 through to the end of 2009, most wetlands have not received the flows required to flush the salt from their sediments.

3.3.3 Evidence to support the Conceptual Model - Comparing hydrology pre- and post-2000

The discharge records demonstrate a reduction in the volume of water flowing past all of the gauging stations in the post-2000 period in comparison to the pre-2000 period (Figure 3-3). The records at the Callendale Regulator and at Mosquito Creek indicate that there were wet years in the early 90s up until 1997; after this flows were much reduced with smaller flows recorded in 2004 and 2005. The record at Rowney Road for the Marcollat watercourse indicates that there were some small flows in 2000, 2001 and 2003 but thereafter it was dry. This suggests that there would not have been sufficient water in wetlands to enable any salt to be flushed from the wetlands.

3.3.4 Evidence to support the Conceptual Model - Comparing surface water and groundwater salinities

There is a strong salinity gradient in the surface water of the wetlands recorded in November 2009 and the groundwater salinities recorded between August and October 2009 (Figure 3-4). The conductivities of the unconfined aquifer are less than 500 µS cm-1 (dark blue) in the southern region of the South East increasing to greater than 10000 µS cm-1 (red) in the north. Table 3-3 documents the conductivities recorded in wetlands in October and November 2009 and January 2010. The increase in salinities in all wetlands as the season progresses is evident with salinities more than doubling in Bloomfield Swamp, Wrongway Wetland, Smiths Swamp and Double Swamp. This is due to evapoconcentration. The conductivities recorded between August and October 2009 at Obswell bores located close to the wetlands are also displayed in Table 3-3. Bool Lagoon is an extensive wetland and therefore both the minimum salinity reading, which was recorded in the north east section of the main basin, and the maximum salinity which was recorded at the outlet are included. For some wetlands, where there was more than one Obswell bore close to the wetland, salinity readings from both are provided. The table allows direct comparison between wetland salinities and that of the nearest observation bore. A majority of wetlands have conductivities greater than that of the associated bore except for Bloomfield Swamp, Dine Swamp and some areas of Bool and Hacks lagoons.



A 2390563 Rowney Rd, Marcollat Water Course

AS 2390519 Mosquito Creek - Upstream of Hacks and Bool Lagoon

A 2390514 DS Callendale Regulator - Bakers Range Water Course

Figure 3-3: Discharge recorded at gauging stations at; a) Rowney Rd on the Marcollat Water Course, b) Mosquito Creek and c) Callendale Regulator, upstream from the Bakers Range water course

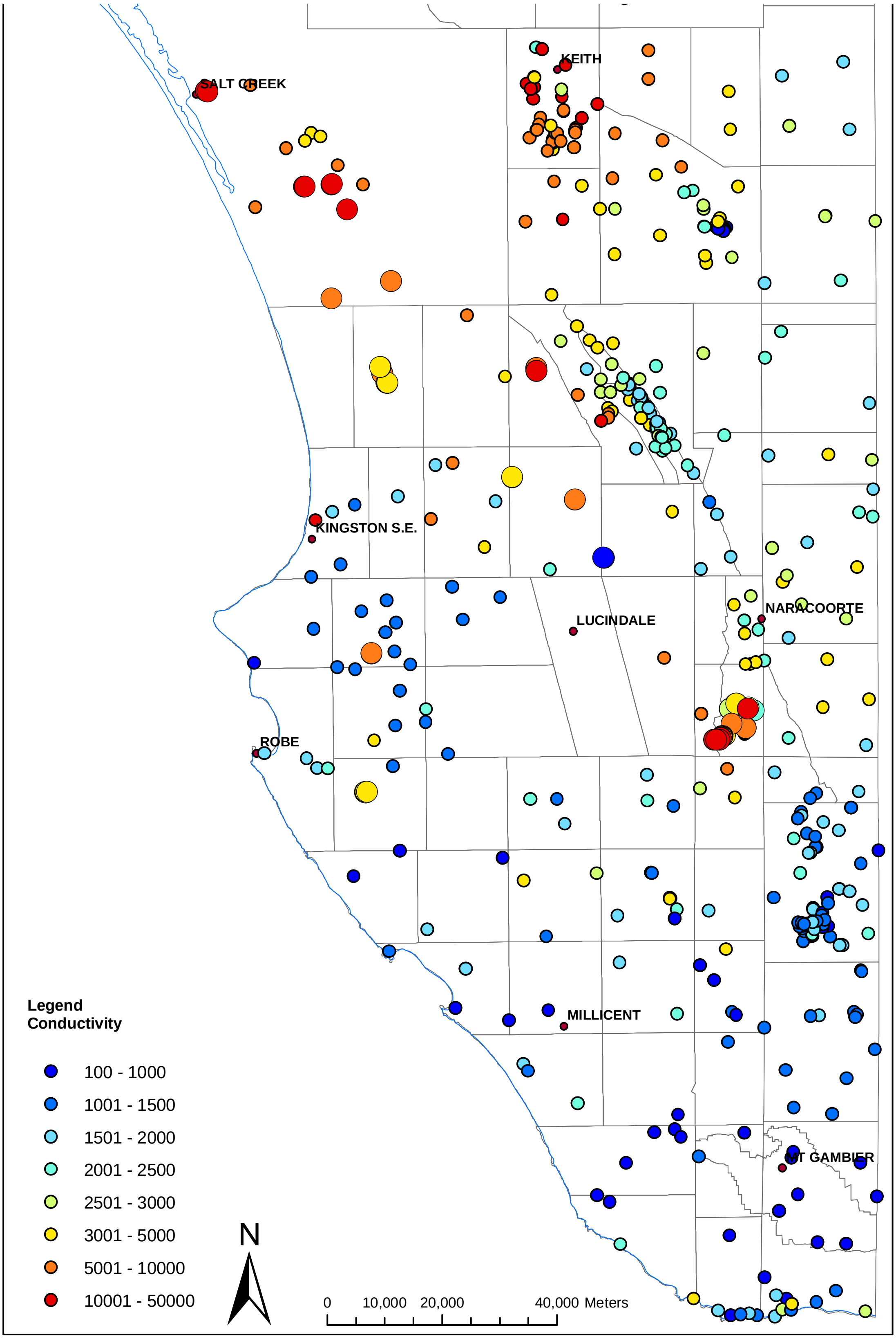


Figure 3-4: Map of the South East region displaying the conductivity (µS cm^{-1}) of the groundwater (small circles) recorded between August and October 2009 and wetlands (large circles) recorded in November 2009.

Table 3-3: Conductivity readings of the surface water of wetlands and unconfined aquifer from neighbouring Obswell bores recorded in the 09/10 season.

SURFACE WATER SALINITIES					GROUNDWATER SALINITIES			
WETLAND	**Easting**	**Northing**	**November Salinity (µS cm^{-1})**	**January Salinity (µS cm^{-1})**	**Obswell Unit No**	**Easting**	**Northing**	**August-October Salinity (µS cm^{-1})**
Big Telowie Swamp	402207	5965161	580	7100				
Bloomfield Swamp	449680	5920019	590	1200	WLM011	440346	5918000	1100
Hacks Lagoon	475840	5893502	1600	DRY	JOA026	481846	5888674	1400
Bool Lagoon	471598	5893726	1500	DRY	ROB004	466663	5892895	2900
Bool Lagoon	469201	5888364	14000	DRY	ROB013	474267	5889402	2100
Bunbury CP	406729	6001204	121700	DRY	MCN022	400250	5993277	1700
Canes Swamp	433729	5934043	7300	DRY	LOC013	461664	5928040	2700
Dine Swamp	408394	5879308	345	420	JES004	491878	5909420	1600
					JES054	488622	5902345	1800
Wrongway Wetlands	397390	5984599	15600	35400	MSN001	388039	6002195	2600
					NVL001	388910	5981038	4100
Mandina Lake	402182	5984966	12400	22400	WEL001	407770	5984992	3500
Mandina Marshes	404936	5980685	15900	51100				
Lake Hawdon South	408014	5879282	3700	DRY	ROS005	409645	5888220	1800
					WAT011	399720	5883431	1100
					WAT027	401594	5883338	700
Morella Basin	380332	6001224	25900	19600	MSN001	388039	6002195	2600
North Swamp	437882	5953002	9000	DRY	MAR027	442170	5957695	1400
Pretty Johnnys	412622	5968185	7600	DRY				
Rushy Swamp	409193	5903360	6100	DRY	BOW006	403224	5901031	550
					MTB009	399100	5907679	570
Rocky Swamp	411048	5952156	5300	7400				
Reedy Swamp	437973	5952504	16000	DRY	MAR029	446715	5952790	1100
Schofield Swamp	433727	5934052	4200	DRY				
Smiths Swamp	411882	5950481	5300	8700				
Double Swamp	410765	5953228	5300	10700				

The difference between the salinity of the surface water in the wetlands and the salinity of the groundwater was plotted against the latitude using the WGS84 global reference frame (Figure 3-5). The correlation between the average annual rainfall and the distance north was also plotted. At a northing of 5894100, the annual rainfall is 640 mm and the conductivity difference is close to zero. As the distance north increases, average annual rainfall decreases and the difference in salinity increases.

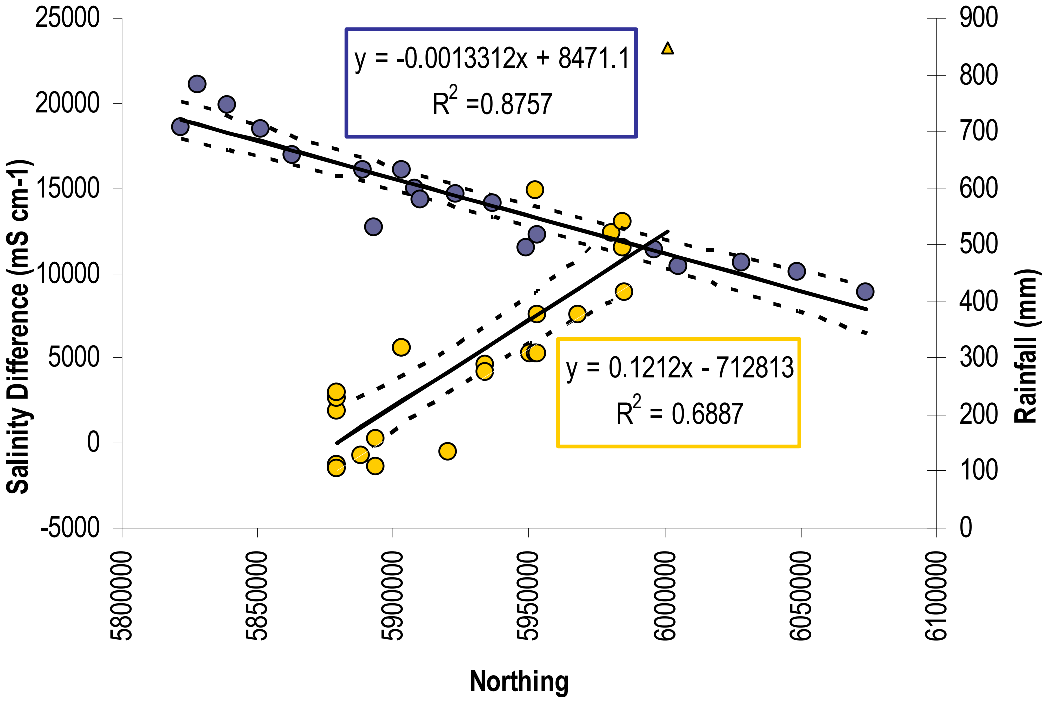


Figure 3-5: Correlation of difference in salinity between surface water in wetlands and the surrounding groundwater and distance north (yellow circles) and average annual rainfall and distance north (blue circles) with the dashed lines indicating the 95% confidence intervals.

3.4 Discussion

3.4.1 Change in species composition

Compared with the pre-2000 surveys, the post-2000 surveys indicate an overall change in species composition. This change is accompanied by a shift from fresher to saltier conditions and from wetter to drier conditions. Species requiring fresh conditions are rarer or not recorded and are replaced by species preferring more saline conditions. These changes in species composition are analogous to those reported in the Wheatbelt of Western Australia (Lyons, et al. 2004); *Azolla filiculoides, Myriophyllum propinqum, Utricularia sp, Spirodela sp* and *Wolffia angustata* which are considered to be salt sensitive (Morris, et al. 2009) and these species have been lost from the wetlands in this study whilst previously un-recorded species that are salt tolerant (*Lepilaena cylindrocarpa, Lepilaena patentifolia, Lepilaena pressii* and *Ruppia tuberosa)* have colonised the wetlands. The quality of water in wetlands in Lower South East region of South Australia such as Pick Swamp, Piccaninnie Ponds, Ewans Ponds, The Marshes and Honans Scrub remains very fresh (<1000 µS cm-1) and these wetlands are a stronghold for freshwater macrophytes in the region (Ecological.Associates 2010) and are therefore very important and valuable sites. The change in species composition in relation to increasing salinity is consistent with the other studies (Brock 1981, Smith, et al. 2009) and work that has documented the reduction in germination, plant growth, biomass and survival at elevated salinities (James and Hart 1993, Macek and Rejmánková 2007, Morris and Ganf 2001, Nielsen, et al. 2003, Salter, et al. 2008, Warwick and Bailey 1997).

A survey of 96 species in the Wimmera district of Victoria found that 79% were negatively associated with increasing salinity (Smith, et al. 2009). Geographically the Upper and Lower South East wetlands are relatively close to the Wimmera district of Victoria where Smith et al. (2009) investigated the relationship between the probability of occurrence of 96 aquatic macrophytes across a salinity gradient that ranged from <1000 to > 100000 µS cm^{-1}. They concluded that two species *Lamprothamnium macropogon* and *Ruppia polycarpa* were positively correlated and 75 were negatively correlated with salinity.

There is evidence that adult freshwater macrophytes can survive at elevated salinities for short periods of time (Goodman, et al. 2010) and germination from seed banks that have been exposed in elevated salinities for short pulses is unaffected (Nielsen, et al. 2007). The loss of plants from the wetland complexes in the South East indicates that salinity effects have not been a once off, short term effect but rather a factor which has been acting over a longer period of time.

3.4.2 Changes in hydrology and comparison of surface water and groundwater salinities

The volume of water flowing through the water courses in the South East and filling wetlands was much less in the period 2000-2009 than it was from 1990-1999. This decrease, coupled with the elevated salinity concentrations recorded in the wetlands in comparison to the surrounding groundwater, supports the processes described in the conceptual model. The reduced volume of water received by the wetlands from 2004 to the end of 2009 was insufficient to flush salt from the sediments. Therefore it is probable that every time the wetland is inundated, salt from the sediment comes out of solution and more salt enters the wetland from the unconfined aquifer. This has caused the concentration of salt in the wetland to increase. The effects of these changes can be seen in the changing floristic composition of wetlands in the South East region; salt sensitive species present pre-2000 are being lost and salt tolerant species are occurring. With the continual accumulation of salts in wetland sediments, increases in salinity may become exponential through time (James, et al. 2009).

Groundwater that is low in salinity has a beneficial impact on wetland ecology which can be diminished in dry periods when groundwater levels, and hence, inflows to wetlands are reduced or even cease. Conversely, if groundwater is saline, and inflows increase due to raised groundwater levels caused by factors such as land use change and river regulation, then this may have a detrimental impact on the ecology of a wetland and its surrounding areas (Jolly, et al. 2008). Whilst a majority of the points indicating the difference in salinity between the surface water and the groundwater fall within the 95% confidence intervals (Figure 3-5) there were some points that were outside. Those that were above the upper 95% confidence interval are wetlands where the difference in the groundwater and surface water salinity is greater than that which would be predicted by the regression line and this may indicate that these wetlands have an increased chance of suffering salinisation. Conversely, wetlands the fall below the lower 95% confidence interval are wetlands that are not at risk of salinisation. A combined approach of soil salinity monitoring (to determine if the salt concentration in the soil is increasing through the processes described in the conceptual model), groundwater salinity and depth monitoring (to determine if the groundwater is close enough to the surface for salt to be drawn to soil surface through capillary action), and monitoring of wetland water quality may give early warning signs of wetlands that are at risk of secondary salinity effects. Currently there is no index to quantify the relationship between the difference in groundwater salinity and wetland salinity and the response of non-halophytes. To build such an index more data indicating the rate at which saline groundwater causes increased salinisation of wetlands would be required. A greater knowledge of the salt tolerance of non-halophytic species would be necessary and subsequent chapters of this thesis address this.

4 Predicting probability of occurrence of wetland plants under elevated salinity regimes

4.1 Introduction

World wide, fresh waters are experiencing declines in biodiversity (Darwall and Vie 2005) far greater than those in the most affected terrestrial ecosystems and if trends in human demands for water remain unaltered and species losses continue at current rates, the opportunity to conserve much of the remaining biodiversity in fresh water systems will vanish (Dudgeon, et al. 2006). Methods used for identifying areas for conservation include: prioritising areas where high levels of human threats and biodiversity coincide (Ricketts and Imhoff 2003); protecting areas because of their unique, rare or endangered flora and fauna (Maltby 1991); identifying `hotspots' such as areas featuring exceptional concentrations of endemic species and experiencing exceptional loss of habitat based on species endemism; and degree of threat (Norman Myers, et al. 2000). It is also important to conserve areas that although might be degraded, support a large proportion of the biodiversity and contribute greatly to available habitat.

In many parts of the world, fresh water is subject to severe competition among multiple human stakeholders (Dudgeon, et al. 2006). In the United States the greatest wetland losses have occurred as a result of agricultural conversion which has accounted for 87% of wetland loss since colonial times, and furthermore agricultural conversion has been the force behind the reduction of wetland area in Europe over a much larger timescale (Maltby 1991, Ricketts and Imhoff 2003). In the South East region of South Australia, land use changes including agriculture and the accompanying drainage schemes and forestry are impacting on wetlands. It is a landscape which is characterised by a strong north-south surface water and groundwater salinity gradient. Based on floristic composition and structure, and water and salinity requirements twenty different wetland types have been described for wetlands in the region (EASSOC. 2009). These range from naturally occurring hypersaline wetlands in the northern areas, with salinities gradually declining with distance south, through seasonal brackish aquatic beds to freshwater emergent sedgelands and aquatic beds. In this region, if only ‘hotspot’ areas or those under direct threat are conserved, it is likely that many of the wetland types currently identified will not be protected and may be lost from the landscape.

Macrophytes contribute greatly to the structural diversity of wetland environments (Reid and Brooks 2000), however most freshwater aquatic plants are intolerant of high salinity levels (Hart, et al. 1991, James, et al. 2003, Nielsen, et al. 2003). Secondary salinisation is an important issue in parts of central and South America, large tracts of northern and southern Africa, the Middle East and central Asia, and many parts of Australia (Williams 1999). In the South East region of South Australia, changes in the salinity and water regime resulting from secondary salinisation, are already affecting the floristic composition of the wetland complexes (Chapter 1, (Taylor 2006). Although the effects of increasing salinisation on aquatic biota have been extensively reviewed, the ecological consequences of salinisation in Australian freshwaters are not well understood (Hart, et al. 1991, Morris, et al. 2009, Nielsen, et al. 2003) and our understanding of the effects of secondary salinisation on the biodiversity of non-riverine wetlands is limited (James, et al. 2003). The effects of even small increases in the salinity of fresh waters can be profound (Williams 1999) and therefore secondary salinisation of freshwater wetlands is likely to markedly and negatively impact upon non-riverine wetland macrophyte assemblages.

A continuation of forestry and associated infrastructure such as pulp mills, continued drainage, agriculture including vineyards and likely climate change effects (Kingsford 2011) will ensure that there are continued changes in both salinity and water regimes in the South East region. Comparisons between survey data collected pre-2000 and post-2000 show that salt sensitive glycophyte species have been lost from some areas in the post-2000 surveys, and that salt tolerant halophyte species that were not present in the pre-2000 are now recorded (Chapter 3). Therefore, tools that can help predict the ecological response to these changes are important. This study will examine the probability of occurrence of species across a salinity range. Smith, et al.(2007) produced curves relating species occupancy at a site to the salinity of that site for species found in the Wimmera region of Western Victoria. This study aims to employ a similar approach so that curves predicting the probability of occurrence in relation to salinity can be produced for species common in wetlands in the South East of South Australia.

4.2 Materials and Methods

4.2.1 Wetland Surveys

In November and December 2009, 26 wetlands in the South East were sampled to assess the occurrence of macrophytes in relation to salinity (Figure 2-1, Table 4-1). In January 2010, nine of the wetlands (those still holding water) were re-surveyed. Where a salinity gradient was present or there existed obvious spatial differences in the macrophyte community within the wetland, multiple sites were surveyed in the wetland. The sample sites were selected to ensure that all the species present at each wetland were recorded. At each site, a twenty metre long quadrat, consisting of twenty 1 m×0.5 m cells was surveyed. In each cell, the presence of macrophyte species was identified and recorded. For each species, the number of cells in each transect containing that species was calculated as a frequency of occurrence (e.g. 4 of 20). Conductivity readings were taken at each site at approximately 10 cm above the soil surface using a TPS WP-81 conductivity meter correct at standard temperature of 25°C. Within each time period, species data from each wetland were pooled and conductivity readings averaged. Using the package PCOrd version 5 (McCune and Mefford 2006) relationships between the plant community and conductivity between November/December 2009 and January 2010 were analysed by NMS ordination.

4.2.2 Probability of Occurrence Curves

A Generalised Linear Model (GLM) is an extension of linear regression but unlike regression models, GLMs can be used when the variance is not constant and the errors are not normally distributed (Crawley 2007). There are three important properties of a GLM: the error structure, which is defined by a probability distribution; the linear predictor, which is the linear sum of the effects of the explanatory variables; and the link function, which relates the mean value of the response variable to its linear predictor (Crawley 2007, Zuur, et al. 2009). For this data set, the response variable (Y_i), was the proportion out of 20 that a species was present in each quadrat. Proportion datasets are strictly bounded as it is not possible to have a proportion greater than one or less than zero and therefore binomial distributions are used to describe the errors.

Taking this into account, the general form for a GLM for proportional data is:

$Y_i \sim B(n_i, \pi_i)$, (describing the binomial error distribution)

$E(Y_i) = \pi_i \times n_i$, (the linear predictor)

logit $(\pi_i) = \alpha_i + \beta_I \times X$ (the link function)

where: n_i is the number of quadrats sampled;

π_i is the probability of success;

$E(Y)$ is the expected value of Y;

α is the intercept parameter (the mean probability of occurrence) and β is the slope parameter (slope of the change in occurrence with 1 unit change in (log) salinity) which are estimated by the model and;

X is the independent variable, in this case salinity (Zuur, et al. 2009).

In this dataset, the independent variable (X), represented salinity and was a fixed effect. The random effect of the wetlands also needed to be accounted for and therefore, a type of GLM called a Generalized Linear Mixed effect Model (GLMM) was used (Crawley 2007, Zuur, et al. 2009).

Probability curves were generated for the 44 species that were recorded, however due to a lack of positive recordings, only 15 of the curves were informative. The curves for the remaining 29 species were uninformative, showing no or little change from a probability of zero with increasing salinity. To produce curves for *Myriophyllum meulleri, Stuckenia pectinatus, Ruppia megacarpa, Ruppia polycarpa, Ruppia tuberosa, Sarcocornia quinqueflora, Triglochin procerum* and *Triglochin striatum,* data points that were responsible for causing false convergence for the curves were removed. In most cases, these data points were all from a wetland in which the species was not found at all, and when this was the case, one data point from that wetland was left in the data set and the others removed.

4.3 Results

The conductivity of the wetlands sampled varied from 350–121700 μS cm^{-1} and 44 macrophyte species were recorded (Table 4-1). In the November/December 2010 surveys a maximum diversity of 32 species was found at Bool and Hacks Lagoon with conductivities ranging from 1440–14000 μS cm^{-1}. The highest diversity in a single quadrat occurred in Rushy Swamp, where a total of 16 species were recorded at a conductivity of 6440 μS cm^{-1} (Figure 4-1a). Nine wetlands in the Marcollat water course ranging in conductivity from 910-4500 μS cm^{-1} contained no aquatic macrophytes and *Lemna minor* was the only species found at Dine Swamp with a salinity 350 μS cm^{-1}. The highest conductivity reading occurred in a quadrat in Bunbury Conservation Park, where three species where recorded; *Lepilaena cylindrcarpa*, *Ruppia tuberosa* and *Sarcocornia quinqueflora*. Except for Morella basin, in all wetlands sampled in both November/December 2009 and January 2010, there was an increase in conductivity from November/December to January. There was a release of water from Morella Basin at the time of the January sample and it is likely that this release was flushing salt from the Basin, resulting in a decrease in conductivity.

Table 4-1: Summary of wetland salinity and diversity where "Total No. Species" is the total number of species recorded across all quadrats in each wetland over both survey periods.

Wetland	Total No. Species	Nov/Dec Conductivity Range (μS cm^{-1})	Jan Conductivity Range (μS cm^{-1})
Schofield Swamp	0	4190	-
Bullocky Swamp	0	3890	-
Little Reedy Swamp	0	3560	-
South Reedy Swamp	0	3220	-
The Muddies	0	1280	-
The Sisters	0	910	-
Park Hill	0	4000	-
Lever Swamp	0	960	-
Jaffray Swamp	0	1480	-
Dine Swamp	1	350	420
Bunbury CP	3	113000 - 121700	-
Bloomfield Swamp	4	530 –610	1250
Pretty Johnnys	5	7560	-
Morella Basin	6	25040 - 27100	19600
Canes Swamp	9	7210 - 7380	-
North Swamp	9	9050 - 10000	-
Reedy Swamp	11	15880 - 16910	-
Lake Hawdon	12	3380 - 4550	-
Snuggery Swamp	14	4760 – 5250	10670-10930
Rocky Swamp	15	4740 – 5280	7370-7410
Big Telowie Swamp	16	5310 – 5840	6880-7090
Wrongway Wetland	16	14940-15960	13470-35400
Rushy Swamp	16	6120 - 6440	-
Smiths Swamp	17	4650 - 5500	8720-8750
Mandina Marshes	20	11740 - 15880	22400-51100
Bool and Hacks Lagoon	32	1440 - 14050	-

The position of the wetlands in the 2-D NMS ordination (stress=16.5) from both time periods is scattered (Figure 4-2). There is no pattern in the movement of wetlands from the November/December 2009 survey to the January 2010 survey. The positioning of the species does not show any correlation with the conductivity vector. Even for wetlands such as Snuggery Swamp, Smiths Swamp, Rocky Swamp and Mandina Marshes, which had the greatest percentage increase in conductivity, there was not a strong correlation with the vector.

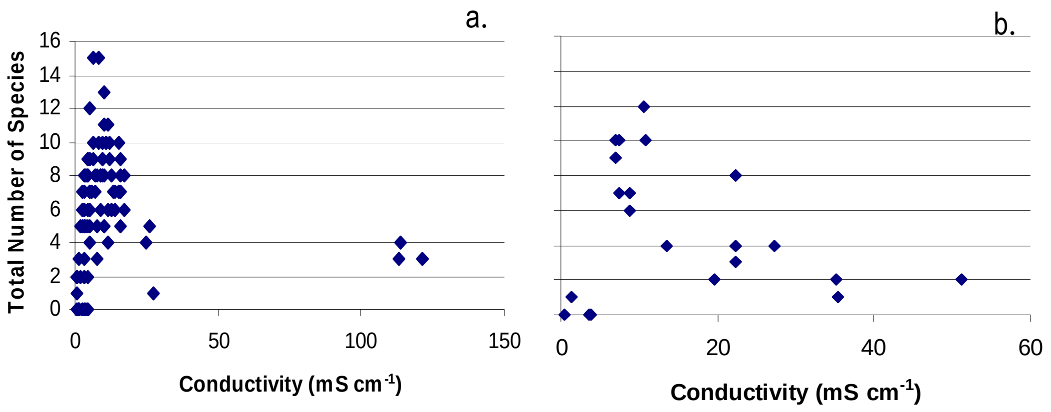


Figure 4-1: Number of species recorded in a single quadrat for wetlands of different conductivities in (a) November and December 2010; and (b) January 2011.

Informative probability curves were produced for 15 out of the 44 species detected in the study (Figure 4-3). The probability curves are the population level predicted mean relationship between salinity and occurrence, thus they represent the mean relationship averaged over all wetlands. The additional random effect variance, which is due to between wetland differences, accounts for mean differences in the occurrence-salinity relationship in the model and this means that the fitted curve would shift up or down (on the logit scale) according to each specific wetland. If a curve were plotted for each wetland (though this is not estimated directly) they should fit the observed data more closely. Similarly, the confidence intervals do not include the between-wetland variance; they are confidence intervals for the population level effect only of salinity.

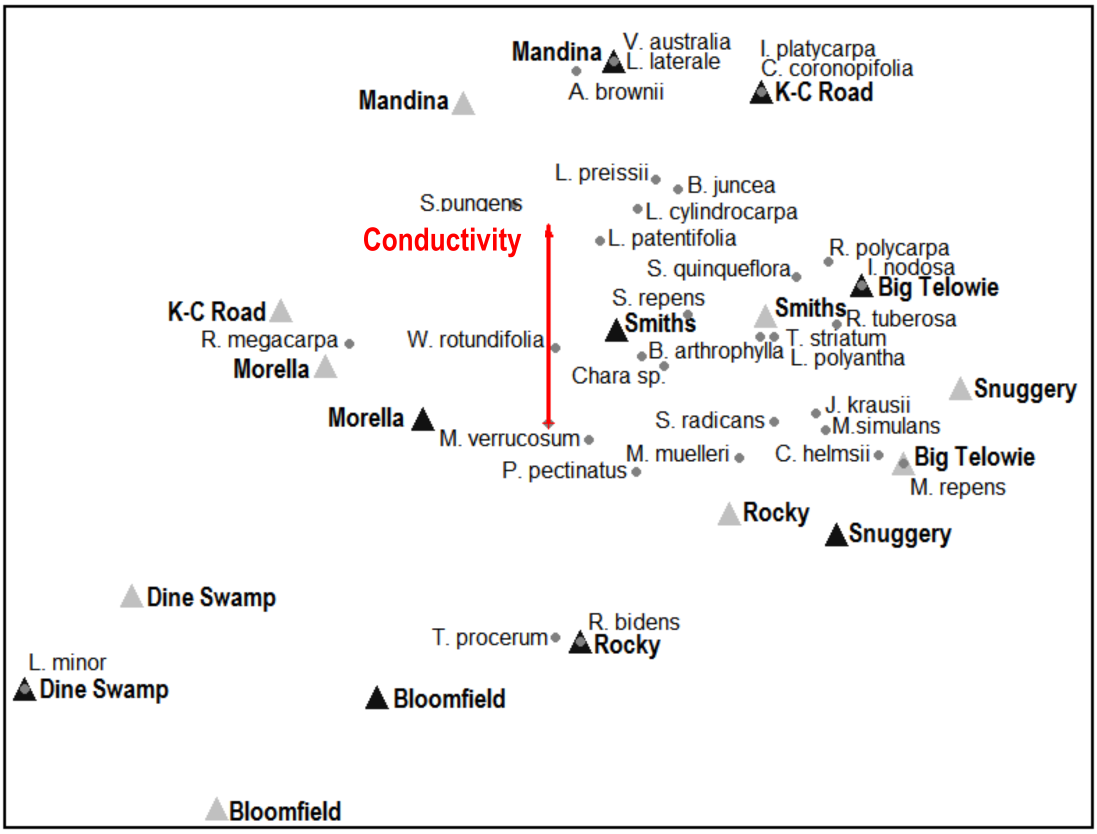


Figure 4-2: 2-D NMS ordination (stress equals 16.5) comparing the plant community of nine wetlands surveyed in November/December 2010 (black triangles) and January 2011 (grey triangles) with the conductivity vector displayed in red.

Lemna minor, *Lileaopsis polyantha*, *Myriophyllum verrucosum*, *Stuckenia pectinatus*, *Ruppia polycarpa*, *Schoenoplectus pungens* and *Triglochin procerum* were all negatively associated with increasing salinity, while *Lepilaena cylindrocarpa*, *Lepilaena preissii*, *Myriophyllum meulleri*, *Ruppia megacarpa*, *Ruppia tuberosa*, *Sarcocornia quinqueflora*, *Selliera radicans* and *Triglochin striatum* were all positively associated with increasing salinity. *Stuckenia pectinatus* and *Ruppia polycarpa* were predicted to occur at salinities between 3000 µS cm^{-1} and 20000 µS cm^{-1} and *Ruppia tuberosa* and *Triglochin striatum* up to 54000 µS cm^{-1}. These species have the broadest range of salinity tolerance.

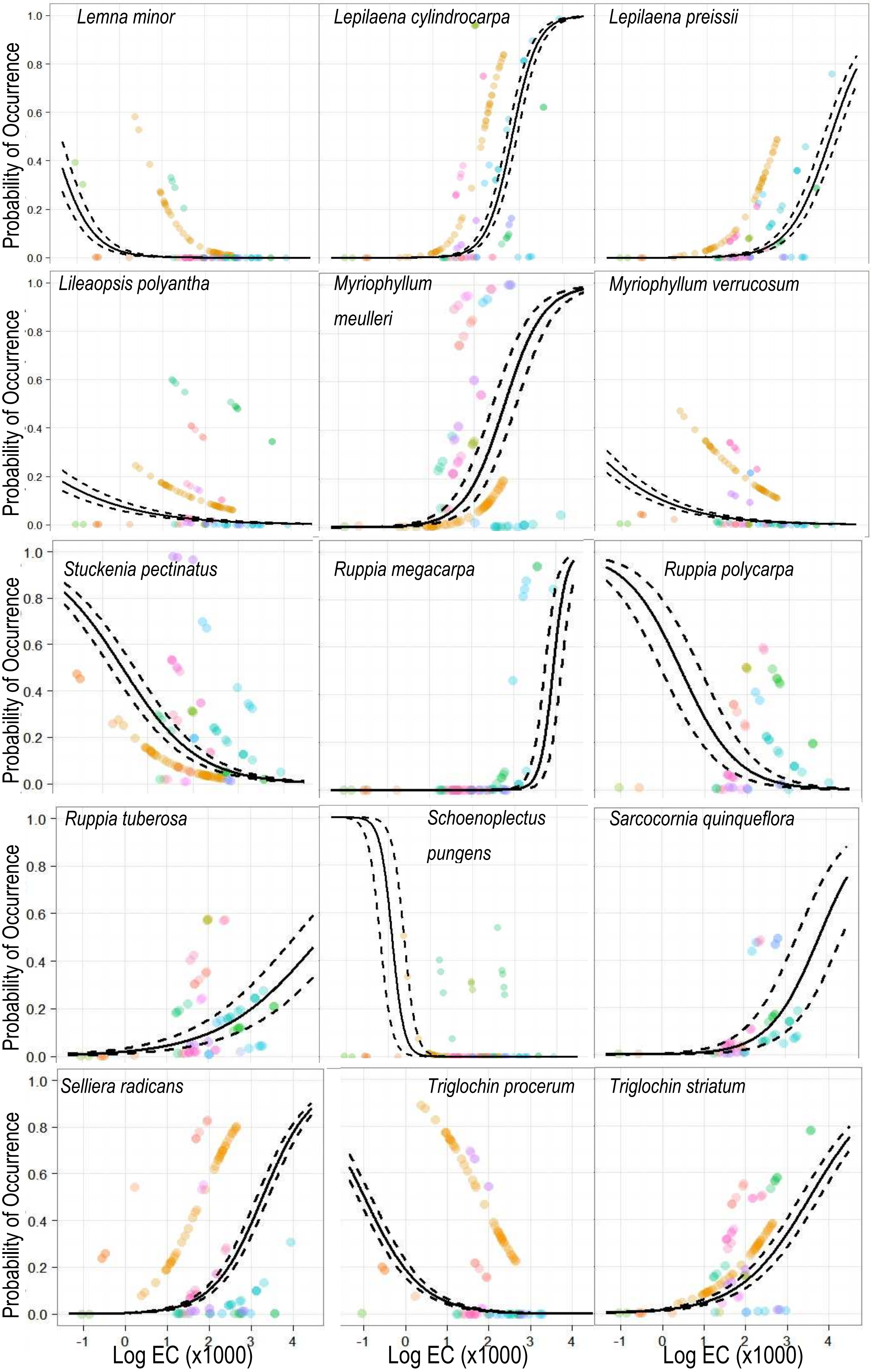


Figure 4-3: Curves predicting the probability of occurrence vs log conductivity for macrophyte species where dashed lines are the 95% confidence intervals and the different colour dots represent different wetlands and the modelled occurrence of the species at those wetlands.

4.4 Discussion

The curves display a wide range in tolerances across the 15 species and highlight the variance due to between wetland differences. The curves predicting the mean relationship between salinity and occurrence (averaged over all wetlands) indicates that *L. minor* and *L. polyantha* are the most salt sensitive species and that they have a probability of occurrence of less than 10% at salinities greater than 600 and 1000 µS cm^{-1} respectively. The response of these species at the scale of the individual wetland, however, indicates that they may be able to tolerate higher salinities (Figure 4-3). For *L. polyantha*, the modelled points (light green dots) indicate that at Wrongway Wetland this species could be found at salinities close to 16000 µS cm^{-1} with a probability of 50%. Similarly, the modelled points predict that *L. minor* can persist at salinities of up to 4500 µS cm^{-1} at Bool Lagoon (orange dots) and Lake Hawdon South (light blue dots) and the Australian salt sensitivity database (Morris, et al. 2009) lists 10000 µS cm^{-1} as the upper salinity tolerance. The salt sensitivity database also lists 10000 µS cm^{-1} as the upper salinity tolerance for *T. procerum* (Morris, et al. 2009), and James and Hart (1993) reported sublethal effects but no deaths at a salinity of 7000 µS cm^{-1}. In this study the curve indicates that *T. procerum* has less than a 10% chance of occurrence at salinities greater than 2700 µS cm^{-1}, but the modelled points from Bool Lagoon (orange dots) and Rocky Swamp (light pink dots) show that in these wetlands there is a 60% chance of finding *T. procerum* at a salinity of 6250 µS cm^{-1}. The curve for *S. pungens* indicates a less than 10% probability of occurrence at salinities above 1600 µS cm^{-1}, yet this species is reported to have a wide salinity tolerance (King, et al. 1990, Smith, et al. 2009). The modelled points for the individual wetlands of Canes Swamp (green dots), Lake Hawdon South (light blue dots on the right) and Wrongway Wetland (light blue dots on the left) support a wide tolerance for this species; at Wrongway Wetland there is a greater than 50% probability of occurrence at a salinity of 12000 µS cm^{-1}.

For all species, the data clearly show a difference between the curves predicting the mean relationship between salinity and occurrence, which is averaged over all wetlands, and the modelled points for the individual wetlands. These differences are particularly large for the salt sensitive species and there could be several reasons for this. This study uses a dataset from 26 wetlands and whilst these wetlands encompass a broad salinity range, they do not allow for replication of salinity conditions at different wetlands. Therefore, if a species is not present at a wetland, in this study it is considered to be as a consequence of the salinity of the surface water in the wetland. As the probability curves are calculated using the average from all the wetlands, in a small dataset the absence of a species at each wetland has a large effect on the calculation of the curves. If a greater number of wetlands with overlapping salinity conditions had been surveyed, the absence of a species at a wetland within a particular salinity range would not have such a large impact. It is also important to consider that the salinity of the surface water in a wetland is not the only factor that determines the distribution of a species. Factors such as soil salinity, water regime (Blanch, et al. 1999, Brownlow 1997, Casanova and Brock 2000, Leyer 2005, Nicol and Ganf 2000), and grazing effects (Blanch and Brock 1994, Nicol, et al. 2007) also have a role.

The halophytic species *L. cylindrocarpa, L. preissii, R. megacarpa, R. polycarpa* and *R. tuberosa,* as well as *T. striatum,* have all been reported to have a positive association with increasing salinity (Brock 1981, Brock 1981, Sim, et al. 2006, Smith, et al. 2009). Reported salinity tolerances are: up to 78000 μS cm^{-1} for *L. cylidrocarpa*, 11000-250000 μS cm^{-1} for *L. preissii,* 20000-350000 μS cm^{-1} for *R. tuberosa* and 7000-72000 μS cm^{-1} for *R. megacarpa* (Brock 1981), and the curves in this study support these salinity tolerances, indicating that the probability of occurrence of these species is high at salinities above 60000 μS cm^{-1}. *Ruppia sp.* and *Lepilaena sp*. have lifecycle patterns and morphology enabling them to survive in ephemeral saline environments; during the aquatic phase they produce large numbers of viable seeds that can survive desiccation and extreme salinity (Brock and Lane 1983). Brock (1981) measured an increase in the proline concentration in the tissue of *R. tuberosa, R. megacarpa* and *R. polycarpa* and found that it occurred with an increase in habitat salinity for all three species. This suggests that proline could make a significant contribution to the cytoplasmic osmotic potential and be a mechanism of salt tolerance within the genus. In contrast to other studies, both *R. polycarpa* and *S. pectinatus* displayed negative associations. Findings by Sim, et al. (2006) showed that the survival of *R. polycarpa* was negatively affected by increased salinity and that 60000-70000 μS cm^{-1} was the critical threshold for adult survival however the curves in this study indicate that the probability of occurrence of *R. polycarpa* is much lower with an upper tolerance of 8000 μS cm^{-1}.

Despite the discrepancies between the averaged curve and the modelled points for the individual wetland, for most species the salinity ranges found in this study fall within those reported in other studies. The differences are most likely a reflection of the field conditions present at time of sampling. Salinities can demonstrate considerable temporal and spatial variability and therefore the reported salinities that organisms have been found at in the field on single occasions may not always accurately describe the salinities they are exposed to over the longer term (Morris, et al. 2009). Observations made at a time when salinities are lower than usual will result in an artificially lower tolerance limit for the existing species (Morris, et al. 2009). Conversely, the maximum field salinity at which a species has been found is reflective of its salinity threshold, but may not be the absolute maximum salinity that the species can tolerate. This may be due to a limited survey effort or lack of suitable habitat at higher salinity sites (Kefford, et al. 2004).

The curves produced in this study support work that suggests that increasing salinity beyond 1500 μS cm^{-1} will adversely affect many freshwater plants and that 6250 μS cm^{-1} is the upper tolerance level for most freshwater species (Nielsen, et al. 2003, Smith, et al. 2009). Unlike the predictions made by Smith, et al. (2009) that the number of macrophyte species would decrease with increasing salinity, in this study, the number of species increased with increasing salinity up to 6440 μS cm^{-1}, after which they declined. Smith, et al. (2009) suggested that the number of species observed in low salinity wetlands was less than the predicted due to species being present but not detected. However, in the low salinity wetlands sampled in this study, such as those in the Marcollat water course, observations of: methane in the sediment; water colour indicative of high dissolved organic carbon; low numbers of phytoplankton; and high numbers of zooplankton were made. These observations indicate that the levels of dissolved oxygen in the water may have been very low. Low dissolved oxygen inhibits germination and this may be why species numbers were low in these wetlands (Leck 1996).

Of the 44 species recorded, curves for 29 of them were uninformative, showing no or little change from zero in probability of occurrence with increasing salinity. This was because these species were only recorded a few times in the surveys, providing insufficient data points to fit a model to. In a study by Brock and Lane (1983), all macrophytes recorded from fresh water were from permanent habitats, not from ephemeral habitats and this may indicate that the freshwater species of submerged macrophytes are in general not adapted to survive fluctuations of salinity and permanence. However, the wetlands surveyed in this study were recovering from a period of extended drought and for many of them, the winter of 2009 was the first they had held surface water in up to eight years. Figure 3-1 indicates that over a 15 year period, conditions have got drier and saltier driving a shift in species composition from species preferring wetter and fresher conditions to those favouring drier and more saline conditions. There was not a consistent pattern in the position of November/December 2009 survey points and the corresponding January 2010 points in the ordination (Figure 4-2). Despite an increase in salinity at all sites except Morella Basin, the points showed no correlation with increasing salinity. As the season progressed and the wetlands dried and salinities increased, the species composition in the wetlands underwent little change. This adds further evidence that the loss of plants from the wetland complexes in the South East is not the result of a ‘one off’ or short term salinity effect but a factor which has been acting over a longer period of time. It also suggests that the wetlands in the South East are ‘slow response’ wetlands for which seasonal variability has a weak impact on the ecosystem properties of stability, resilience and species richness–mean salinity relationship (Jin 2008).

These curves, used in combination with knowledge gained from other studies (eg (Morris, et al. 2009, Smith, et al. 2009) enable salinity thresholds to be set for many of the common species found in the South East region. Employing these thresholds to drain operation will allow wetlands to be managed in a way that will promote the occurrence of target species. In this way, it may be preferable for some of the northern wetlands to have salinities of greater than 20000 $\mu S\ cm^{-1}$ so that salt tolerant species such as *T. striatum, R. megacarpa, R. tuberosa, L. cylindrocarpa* and *L. preissii* can be maintained in the landscape. A target of salinities less than 3000 $\mu S\ cm^{-1}$ may be set for wetlands further south so that appropriate conditions are maintained for species such as *M. verrucosum, L. polyantha, L. minor* and *S. pungens.* This study has only considered the factor of salinity in predicting the occurrence of aquatic macrophytes, however other variables such as dissolved oxygen, dissolved organic carbon and pH will also affect their distribution. The interaction between hydrology and salinity regimes is also likely to have a large affect.

While preservation of intact freshwater bodies and their biodiversity remains a priority, it is important to recognize the potential that partly degraded habitats may have to support significant portions of their original biodiversity (Dudgeon, et al. 2006). While saline systems may not be as species-rich as their ‘fresh’ counterparts, they do possess a distinctive flora and fauna and have retained their ecological function despite their reduced diversity (Strehlow, et al. 2005). The future management and prioritisation of wetland ecosystems needs to strike a balance between conserving the most pristine and non-impacted environments and protecting habitats which maybe degraded but contribute greatly to regional biodiversity; with the goal of sustaining all habitat types in the landscape.

5 Investigating the effects of seasonal evapo-concentration on three aquatic macrophytes

5.1 Introduction

Many Australian aquatic systems are characterised by high temporal variability in dissolved salt concentrations (Hart, et al. 2003), however wide and irregular fluctuations of environmental parameters has made water and salinity regime generalizations difficult (Brock and Lane 1983). In non-modified systems, periods of high flow often coincide with low salinity and low flow with high salinity (Hart, et al. 2003, Nielsen, et al. 2003) resulting in the occurrence of both high-flow flushing events, and occasions of low-flow events in which salt concentrations may exceed thresholds critical for biota (Nielsen, et al. 2003). The alteration of flow through modification of temporal and spatial patterns coupled with secondary salinisation, has diminished this relationship such that, with the continual input of salt but no flushing flows, the concentration of salt in the sediments increases (Nielsen, et al. 2003).

Intermittent and temporary wetlands are particularly vulnerable to salt impacts because of the concentrating of salt in the water column during drawdown and the subsequent build up of salt in the sediment profile (Hart, et al. 1990). Soil salinities can be considerably higher than water column salinities even in waterlogged soil due to transpiration (Salter, et al. 2007). Although plants of temporary wetlands are generally considered to be well adapted to dynamic water regimes, human-induced changes such as secondary salinisation may impose a new set of limits on the ability of a given species to tolerate fluctuating water levels or markedly different wetting and drying cycles (Salter, et al. 2008). Determining impacts of secondary salinisation on aquatic ecosystems has become an increasingly important issue because of the widespread occurrence of secondary salinisation in Africa, the Middle East and central Asia, and many parts of Australia (Williams 1999), particularly in southern Australia (Strehlow, et al. 2005).

Salt stress in plants affects all the major processes such as growth, photosynthesis, protein synthesis and energy and lipid metabolism. The detrimental effects are observed at the whole plant level as death or a decrease in productivity (Parida and Das 2005). It is thought that juveniles are far more intolerant than adults to elevated salinity conditions (James, et al. 2003). However once they are established, plants become increasingly tolerant to salinity during later stages of growth (Maas 1993) and this has important management implications.

To better manage and rehabilitate degraded wetlands, the interactive effects of wetting and drying need to be examined in combination with changes that take place simultaneously in other key environmental variables; in Australia the most important of these is likely to be salinity (Salter, et al. 2008). The aims of this study were; to assess salinity evapoconcentration effects on three species of freshwater aquatic plants; to determine the consequences of longterm exposure to elevated salinity conditions; and to investigate the difference between adult and juvenile salt sensitivity. In order to assist with this goal, *Baumea arthrophylla, Bolboschoenus medianus* and adult and juvenile *Triglochin procerum,* were grown in soil from two wetlands with differing previous salinity history, and exposed to four different starting salinities.

5.2 Materials and Methods

5.2.1 Species and soil description and collection

Three species were investigated: the strap leafed perennial *Triglochin procerum* (both adult and juvenile plants)*;* the slow growing sedge *Baumea arthrophylla*; and the fast growing sedge *Bolboschoenus medianus.* All three species prefer stationary or slow moving water and have an emergent growth form; rooted below the surface of the water with leaves or stems that grow up through the water column to either float or be held above the water's surface (Sainty and Jacobs 2003). Soil was collected from Big Telowie and Snuggery wetlands on the 10th and 11th of November 2008 (Figure 2-1). In October 2008 the salinity of the Big Telowie and Snuggery wetlands were 4000 and 13000 µS cm^{-1} respectively and 5000 and 20000 µS cm^{-1} just before they were completely dry. To minimise disturbance to the soil, it was cut out of the ground in blocks which could be easily slipped into potting bags. *B. arthrophylla* and *B. medianus* were sourced from stands growing in ponds at The University of Adelaide on the 9th and 23rd of December 2008 respectively. *T. procerum* plants and seed were collected from the Laratinga wetlands in Mount Barker, South Australia on the 3rd of December 2008.

5.2.2 *Experimental design*

Plant performance was assessed under four starting salinities; 1500, 6250, 12500 and 18750 µS cm^{-1}, and from soils sourced from two different wetlands; Big Telowie and Snuggery. These salinities were selected because: 1500 µS cm^{-1} is considered freshwater, 6250 µS cm^{-1} is recognised as a threshold salinity above which normally widespread freshwater aquatic macrophytes disappear from wetlands (Brock 1981); 12500 µS cm^{-1} was the starting salinity in Snuggery wetland in 2008 and therefore representative of salinities experienced in the South East region; and 18750 µS cm^{-1} (one third seawater) which is not uncommon in brackish-water wetlands (Salter, et al. 2008). Soil analyses were conducted by CSBP Soil and Plant Analysis Laboratory and are shown in Table 5-1. There was a significant difference in the soil conductivity ($EC_{1:5}$ dry soil:water extract) between the two wetlands; F=36.03, p=0.0039. There were also significant differences in the nitrogen, phosphorus, potassium and sulphur content of the soils, however in order to mitigate these differences, complete nutrient slow release fertilizers Osmocote® and Osmocote Plus® were added to both soils in the proportion of 7:3 to achieve a nutrient loading equivalent to 100 g N m^{-2}. For all species, on the day of collection, fresh weights and leaf (or stem) lengths and numbers were recorded. Individuals were planted directly into the wetland soil. *T. procerum* seed was germinated on damp sand and on the 26th of December 2008 (when the seedlings were approximately 2 weeks old) they were transplanted into the wetland soil.

Table 5-1: Analysis of soil from Big Telowie and Snuggery wetlands

Wetland	Texture	Nitrogen	Phosphorus	Potassium	Sulphur	Org. Carbon	Conductivity	pH
		mg/kg	mg/kg	mg/kg	mg/kg	%	µS cm^{-1}	
Big Telowie	3.5	3.3±0.6	6.7±2.1	412.0±23.3	48.0±19.4	1.9±0.4	800±200	8.2±0.1
Snuggery	2.5	20.7±5.7	16.7±1.2	331.3±31.1	170.3±35.0	1.6±0.4	29000±600	8.0±0.0

To provide replicate salinity treatments, clear rigid PVC chambers, 60 cm× 60cm × 57cm high were used. Four replicate chambers were used for each salinity and wetland soil combination. To allow for possible plant deaths, three individual plants of each species were placed in each chamber; a total of 12 plants per chamber (three adult *T. procerum*, three juvenile *T. procerum,* three *B. medianus* and three *B. arthrophylla*). The 12 pots were a tight fit and as a result, there were minimal gaps between the pots and the bottom of the chamber was effectively filled with soil. The chambers were randomly divided between three outdoor ponds (4.5 m × 3.5 m × 1.2 m deep) in which they were semi-immersed to minimise temperature fluctuations. Seawater was diluted to the required salinity with reticulated water. On the 29th December 2008, water of the appropriate salinity was added to each of the chambers. The chambers were filled to a depth of 18 cm so that the soil was covered with 1-2 cm of water. The water depth was maintained by replacing water lost from the chamber via evaporation with reticulated water. Initially the salinity increased as salt came out of the soil and into solution. Once it had stabilised, the salinity was held constant. Where necessary, salt was added to some chambers to ensure that replicate treatments remained similar to each other. After the 3rd of March 2009, water levels were no longer maintained and due to evapoconcentration occurring under ambient conditions, water levels in the chambers began to decrease and salinity increased. By the end of the experimental period, the water level had dropped to 15-17cm below the soil level.

A data logger recording salinity (temperature adjusted) and depth was deployed in Snuggery wetland from the 19th of September 2008 until the wetland dried on the 20th October 2008. At the start of this period, the wetland was 50 cm deep and had a salinity of 13200 μS cm^{-1}. Over the course of 30 days, the wetland completely dried and the final salinity was 19700 μS cm^{-1}, an increase of more than 30%. The salinity profile recorded in Snuggery was replicated in this experiment. In order to reproduce the 30% increase in salinity over the 30 day time period, salt was added to the chambers as necessary. On the 20th of March, the water level dropped to the surface of the soil and no more salt was added. After this time, water level and salinity fluctuated as a result of the ambient conditions.

Throughout the experimental period, leaf number and length were recorded approximately every three weeks for adult and juvenile *T. procerum* and stem number and length were recorded for *B. arthrophylla* and *B. medianus.* Commencing on the 12th of March 2010 all plants were harvested and the final dry weight biomass was measured. Where more than one plant of each species was present, the final dry weight was averaged. Two soil samples from each pot were collected; a sample from the top 0-5 cm and a sample from 5-10 cm. Samples were oven dried at 65°C to constant weight and the replicates were then ground with a mortar and pestle to <2mm and analysed for electrical conductivity. The electrical conductivity was determined following methods of Slavich and Petterson (1993), whereby soil was overlain with deionised water to 1:5 soil:water. After 24 hrs on an orbital shaker the electrical conductivity was measured ($EC_{1:5}$). Soil was only sampled from pots which had a live plant present at the end of the experiment.

5.2.3 Statistical analysis

The statistical software package JMP IN® (version 4) was used to carry out the statistical analyses. Two-way ANOVA with the factors of salinity regime and soil type and an interaction term (salinity regime × soil type) were used to determine if there were differences in the final dry mass of *B. arthrophylla* and *B. medianus* between treatments. To test the difference between the *T. procerum* treatments, three factors were considered: salinity regime, soil type and lifestage (adult or juvenile), and a three-way ANOVA was used. Two-way ANOVAs with the factors salinity regime and soil type and an interaction term (salinity regime × soil type) were used to determine if there were differences between treatments in the conductivity ($EC_{1:5}$) of the soil at the end of the experimental period. To compare the conductivity ($EC_{1:5}$) of the 0-5 cm soil sample and the 5-10 cm sample within each treatment, a one-way ANOVA was used. For all data, normality was tested using a Shapiro-Wilk test, homogeneity of variance with the O’Brien test and the Tukey HSD test was used to compare means.

5.3 Results

In the first week of the experiment, salinity levels increased as salt came out of the soil and into solution (Figure 5-1). As summer progressed and the water level dropped, the salinity continued to increase. Over the winter period, the water level increased and salinity levels decreased for all treatments. By July 2009, for corresponding salinity treatments, the salinity concentration in soils from Big Telowie and Snuggery were very similar. In the summer of 2009/2010, as evapotranspiration occurred and water levels dropped, salinity again increased. As the water levels continued to drop, the salinity concentration peaked, but not as high as the previous summer, and then reduced rapidly as the water level approached 0 cm.

5.3.1 Survival

For all species, there was generally higher mortality in the 18750 $\mu S\ cm^{-1}$ salinity treatment than in the other salinity treatments. Adult *T. procerum* plants had a higher survival rate in soil from Big Telowie than from Snuggery, and across both wetland soils; the adult plants had higher survival rates than the juvenile *T. procerum* plants (Figure 5-3).

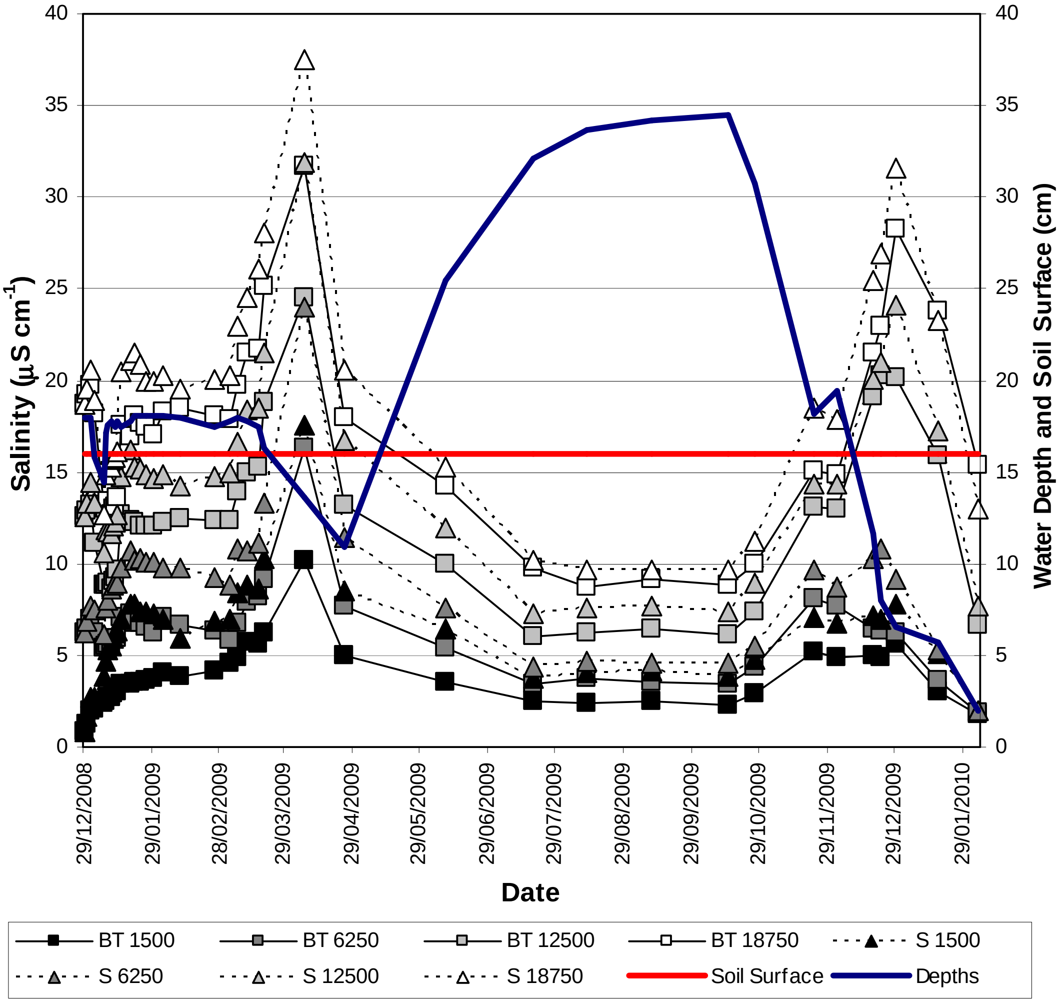


Figure 5-1: Salinity and water depth profile over the course of the experiment where black, dark grey, light grey and white represent 1500, 6250, 12500 and 18750 µS cm^{-1} respectively and the squares and continuous line represent Big Telowie treatments (BT) and the triangles and broken line represent Snuggery treatments (S).

Triglochin procerum

There was a three-way interaction between lifestage, soil type and salinity regime for the number of leaves per plant (Figure 5-2 and Table 5-2). The soil had a significant effect; there were more leaves on plants grown in Big Telowie soil than for those grown in Snuggery soil. Adults and juveniles performed similarly under the salinity regimes of 1500 and 6250 µS cm^{-1} but adults had higher leaf numbers at 12500 µS cm^{-1} and both lifestages had reduced leaf numbers at 18750 µS cm^{-1}. Under 1500, 12500 and 18750 µS cm^{-1}, all plants had less leaves when grown in soil from

Snuggery compared to Big Telowie, except for those in the 6250 μS cm^{-1} salinity treatment where the leaf number stayed constant. Overall, while adults performed better than the juveniles at higher salinities, their ability to do so was moderated by the wetland soil in which they were grown.

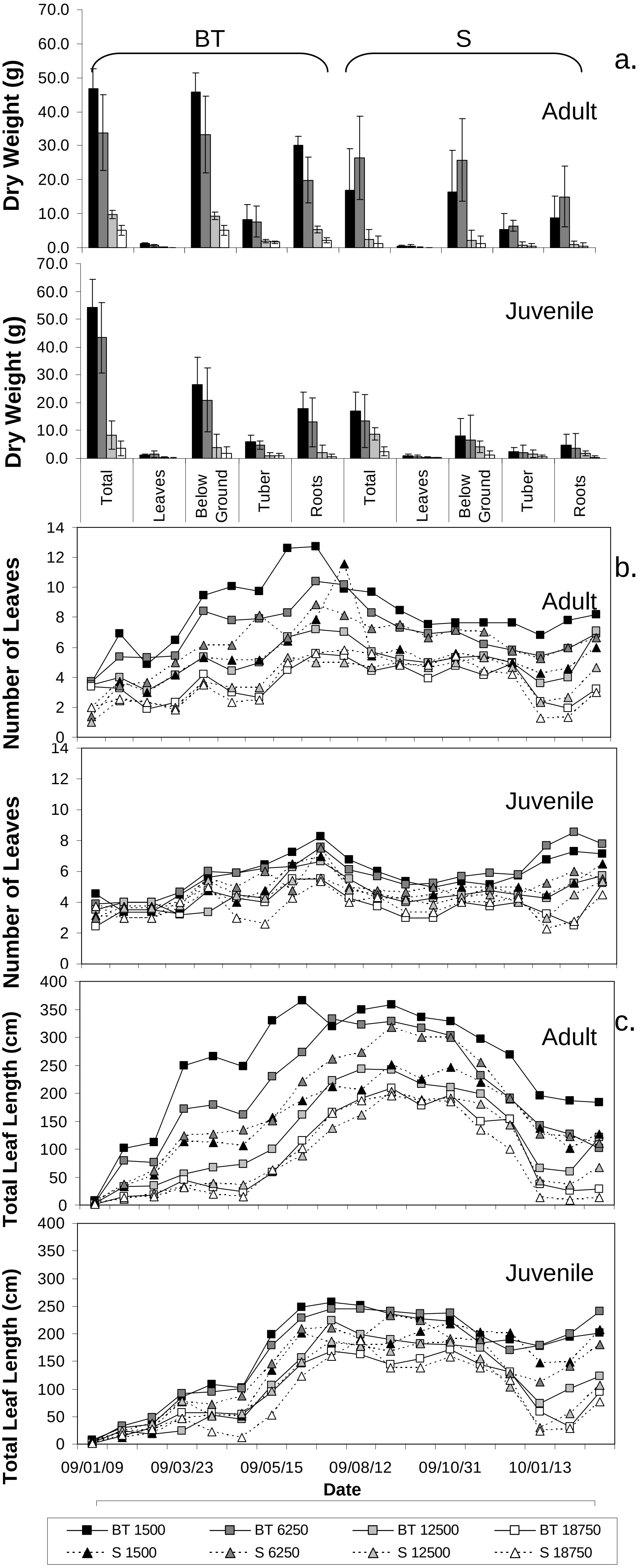


Figure 5-2: Final (a) dry weights of morphological features, (b) number of leaves and (c) total length of leaves for adult and juvenile *Triglochin procerum* exposed to starting salinities of 1500 µS cm^{-1} (black); 6250 µS cm^{-1} (dark grey); 12500 µS cm^{-1} (light grey); and 18750 µS cm^{-1} (white) in soil from two wetlands; Big Telowie (BT) and Snuggery (S), represented by squares and triangles respectively (Table 5-2).

Table 5-2: *F* and *p* values for the three-way ANOVA (salinity regime×soil type×lifestage) for *Triglochin procerum* and the two-way ANOVA (salinity regime×soil type) for the dry weights and measures *Baumea arthrophylla* and *Bolboshoenus medianus.*

	Total		**Leaves**		**Below Ground**		**Tuber**		**Roots**		**Leaf No.**		**Leaf Length**	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Triglochin procerum														
Salinity	37.23	**<0.0001**	12.22	**<0.0001**	37.60	**<0.0001**	17.59	**<0.0001**	39.36	**<0.0001**	10.09	**<0.0001**	13.59	**<0.0001**
Soil	30.27	**<0.0001**	6.76	**0.012**	30.94	**<0.0001**	7.13	**0.01**	36.18	**<0.0001**	9.22	**0.0039**	5.97	**0.018**
Lifestage	17.87	**0.0001**	2.79	0.10	20.26	**<0.0001**	9.12	**0.004**	17.34	**0.0001**	1.34	0.25	2.69	0.11
Salinity×Soil	7.11	**0.0005**	1.37	0.26	7.36	**0.0004**	1.27	0.29	10.04	**<0.001**	0.4	0.75	0.52	0.67
Salinity×Lifestage	3.85	**0.015**	0.41	0.75	4.31	**0.0091**	2.39	0.08	3.71	**0.018**	0.54	0.66	0.4	0.75
Soil×Lifestage	0.87	0.35	0.061	0.81	0.98	0.33	0.018	0.89	1.03	0.314	1.11	0.3	0.0066	0.94
Salinity×Soil×Lifestage	1.19	0.32	1.79	0.16	1.11	0.35	0.43	0.73	1.31	0.28	3.73	**0.017**	2.73	0.054
Baumea arthrophylla														
	Total		**Stem**		**Below Ground**		**Rhizome**		**Roots**		**No. Stems**		**Stem Length**	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Salinity	9.89	**<0.001**	12.75	**<0.001**	9.95	**<0.001**	11.90	**<0.001**	8.45	**<0.001**	26.16	**<0.001**	21.02	**<0.001**
Soil	6.76	**0.016**	6.61	**0.017**	7.17	**0.013**	8.62	**0.007**	6.02	**0.022**	7.85	**0.01**	7.99	**0.009**
Interaction	0.58	0.64	0.46	0.71	0.49	0.70	0.37	0.78	0.67	0.58	1.60	0.21	0.51	0.68
Bolboschoenus medianus														
	Total		**Shoot**		**Below Ground**		**Corm**		**Roots**		**No. Shoots**		**Shoot Length**	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Salinity	12.94	**<0.001**	10.94	**<0.001**	11.86	**<0.001**	14.19	**<0.001**	5.31	**0.006**	5.73	**0.004**	9.99	**<0.001**
Soil	0.22	0.65	0.99	0.33	0.10	0.76	0.15	0.70	0.10	0.76	0.03	0.87	2.22	0.15
Interaction	1.48	0.25	0.63	0.60	1.57	0.22	1.41	0.26	1.04	0.39	1.02	0.40	0.57	0.64

The interactive effect of salinity regime×lifestage and salinity regime×soil type was significant for the total, below ground and roots dry weights. In all cases the salinity regime×lifestage interaction resulted in similar dry weights for both adults and juveniles at 12500 and 18750 µS cm^{-1} but much higher dry weights for the adult plants than for the juveniles at 1500 and 6250 µS cm^{-1}. Overall, the salinity regime×soil type interaction resulted in higher dry weights for plants grown in Big Telowie soil, than for those grown in Snuggery soil. However, in Big Telowie soil, plants grown at 1500 µS cm^{-1} had the largest dry weight but in the Snuggery soil, plants at 6250 µS cm^{-1} had the largest dry weight.

5.3.2 Baumea arthrophylla

For this species there was no interaction between the primary factors. Wetland soil and salinity had significant effects for all measures (Figure 5-3, Table 5-2). The total, stem and roots dry weights from the 1500 µS cm^{-1} treatment were significantly higher than those from the 12500 and 18750 µS cm^{-1} treatments, and the 18750 µS cm^{-1} treatment which was the lowest, was only similar to the 12500 µS cm^{-1} treatment. The 6250 µS cm^{-1} salinity treatment is transitional; the dry weights from this treatment are not significantly different from those in the 1500 and 12500 µS cm^{-1} treatments. The dry weights of the rhizomes and the combined below ground structures responded differently to the effect of salinity regime. The dry weights from the 1500 and 6250 µS cm^{-1} treatments were significantly heavier than those in the 12500 and 18750 µS cm^{-1} salinity treatments. The response of stem number and length is similar; plants grown in the 1500 and 6250 µS cm^{-1} treatments had a greater number of stems with a longer total length, than those grown in the higher salinity regimes.

5.3.3 Bolboschoenus medianus

The wetland soil the plants were grown in did not have a significant effect on any of the dry weight measures or the shoot length and number and there were no interactions. Salinity regime had a significant effect on all dry weight measures and the shoot length and number (Figure 5-4, Table 5-2). For the total, shoot and below ground dry weights, plants grown at 1500 µS cm^{-1} had the highest dry weights and were only similar to those at 6250 µS cm^{-1}. Those grown at 12500 µS cm^{-1} were similar to plants from both the 6250 µS cm^{-1} and the 18750 µS cm^{-1} treatment. This response was mirrored in the final total shoot length where there was a distinct difference with the longest total shoot length recorded for plants in the lowest salinity treatment and plants in the highest salinity treatment recording the shortest total shoot length. For the corm dry weight, the salinity effect resulted in those grown at 6250 µS cm^{-1} being similar to both the 1500 and 12500 µS cm^{-1} treatments but those grown at 18750 µS cm^{-1} were significantly different to all the others. For the roots and the shoot number, the significant salinity effect indicated that the 6250 µS cm^{-1} salinity treatment was similar to both the 1500 and 12500 µS cm^{-1} treatments while the 18750 µS cm^{-1} treatment was significantly different to all but the 12500 µS cm^{-1} treatment.

5.3.4 Soil Salinity

A 2-Way ANOVA was used to determine if the salinity regime or the wetland soil influenced the amount of salt deposited in the soil as the water level dropped Table 5-3). For all of the species the salinity regime had a significant impact on the concentration of salt left in the top 5 cm of soil in the pots (F=10.73, p<0.001 for adult *T. procerum*, F=5.14, p=0.017 for juvenile *T. procerum*, F=9.29, p<0.001 for *B. artrhophylla* and F=15.20, p=<0.001 for *B. medianus*). For adult *T. procerum* and *B. arthrophylla* and *B. medianus*, soil from the 1500 and 6250 µS cm^{-1} salinity treatments had the lowest conductivity. Soil salinity of the 12500 and 18750 µS cm^{-1} treatments were significantly higher. For juvenile *T. procerum*, the salinity effect resulted in the 62500 µS cm^{-1} treatment being similar to both the 1500 (which had the lowest soil conductivity) and 12500 µS cm^{-1} treatments but the conductivity of soil from the 18750 µS cm^{-1} treatment was significantly higher than all the others. *B. medianus* was the only species for which wetland soil did have a significant effect (F=9.55, p=0.005); for each salinity treatment, the soil conductivity from pots containing Big Telowie soil was higher than those containing Snuggery soil. There were no interactions.

For all species in all salinity treatments, there was a significant difference between the soil salinity in the 0-5 cm layer of soil and the 5-10 cm layer of soil (Table 5-3). The soil in the 0-5 cm layer was significantly higher in cases.

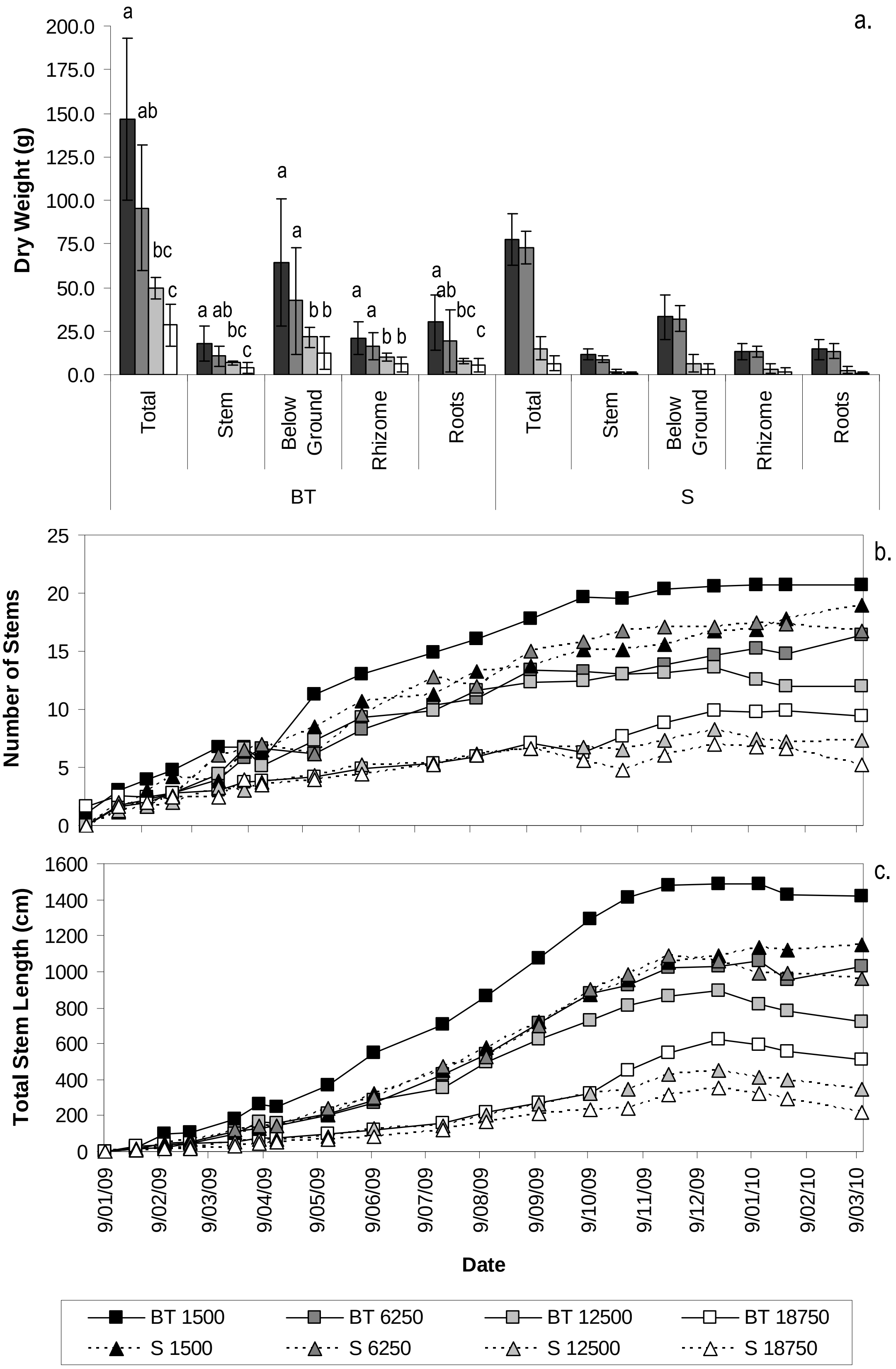


Figure 5-3: Final (a) dry weights of morphological features, (b) number of leaves and (c) total length of leaves for *Baumea arthrophylla* exposed to starting salinities of 1500 µS cm^{-1} (black); 6250 µS cm^{-1} (dark grey); 12500 µS cm^{-1} (light grey); and 18750 µS cm^{-1} (white) in soil from two wetlands; Big Telowie (BT) and Snuggery (S) (Table 5-2).

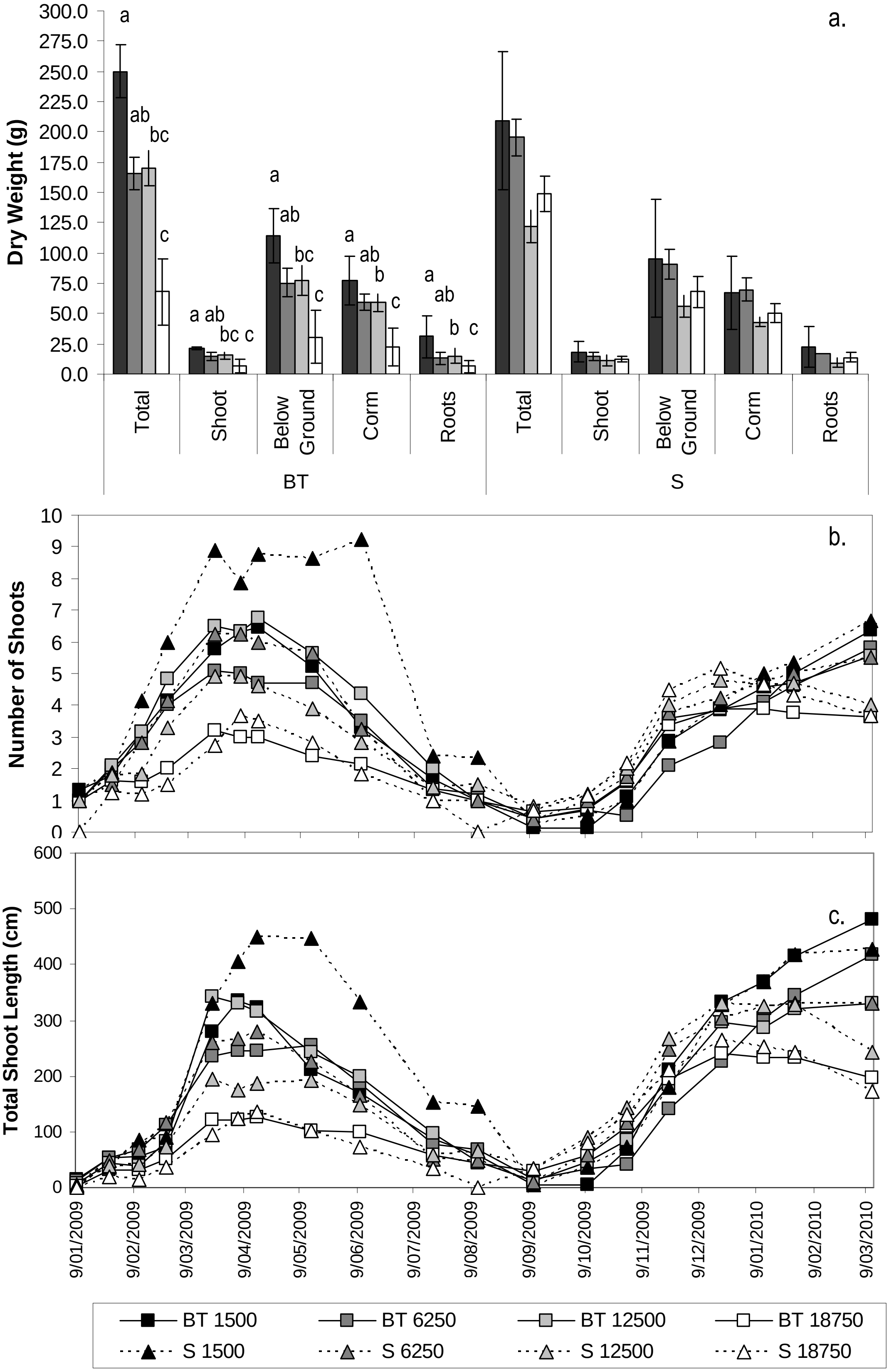


Figure 5-4: Final (a) dry weights of morphological features, (b) number of leaves and (c) total length of leaves for *Bolboschoenus medianus* exposed to starting salinities of 1500 µS cm^{-1} (black); 6250 µS cm^{-1} (dark grey); 12500 µS cm^{-1} (light grey); and 18750 µS cm^{-1} (white) in soil from two wetlands; Big Telowie (BT) and Snuggery (S) (Table 5-2).

Table 5-3: Results of one-way ANOVA comparing 0-5cm sample to the 5-10cm sample for each salinity treatment

Species	Salinity (µS cm^{-1})	F	P
Triglochin procerum - Adult	1500	15.02	0.0017
	6250	13.38	0.0026
	12005	7.66	0.015
	18750	31.80	0.0013
Triglochin procerum - Juvenile	1500	8.70	0.012
	6250	7.76	0.015
	12005	3.89	0.069
	18750	10.55	0.018
Baumea arthrophylla	1500	15.35	0.0015
	6250	5.98	0.028
	12005	10.22	0.0065
	18750	9.83	0.0073
Bolboschoenus medianus	1500	7.38	0.017
	6250	5.98	0.028
	12005	15.23	0.0016
	18750	6.07	0.027

5.4 Discussion

5.4.1 Depth-Salinity Regime

The distinctive inverse relationship between water depth and salinity in this experiment is similar to those recorded by (Strehlow, et al. 2005) in Western Australia lakes and reflects the pattern described by Nielsen et al. (2003). The lower salinity peak in the second summer compared with the first indicates that salt was lost from the water column, most likely to the sediments and /or incorporated into plant biomass to balance to the loss of salt from the water column as suggested by James, et al. (2009). The bathymetry of each wetland is different and it is this which dictates the depth-salinity relationship and hence the rate and degree of salinity increase experienced as the wetland dries. The period of acclimation can influence a plants salinity tolerance; generally, the longer an animal has been acclimated to a particular salinity regime, the more tolerant it becomes (Hart, et al. 1991). In this study the rate of salinity increases was modelled on the salinity changes observed at Snuggery wetland as it dried up during September and October 2008, but it is important to recognise that this rate of change will be different at each wetland and consequently the response of the plants may be different.

5.4.2 Salinity Treatment

There are many examples of the sub-lethal affects of salinity exposure on aquatic macrophytes and in particular, the reduction in leaf or shoot length and number (James and Hart 1993, Macek and Rejmánková 2007, Morris and Ganf 2001, Salter, et al. 2008, Warwick and Bailey 1997). Munns and Termaat (1986) state that under elevated salinities, root growth is almost always less affected than shoot growth and so the root:shoot ratio is increased. *Vallisneria australis* plants submerged at a salinity of 18 mS cm^{-1} showed a decline in leaf lengths but an increase in the number of ramets per pot (Salter, et al. 2008). *Bolboschoenus medianus* has been shown to reallocate biomass in response to salinity by producing fewer leaves and shorter culms and simultaneously increasing tuber biomass (Morris and Ganf 2001). They reported that under a salinity of 13 mS cm^{-1} and a nutrient loading of 100 g N m^{-2}, below ground biomass accounted for 64% of the total biomass. In this study, the percentage of biomass allocated to below ground structures was < 95, < 90, < 75 and < 80% for adult and juvenile *T. procerum, B. arthrophylla* and *B. medianus* respectively, across all salinity treatments suggesting that long term exposure to elevated salinity conditions results in a large investment in below ground biomass by all species. This is consistent with work done by Salter, et al. (2008) who suggest that the shift in biomass allocation by *Vallisneria australis* from leaf growth to vegetative reproduction may represent a tolerance mechanism, which would allow it to persist in saline environments.

The earliest response to salt stress is a reduction in the rate of leaf expansion (Munns 1993)and this was seen in adult and juvenile *T. procerum* and in *B. medianus* at all salinity levels in the first season. Growth of leaves and shoots resumes when the stress is relieved (Parida and Das 2005) and this was seen in across all salinity treatments for all species when the water level increased and salinity was reduced. In the second season, as the salinity level increases, reduction in the leaf or shoot length and number only occurred in the 12500 and 18750 µS cm^{-1} treatments fro juvenile *T. procerum* and *B. medianus* and there was no reduction in stem length of number for *B. arthrophylla* for any of the treatments. This may indicate increased salinity tolerance.

Salinity effects occur at various stages in the life history of a plant (Hart, et al. 1991, James, et al. 2003, Nielsen, et al. 2003). Studies on the effects of salinity have generally focused on impacts on adult life stages, which are potentially the most tolerant life stages (Nielsen, et al. 2003). This study shows that the initial lifestage at exposure to the salinity regimes had a significant effect on the final dry weights of the *T. procerum* plants. The differences in the dry weights and leaf length and number were greatest between adults and juveniles at 1500 and 6250 μS cm^{-1}, with the adults having much larger weights and measures. At higher salinity, adults and juveniles performed similarly and this suggest that the juveniles are only more salt sensitive than the adults at lower salinities. At salinities greater than 6250 μS cm^{-1}, the sensitivities of adults and juvenile *T. procerum* plants are very similar.

5.4.3 Wetland Soil and Salinity Interaction

For both adult and juvenile *T. procerum* and *B. arthrophylla,* almost all dry weight and morphological measures were significantly affected by the wetland soil they were grown in with plants grown in soil from Big Telowie having higher dry weights. The major difference between the two soils was the initial salt content. Despite both soils having the same starting salinity treatments imposed, this difference caused salinity concentrations to be higher in treatments with soil from Snuggery than in Big Telowie for a majority of the experimental period. However by July 2009, the salinities for both soils were very similar. Therefore, the effect of soil really indicates that even when exposed to the same salinity conditions for a growing season, the elevated salinity effects of the previous season will still result in plants with a reduced biomass.

The interaction between salinity regime and wetland soil was only prominent in dry weight measures for *T. procerum* and this interaction is reflective of the effect that the soil salt had on the salinity regimes.

5.4.4 Soil Salinity

Increases in salinity may become exponential through time due to the continual accumulation of salts in wetland sediments (James, et al. 2009, Nielsen, et al. 2003). The results from this study support this with the highest soil salt concentration occurred in the top (0-5cm) of soil in the highest salinity treatment for all species. The significantly higher soil salt concentrations in the top layer of soil than in the 5-10 cm layer support the conceptual model outlined in Chapter 1 in which the process of capillary action continues to bring salt to the surface even once the water level has dropped below the surface of the soil. Watt, et al. (2007) have recorded the effects of this in a seasonally flooded Mediterranean wetland. They observed that although the distribution of macrophytes and the emergent vegetation was most likely influenced by the direct effects of water levels rather than by soil salinity, brackish assemblages were found where water levels were near the surface in summer and autumn leading to increased salinity.

6 The response of freshwater plants to salinity pulses

6.1 Introduction

Despite being recognised as areas of ecological complexity and conservation importance (Davis, et al. 2006), wetlands continue to be among the world's most threatened ecosystems (Zedler and Kercher 2005). One of the threats to wetlands is salinisation, which is the process by which the concentration of solutes, such as the dissociated cations Na^+, K^+, Ca^+ and Mg^+, and the anions Cl^-, SO_4^-, NO_3^-, HCO_3^-, and CO_3^{2-}, increases. As salinity increases, biota become increasingly stressed, resulting in reduced growth and reproduction (Sim, et al. 2006) and ultimately death (Kefford, et al. 2007, Nielsen, et al. 2003), leading to a decline in species richness (Hart, et al. 1990). Aquatic systems which comprise rivers, floodplains, riparian zones and wetlands, are going to be the most severely affected by salinisation as they occupy the lowest areas in the landscape where salt can accumulate (Brock, et al. 2005, Hart, et al. 2003, James, et al. 2003, NLWR 2001, Walker, et al. 2002)

While salinisation occurs due to natural processes (Ghassemi, et al. 1995), the rate of salinisation has increased significantly as a result of human activity, such as land clearance (Cramer and Hobbs 2002, Halse, et al. 2003, Hart, et al. 1991) and irrigation (Eamus, et al. 2006). The impact of anthropogenic discharges of saline water into wetlands is of particular concern. Such discharges are common in landscapes affected by waterlogging and dryland salinity, in which extensive drainage networks have been constructed to collect saline groundwater (Tanji and Kielen 2002). While this water can potentially be reused for agriculture, cooling of power plants, aquaculture, agroforestry or salt harvest from evaporation ponds (Ghassemi, et al. 1995, Tanji 1990), conditions are often unsuitable for these reuse options and disposal to existing water bodies is the most widely used practice (Lee 1990). Consequently, resource managers responsible for discharging saline water into wetlands require information on the response of biota to increasing salinity in order to develop management regimes that will minimise salinity damage to sensitive species (Warwick and Bailey 1998).

It is known that freshwater ecosystems undergo little ecological stress when subjected to salinities up to 1500 $\mu S\ cm^{-1}$ (Hart, et al. 1991). At salinities above 1500 $\mu S\ cm^{-1}$ freshwater macrophytes have reduced growth rates and reduced development of roots and leaves (Nielsen, et al. 2003) and field surveys have shown that normally widespread freshwater macrophytes are no longer found at salinities of around 6250 μS cm^{-1} (Brock 1981). However, while there have been many studies on the impact of increased salinity on the growth and distribution of freshwater macrophytes, specific knowledge about how to manage the timing, concentration, frequency and duration of releases of salt water in order to minimise the impact on aquatic biota is scarce (James, et al. 2003, Nielsen, et al. 2003) and even less is known about plant recovery after salinity stress (Howard and Mendelssohn 1999).

In this paper, the survival, salinity tolerance and recovery of selected wetland species that are subjected to a pulse of saline water is assessed for the Upper South East region (USE) of South Australia, which is bounded by the towns of Salt Creek (36°12'S, 139°65'E), Keith (36°11'S, 140°37'E), Naracoorte (36°95'S, 140°75'E) and Kingston SE (36°84'S, 139°86'E) and covers an area of over 1 million ha. Only 7% of the original area of wetlands in the region remain and are of high conservation value (USEDS&FMP 1993). The wetland complexes are ephemeral systems that are often dry during summer, and historically they were filled by freshwater during the winter; the majority of which was generated from surface runoff.

In recent years, the wetlands have been isolated from their original source of water by a 650 km network of drains constructed to mitigate the threat of water logging and secondary salinisation. However, flow in the drains is regulated, enabling water in the drains to be discarded to sea or to be directed into the wetlands. Consequently, the drainage discharge has the potential to be of ecological benefit to the wetlands, depending on its salinity. From a management perspective, there is a need to assess the impact of a pulsed discharge of saline drainage water of varying concentrations and durations on key wetland species in an effort to determine how to make best use of the scarce water resources in the region (e.g. is it more beneficial for the wetlands to discharge saline drainage water of a certain concentration to the ocean or to divert it to the wetlands for a certain period of time?).

In this study, *Baumea arthrophylla* Nees. Boeckeler, *Triglochin procerum* R. Br, *Myriophyllum simulans* Orch. and *Cotula coronopifolia* L., were exposed to a pulse of saline water at two salinities, for two durations and their subsequent recovery assessed. Plants were chosen to represent functional groups (Blanch, et al. 1999) inhabiting different areas in the wetlands, and based upon their presence and vulnerability to salinity in wetlands in the USE of South Australia. To address these two salinity effects, the survival, salinity tolerance and recovery of the selected species, subjected to a pulse of saline water were assessed on the following two levels: (a) their overall survival and gross growth parameters including relative growth rates and biomass; and (b) the effect of salinity on individual plant components, including biomass of leaves, stems, roots and tubers and morphological measures. The ‘functional equilibrium’ concept as described by Brouwer (1983) (Poorter and Nagel 2000, Van der Werf and Lambers 1996), will be used to see if a six week salinity pulse followed by a four week recovery period alters the way in which the plants allocate biomass to the photosynthetic organs.

6.2 Materials and Methods

6.2.1 Species description and collection

Baumea arthrophylla is a rhizomatous emergent perennial sedge with cylindrical photosynthetic stems which grow 1-2 m in height (Jessop and Toelken 1986), able to withstand extended periods of flooding and drying. *Triglochin procerum* is a tuberous perennial emergent with strap like leaves up to 2 m in length inhabiting stationary or slow moving water bodies (Sainty and Jacobs 2003). *Myriophyllum simulans* is a submerged perennial with long trailing stems and whorled leaves and emergent flowering stems and is found in all mainland states of Australia in fresh or brackish water bodies (Sainty and Jacobs 2003). *Cotula coronopifolia* is a herb land species often found on flooded soils with bright yellow button-like flowers and can be found in fresh water and brackish tidal areas (Romanowski 1998). All species were collected from the field in September 2007. *Baumea arthrophylla* was collected from Bool Lagoon in the south east of South Australia, *T. procerum* was collected from a disused treatment wetland in Willunga, South Australia and *M. simulans* and *C. coronopifolia* were collected from Tolderol Game Reserve near Lake Alexandrina, South Australia. Plants were potted in tubes in sandy-loam; individual rhizomes of *B. arthrophylla* and *T. procerum,* and stem segments of *M. simulans* and *C. coronopifolia* and allowed to establish.

6.2.2 Experimental design

Plant performance under three conductivity regimes was examined; control (<1500 μS cm^{-1}), 6250 μS cm^{-1} and 12500 μS cm^{-1}. Plants were exposed to salinity pulses of either three weeks and six weeks (hereafter termed 3 or 6 week exposure), followed by a four week recovery in water with a conductivity of less than 1500 µS cm^{-1}. Sea water diluted with reticulated water was used to adjust conductivities. Conductivities of 6250 µS cm^{-1} correspond to the threshold salinity for aquatic macrophytes (James and Hart 1993), and the conductivity of groundwater drains in the USE of South Australia is commonly 12500 µS cm^{-1}. Initial fresh mass of the plants were taken and they were re-potted into bags 19 cm x 19 cm x 20 cm filled with loam containing Osmocote® and Osmocote Plus®, which are slow release, complete nutrient fertilisers in the proportion of 7:3 to achieve a nutrient loading equivalent to 30 g m^{-2} of nitrogen (Morris and Ganf 2001), and topped with clay. For each species, 84 plants were established under fresh (<1500 µS cm^{-1}) water logged conditions for four weeks before the salinity treatments were imposed. At the end of the establishment period, 12 plants of each species were randomly selected and harvested for initial dry mass so that a relationship between leaf length and dry mass could be determined to enable the estimation of the starting dry mass of the remaining plants. The remaining 72 plants were randomly distributed between the eight salinity-duration treatments, each with four independent replicates, with three plants per replicate. At the end of the salinity pulse exposure (either three or six weeks), for each salinity treatment, one plant from each of the four replicates was harvested leaving two plants per replicate. The treatments were freshened to less than 1500 µS cm^{-1} using reticulated water, with the water depth being maintained. The treatments were allowed a four week recovery period after which the remaining two plants were harvested. At harvest, final morphological and biomass measures were taken.

To isolate the different salinity-duration treatments, clear rigid PVC chambers, 60 cm × 60 cm × 57 cm high were used. These were placed in two outdoor ponds (4.5 × 3.5 × 1.2 m deep). Two of the four replicates treatments were assigned to each pond and the chambers arranged randomly within the ponds. The chambers were semi-immersed within the ponds to minimise temperature variations. It was assumed that the salinity of the soil pore water was the same as that of the surrounding water as the plant pots were perforated with numerous holes to enable the free exchange of ions between the sediment and external medium, and sand in which the plants were grown is a coarse and permeable soil. A maximum depth of water of 15 cm above soil level for *B. arthrophylla, T. procerum* and *M. simulans* was imposed. *Cotula coronopifolia* was raised such that the maximum inundation that it experienced was five cm above soil level. This was done to ensure that any stress response would be due to changes in salinity and not caused by an extreme water regime.

Morphological measures including stem or shoot length and number of new stems or shoots were taken at approximately 10 - 14 day intervals.

6.2.3 Growth analysis during exposure and recovery phase

The relative growth rate (RGR) was determined using the following formula (Harper 1977):

$$RGR = \frac{\ln(W_2) - \ln(W_1)}{\Delta T} \ ,$$

Equation 2

where W_1 and W_2 represent plant dry mass (g) at times 1 and 2 respectively and ΔT represents the difference between times 1 and 2 (days). *Triglochin procerum* has a high leaf turn over rate and so the RGR for this species was calculated including the mass of the turned over leaves but the final biomass was not.

For the three and six week exposure treatments, in order to calculate the RGR it was necessary to estimate the dry mass of plants at the start of the experimental period (time 1). This was achieved using the relationship between leaf length and dry mass for *T. procerum* and stem length and dry mass for the other species (Figure 6-1). As there were no differences between the leaf length and dry mass relationship between the salinity treatments, data from all harvested plants were included in the regression.

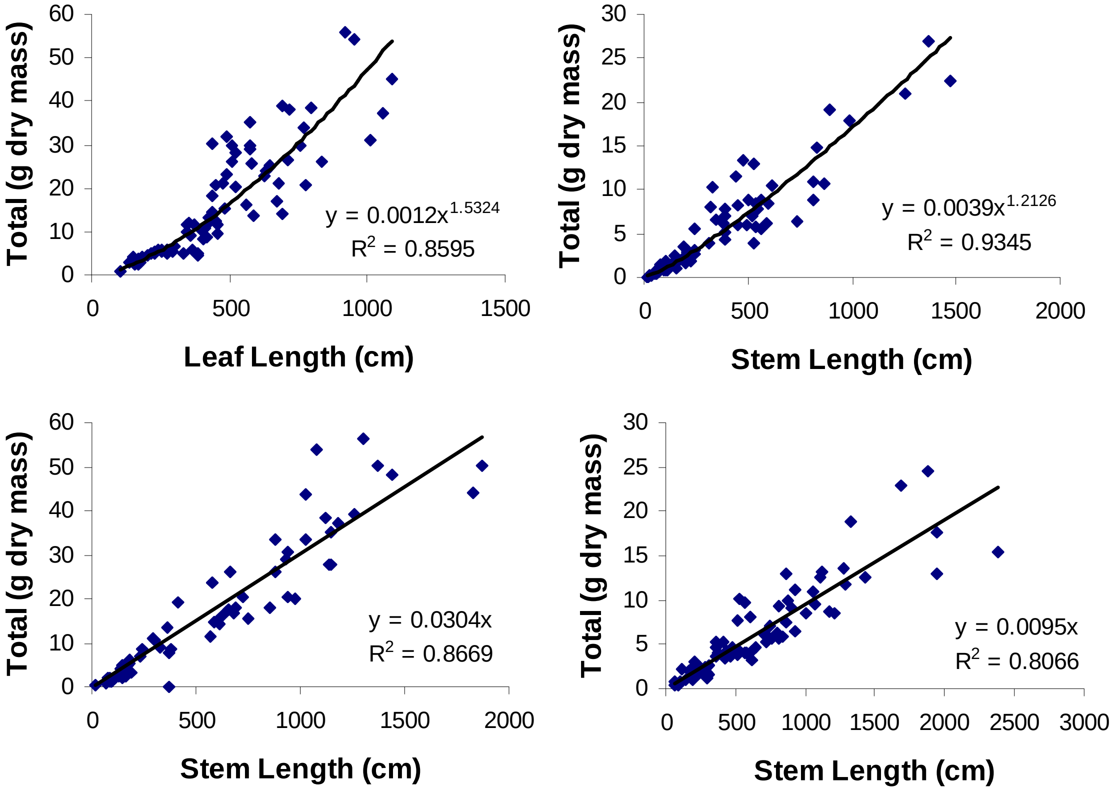


Figure 6-1: Total dry mass (g dry mass) to leaf length (cm) relationship for (a) *T. procerum* and total dry mass (g dry mass) to stem length (cm) relationship for (b) *M. simulans*, (c) *C. coronopifolia* and (d) *B arthrophylla.*

To calculate the RGR during the four week recovery phase the dry mass at the beginning of the recovery phase was estimated via the relationships in Figure 6-1.

6.2.4 Statistical analysis

Statistical analyses were carried out using the statistical software package JMP® (version 4). For each species, one-way ANOVAs were used to determine if there were differences between final plant dry mass after exposure to the three conductivities for either three or six weeks and after plants were transferred to freshwater (<1500 µS cm^{-1}) for four weeks. Two-way ANOVA's, factor one salinity (three levels), factor two duration (two levels) and an interaction term (salinity × duration) was used to determine if there were differences between final dry mass measures for plants exposed to three salinity levels for different duration periods (three or six weeks) and the subsequent plant responses when they were transferred to freshwater for four weeks. Normality of the data was tested using a Shapiro-Wilk test, homogeneity of variance with the O’Brien test and the Tukey HSD test was used to compare means.

6.3 Results

6.3.1 Triglochin procerum

A three week exposure to the three salinities did not influence RGR, total, leaf or tuber dry mass but both below ground mass and root mass were highest at conductivities of 6250 μS cm^{-1} (Figure 6-2). However, an additional effect of salinity became apparent when plants were transferred to freshwater for four weeks; total mass, leaf mass and root mass were highest for plants exposed to 1500 μS cm^{-1} and lowest for plants previously exposed to 12500 μS cm^{-1}.

Exposure to the three salinities for six weeks did not effected plant mass but did RGR because of the rapid leaf turn over which was incorporated into the calculation of RGR. On transfer to freshwater for four weeks those plants that had been exposed to 12500 μS cm^{-1} recorded the lowest mass measurements.

The duration, three or six week, that plants were exposed to salinity influenced the final total dry mass (F=9.88 p = 0.0056), the leaf and root mass (F=14.07. p = 0.0015; F=12.84, p=0.0021) but not the RGR and there was no influence of salinity nor was there evidence for an interaction between salinity and duration (Figure 6-2). During the four week recovery phase tuber mass was influenced by an interaction between salinity and duration (F=4.31 p=0.0295) whereas final dry mass (F= 4.25, p=0.0308) and leaf mass (F=19.36, p=<0.0001) was influenced by salinity (Figure 6-2). Root mass responded to both salinity and duration (F=6.23 p=0.0089; F=4.86, p=0.0408) but below ground mass only responded to duration (F=6.04, p=0.0243). To calculate the RGR of *T. procerum* it was necessary to take into account for its rapid leaf turnover rate. After the four week establishment phase, the number of leaves increased in all treatments but there were no differences among the number of leaves per pot or total leaf length with treatment. After a short lag, leaf numbers at 12500 μS cm^{-1} in both the three and six week exposures responded positively to fresh water. After a four week recovery, the final number of leaves for plants grown at 6250 μS cm^{-1} (11±1.1 and 11.4±4.2) and 12500 μS cm^{-1} (13.6±3.7 and 12.4±3.6) were similar for both the three and six week, respectively.

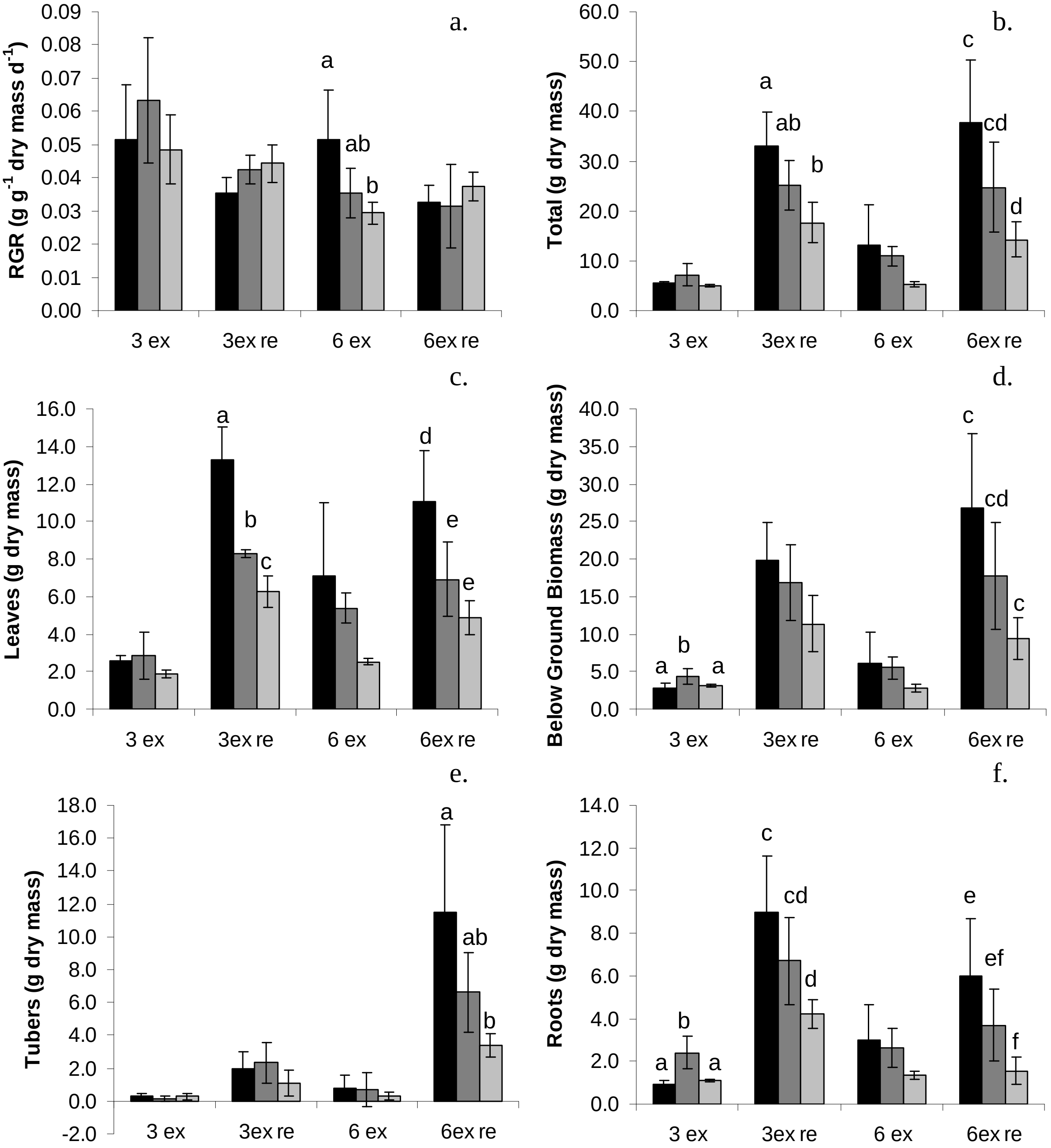


Figure 6-2: (a) RGR (g g^{-1} dry mass d^{-1}), (b) Total (g dry mass), (c) Leaves (g dry mass), (d) Below ground biomass (g dry mass), (e) Tubers (g dry mass) and (f) Roots (g dry mass), for *T. procerum* exposed to conductivities of <1500 µS cm^{-1} (black bars), 6250 µS cm^{-1} (dark grey) and 12500 µS cm^{-1} (light grey) where 3 ex and 6 ex represent the three week and six week exposure periods and 3 ex re and 6 ex re represent the exposure periods plus a four week recovery period. SE bars are shown and where letters are present, they refer to significant differences within a time period, due to salinity effects, as determined by a one-way ANOVA.

Plants at 1500 µS cm^{-1} for the three week exposure plus four week recovery had consistently more leaves than the other treatments. At each salinity level, the final number of leaves was similar for both the three week and six week exposure followed by a four week recovery. For the six week treatments, there was a clear separation of average leaf lengths between the different salinity treatments; however in the recovery phase there was a decrease in average leaf length exhibited by all salinity treatments. For both the three week and six week exposures, by the end of the recovery period the average leaf lengths were similar across salinity treatments with leaf lengths of between 38.2±3.4 and 43.9±4.0.

6.3.2 Myriophyllum simulans

The only influence that a three week exposure to the three salinities had was a small but significant increase in the root mass at the two higher salinities (Figure 6-3). On transfer to freshwater for four weeks, the stem dry mass was significantly lower for those plants that had been exposed to 12500 μS cm^{-1} but there were no detectable differences in the other parameters (Figure 6-3). After exposure to the three salinities for six weeks, RGR, total and stem dry mass were highest at 6250 μS cm^{-1}. On transfer to freshwater for four weeks those plants that had been exposed to 1500 μS cm^{-1} had the highest total dry mass compared to those that had been exposed to the higher salinities. During the exposure period, RGR and stem dry mass was influenced by an interaction between salinity and duration (F= 14.71 p=0.0002; F=3.59, p=0.0490); the origin of the interaction was exposure for three weeks at the three salinities did not influence RGR or stem mass but after six weeks exposure the RGR and stem mass at 6250 μS cm^{-1} was significantly greater than at the other conductivities. During the four week recovery phase the final dry mass was negatively influenced by salinity (F=10.0 P=0.0012), RGR by the duration of exposure ((F=5.34, P=0.033) and stem mass by an interaction between salinity and duration (F=3.80, p=0.0419).

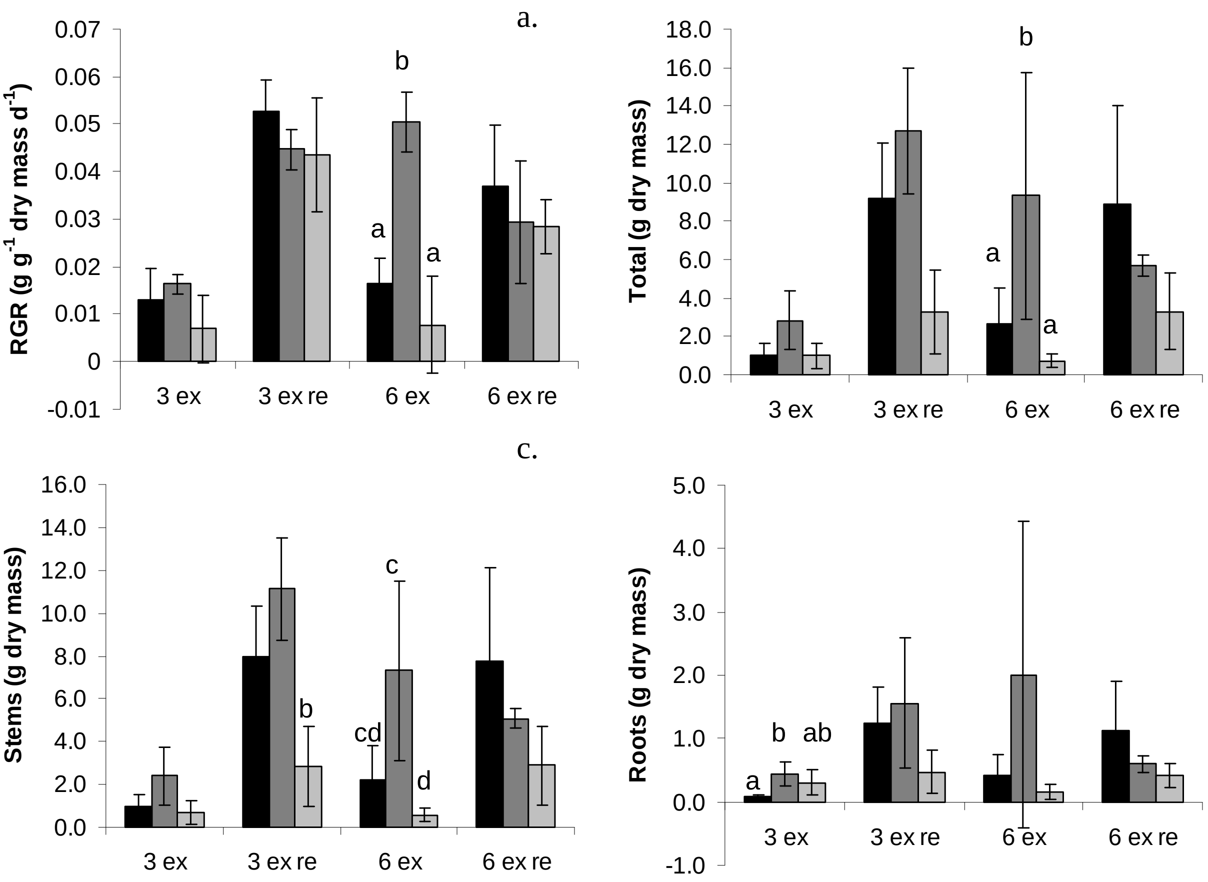


Figure 6-3: (a) Dry weight RGR (g g^{-1} day^{-1}), (b) total dry weight (g), (c) dry weight of stems (g), and (d) dry weight of roots (g), for *M. simulans* exposed to conductivities of <1500 µS cm^{-1} (black bars), 6250 µS cm^{-1} (dark grey) and 12500 µS cm^{-1} (light grey) where 3 ex and 6 ex represent the three week and six week exposure periods and 3 ex re and 6 ex re represent the exposure periods plus a four week recovery period. SE bars are shown and where letters are present, they refer to significant differences within a time period, due to salinity effects, as determined by a one-way ANOVA.

6.3.3 *Cotula coronopifolia*

The experiment was unable to detect any statistically significant differences in the response of any of the plant measurements to salinity within either the three and six week exposures or the four week transfer to freshwater (Figure 6-4). Two-way ANOVA’s showed that during the exposure phase, duration influenced the final dry mass (F=13.90, p=0.002), leaf dry mass (F=14.84, p=0.002) and stem dry mass (F=16.25, p=0.001).

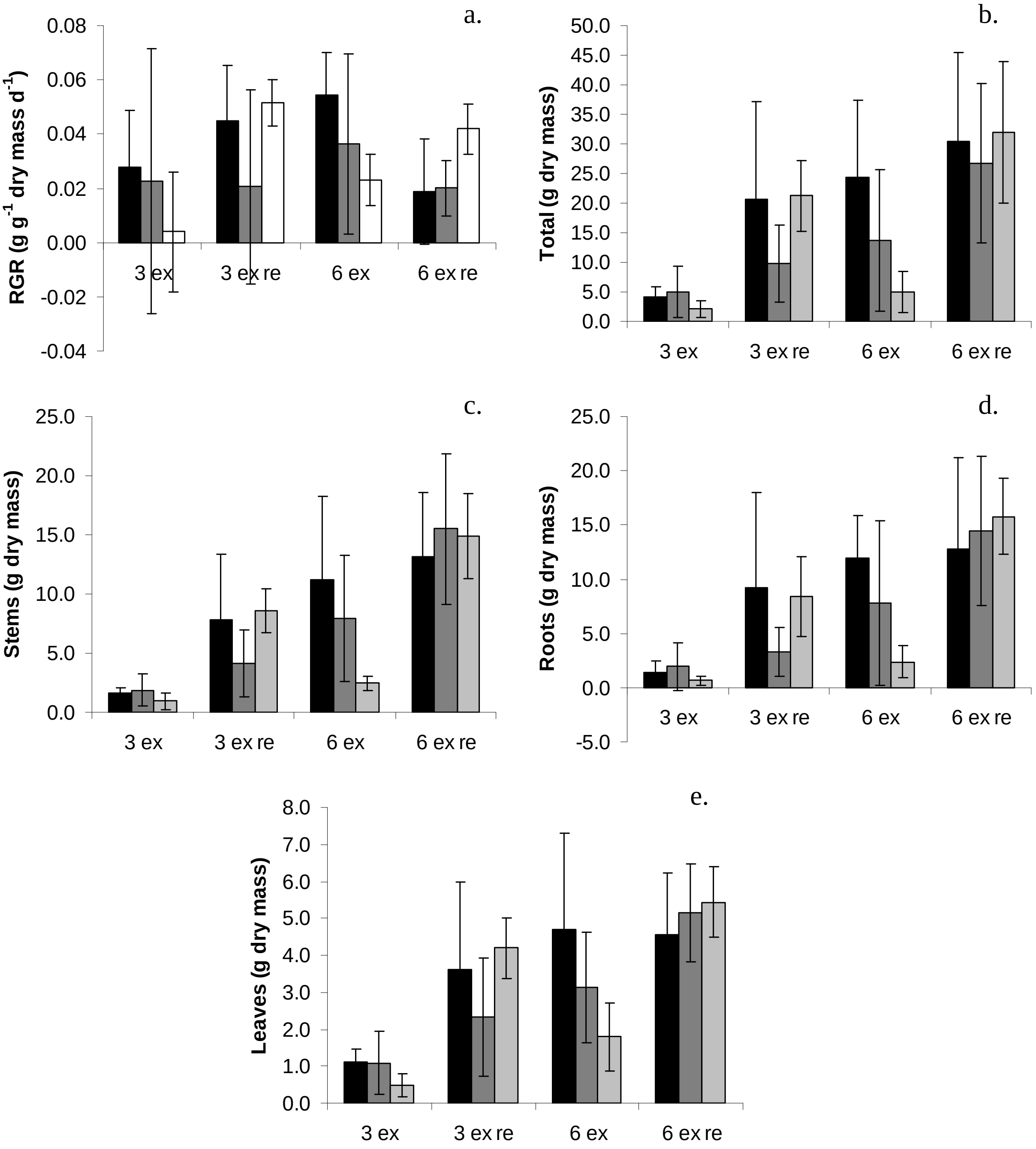


Figure 6-4: (a) Dry weight RGR (g g^{-1} day^{-1}), (b) total dry weight (g), (c) dry weight of stems (g), (d) dry weight of roots (g) and (e) dry weight of leaves (g), for *C. coronopifolia* exposed to conductivities of <1500 µS cm^{-1} (black bars), 6250 µS cm^{-1} (dark grey) and 12500 µS cm^{-1} (light grey) where 3 ex and 6 ex represent the three week and six week exposure periods and 3 ex re and 6 ex re represent the exposure periods plus a four week recovery period. SE bars are shown and where letters are present, they refer to significant differences within a time period, due to salinity effects, as determined by a one-way ANOVA.

Root dry mass was influenced by an interaction between salinity and duration (F=4.25, p=0.036) which was probably as a result of the root mass being highest for plants exposed to 1500 µS cm^{-1} for six weeks whereas there was no difference between the salinities for the three week exposure. After the recovery period there were no detectable differences between the plant characteristics.

6.3.4 *Baumea arthrophylla*

No differences were detected between salinity treatments for each time period (Figure 6-5). During the exposure phase the experiment detected an effect of duration on root dry mass (F=5.04, p=0.039) and during the recovery treatment an influence of duration on stem dry mass (F=4.75, p=0.043).

6.3.5 *Survival and biomass allocation*

All four species survived exposure to salinity pulses of three and six weeks. However, one of the replicate *C. coronopifolia* plants exposed for six weeks to 6250 µS cm^{-1} and 12500 µS cm^{-1} died by the end of the recovery period After a four week recovery period all species showed an increase in the total dry mass compared with the total dry mass immediately before the salinity exposure. These results suggest that the four species can tolerate salinity pulses of between 6250 and 12500 µS cm^{-1}. For each species in the six week exposure followed by a four week recovery treatment the total plant biomass was plotted against the photosynthetic tissue dry mass. The relationships were linear. For *T. procerum,* y=0.23x + 1.66, r^2=0.94, p=<0.0001, n=24; for *M. simulans,* y=0.86x + 0.14, r^2=0.99, p=<0.0001, n=24; for *C. coronopifolia,* y=0.11x + 1.17, r^2=0.81, p=<0.0001, n=21; and for *B. arthrophylla,* y=0.53x + 0.16, r^2=24, p=<0.0001, n=24.

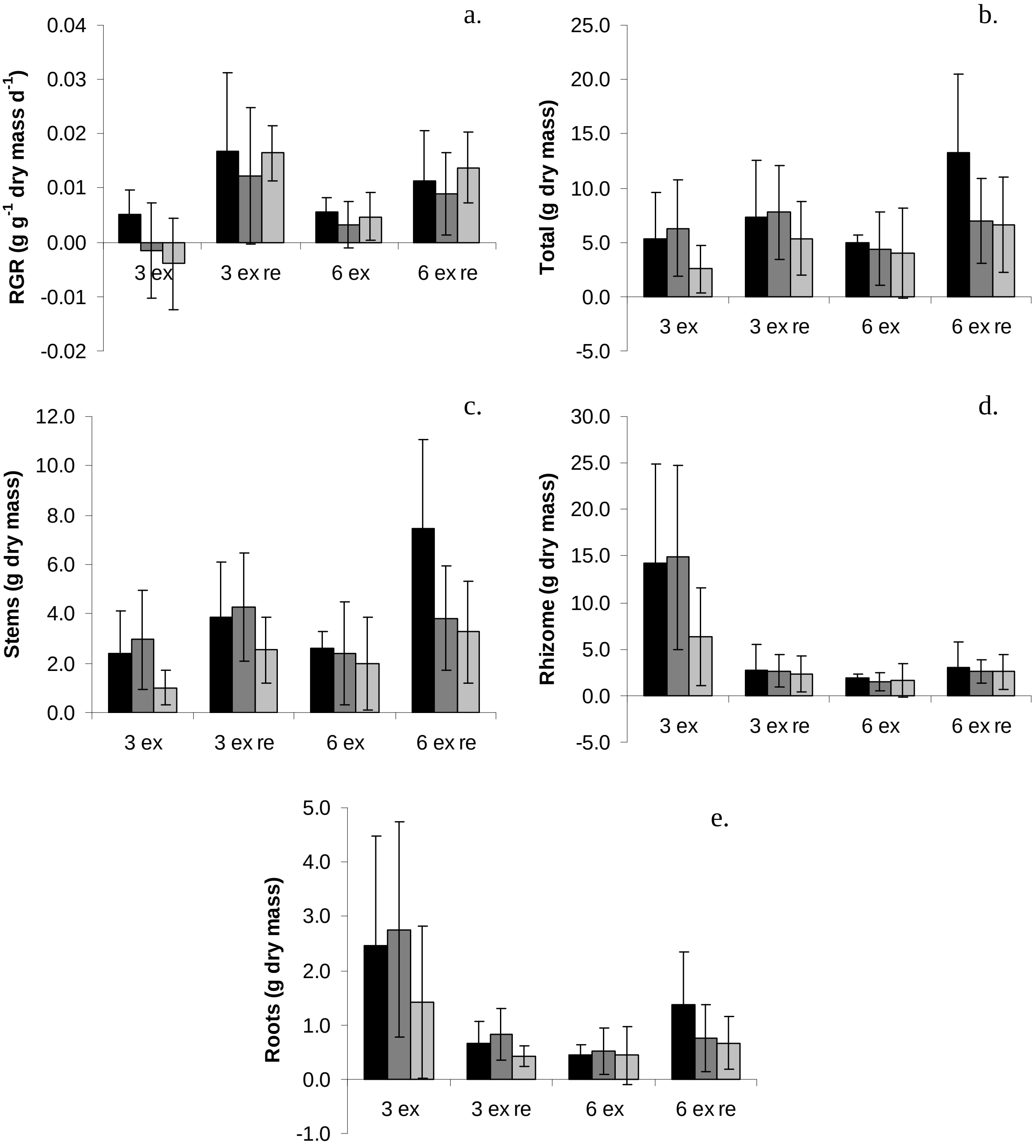


Figure 6-5: (a) Dry weight RGR (g g^{-1} day^{-1}), (b) total dry weight (g), (c) dry weight of stems (g), (d) dry weight of roots (g) and (e) dry weight of leaves (g), for *B. arthrophylla* exposed to conductivities of <1500 µS cm^{-1} (black bars), 6250 µS cm^{-1} (dark grey) and 12500 µS cm^{-1} (light grey) where 3 ex and 6 ex represent the three week and six week exposure periods and 3 ex re and 6 ex re represent the exposure periods plus a four week recovery period. SE bars are shown and where letters are present, they refer to significant differences within a time period, due to salinity effects, as determined by a one-way ANOVA.

6.4 Discussion

6.4.1 Survival

Previous field work has shown that common freshwater macrophytes such as the species used here are not found at or above conductivities of 6250 µS cm^{-1} (Brock 1981). However, in this study plants experienced conductivities of 12500 µS cm^{-1} and the 100% survival exhibited by *T. procerum*, *M. simulans* and *B. arthrophylla* shows that these plants can tolerate exposure for up to six weeks in saline conditions. These results show that the toxic effects of elevated salinities are not immediate, but are due to long term exposure. The fatalities recorded for *C. coronopifolia* occurred in the four week recovery period after six weeks exposure and were plants that had performed poorly throughout the experiment. Therefore it is likely that these deaths can be attributed to an inability of the plants to establish rather than the treatments to which they were exposed.

6.4.2 Above Ground Biomass

Munns and Termaat (1986) state that the earliest response of a non-halophytic plant exposed to an elevated salinity is that leaves grow more slowly. *Triglochin procerum* demonstrated this by producing fewer leaves rather than a reduction in average leaf length, a finding that is contrary to that reported by James and Hart (1993), who recorded a decrease in leaf length but no difference in number of leaves produced. A reduction in leaf length also corresponds to a reduction in the photosynthetic area, resulting in a decrease in the amount of carbon the plant is able to acquire for growth and ultimately the ability to complete its lifecycle. *Myriophyllum simulans* showed a reduction in stem mass as salinity levels increased. Both *T. procerum* and *M. simulans* have a large proportion of their biomass directly in contact with the water in which they grow and it is perhaps this aspect of their morphology that makes it more difficult for them to tolerate saline environments than *C. coronopifolia* or *B. arthrophylla*.

6.4.3 Below Ground Biomass

Differences in the total below ground biomass of *T. procerum* only became apparent in the recovery phase, suggesting that it is capable of withstanding short periods of exposure to saline conditions but its ability to recover is compromised when exposed to conductivities of 12500 µS cm^{-1}. The ability of *T. procerum* to accumulate biomass in the recovery period was compromised at higher salinities at both pulse durations as were the root dry mass. To exist in a saline environment, plants must take up water whilst excluding salt. Under saline conditions the water potential gradient between the external media and the xylem is lower, impeding the uptake of water by the roots and leading to internal water deficits (Colmer 1999). An under-developed root system may also reduce a plant’s ability to obtain nutrients and minerals required for growth and this compromises the long term survival and vigour of the plant.

Triglochin procerum tubers are thickened fleshy underground storage organs which accumulate reserves. The length of time the plants were grown impacted the mass of the tubers and roots. Even under fresh conditions it took the plants more than seven weeks to start producing tubers, however the plants’ ability to do this was also affected by salt exposure. The mass of tubers increased after six weeks exposure and four weeks recovery and the effect of salinity could also be detected. Without these storage organs, the plant has no reserve to draw on and therefore the health and survival of the plant is likely to be reduced. If repeat pulsed salinity releases are to be carried out, the plants would have a much better chance of recovering if they had storage reserves and hence the time it takes for these reserves to be accumulated should be factored into the time between pulse releases. Salinity exposure has been shown to reduce the belowground biomass of other tuber producing wetland plants. (Martin and Shaffer 2005) showed that under controlled salinity, hydrologic regime and substrate type, *Sagittaria lancifolia* L had a reduced belowground biomass production at a conductivity of 8800 μS cm^{-1} in comparison to plants which were grown at 1500 μS cm^{-1}.

6.4.4 Total Biomass

Biomass is often used as a surrogate for carbon gain. The reduced total biomass in the elevated salinity treatments, exhibited by *T. procerum* after the recovery phase, indicate that up to six weeks exposure to saline conditions did not affect the plants’ ability to accumulate biomass. However, their ability to recover from the changing conditions from saline to freshwater was compromised. In contrast, for *M. simulans*, a difference in total dry biomass between salinity treatments was detected after six weeks exposure and after four weeks recovery, indicating that for this species, a greater than three week exposure to saline conditions affects its growth and subsequent recovery. *Cotula coronopifolia* was unaffected by the treatments with large variation in the results making conclusions difficult. This species can be associated with more saline conditions (Sainty and Jacobs 2003) and therefore it is expected to thrive in the higher salinity conditions. It is also a species that may be associated with disturbances and therefore would be less affected by changing conditions. Stress may upset the functional equilibrium of a plant (Brouwer 1983 cited by Poorter and Nagel 2000). (Poorter and Nagel 2000) showed that variations in light and nutrient supply resulted in biomass allocation changes in *Geum urbanum* L. However in this study, after a six week exposure to salinity followed by a four week recovery period, the response of all species indicated that exposure to salinity did not affect the proportion of the total biomass the plants allocated to photosynthetic material.

6.4.5 Relative Growth Rate

The RGRs of *T. procerum* were different after six weeks exposure and a two-way ANOVA showed that the duration of the exposure to salinity was the major factor discriminating between the recovery treatments. For *M. simulans* the variable results suggest that the significant salinity – duration interaction may be a product of this variation. The source of the variation is most probably the initial plant material; although the plants may have appeared ‘equal’ at the beginning of the experimental period, there may have been ontogenic differences resulting in large differences in the final measures. No differences between the RGRs of *C. coronopifolia* were detected within treatments between salinities. Between recovery treatments both salinity and duration were statistically significant factors. However, the variation within and between treatments obscures any biological conclusion. The salinity treatments had very little effect on the growth of the sedge *B. arthrophylla* during exposure and in recovery and this may be attributed to its very low growth rate. Despite the variability in the RGRs measured in this study, they are consistent with the RGRs recorded for aquatic macrophytes in previous studies; 0.02 g g^{-1} day^{-1} for *Vallisneria americana* Michx. (now *Vallisneria australis* S.W.L.Jacobs & Les) (Blanch, et al. 1998), between 0.03 g g^{-1} day^{-1} and 0.042 g g^{-1} day^{-1} for *Bolboschoenus medianus* (V.J.Cook) Sojak (Morris and Ganf 2001), 0.044 g g^{-1} day^{-1} *B. medianus* (Blanch, et al. 1999) and 0.4 g g^{-1} day^{-1} for *T. procerum* and 0.036 g g^{-1} day^{-1} *B. arhtrophylla* (Rea 1992). Rea (1992) also records negative RGRs for *B. arthrophylla* during the summer months.

Plant growth is dependent on leaf expansion to provide the photosynthetic material required for carbon fixation and growth. Growth of root material is important for water and nutrient uptake. As soon as there is an inhibition of these factors, the total biomass of the plant is affected. For *T. procerum,* despite having reduced leaf, root and total dry mass at the higher salinities, there was no difference in RGR. *Triglochin procerum* is a species that has a high leaf turnover rate and the leaves that were grown and then died were included in RGR calculations. Leaf loss at different salinities was similar but plants grown under fresher conditions produced more leaves than those grown in saline conditions. As a consequence although the final masses differed the proportional increase was similar leading to similar RGRs. Therefore, unlike in mangroves where leaf drop has been widely accepted as a salt tolerance mechanism (Thomlinson 1994) in *T. procerum,* increased salinity does not increase leaf drop but reduces new leaf growth. This is in contrast to that reported by Warwick and Bailey (1997) where conductivity exposure up to 8800 μS cm^{-1} had little effect on leaf gain or loss.

6.4.6 Management implications

It is important to assess both the immediate, short term and long term effects of exposure to saline conditions. For the plants tested, this study demonstrates that the immediate effect of high salinity environments on non-halophytic plants is not detectable after three to six weeks of exposure, but the short term impact of the pulse does affect the ability of submerged plants to recover. Nielsen et al. (2007) showed that there was no immediate or short term impact on the emergence of aquatic plants from wetland sediments which were exposed to a high salinity pulse of water of short duration (2 weeks) followed by a return to fresh. Warwick and Bailey (1998) reported that *Potamogeton tricarinatus* A.Benn. exposed to conductivities of up to 8800 μS cm^{-1} immediately after turion emergence survived although were reduced in size, however, those exposed 34 days post-emergence experienced catastrophic leaf loss. Therefore the timing of the pulse release is important and needs to coincide with the life stages of the plants.

Vallisneria australis, an Australian native, freshwater macrophyte has a similar growth form and thrives in similar environmental conditions to *T. procerum*. Research by Salter et al. (2008) showed that *V. australis* grown at 16900 μS cm^{-1}, submerged for 20 weeks had a 100% survivorship but reduced biomass and leaf length compared to those grown at 950 µS cm^{-1}. However, they concluded that under fluctuating or adverse water conditions, such as drying for up to 13 weeks followed by submergence, salinity strongly compromised the resilience of *V. australis.* The initial condition of the plants in the wetland is a critical factor in determining if the application of the water, even in a pulsed regime, will be beneficial to the plants. Work by Morris and Ganf (2001) using *B. medianus* demonstrated that under saline conditions (up to 12200 µS cm^{-1}), increasing the nutrient load increased plant performance. Therefore, if saline water is to be used in wetlands, it should be ensured that the nutrient load of the water is sufficient to mitigate against the toxic effects.

The emergent sedges such as *B. arthrophylla* and the herb land species such as *C. coronopifolia* may be more resilient to short term salinity effects, however the long term consequences of pulsed salinity regimes also need to be considered. In wetlands it is important to ensure that the salt water can indeed be purged from the wetland but due to the ephemeral nature of many of Australia's wetlands, as they dry the salt will accumulate in the sediment. Historically, in wetlands that are linked to watercourses or in flow paths, it would have been flushed out during the next high-flow event however many wetlands no longer receive the high flows required for flushing (Nielsen, et al. 2003). Over time this accumulation would lead to a build up of salt in the basin, which has the potential to be re-dissolved when the wetland next receives water. This would add to the overall salt load of the water in the wetland and could be a potential risk for rivers if a pulse release occurred during a low flow period. The long term impacts of salinity on flowering and viable seed set need further investigation. Plants growing in ephemeral wetlands are at risk because if a saline pulse slows their growth they are unlikely to complete their life cycles before the wetlands dry, and therefore fail to leave behind propagules resulting in a depletion of the seed bank of the wetlands. In this trial, only the *C. coronopifolia* flowered. It is important to recognise that when combined with other stressors that are experienced under field conditions such as herbivory, competition and flooding, the results from this controlled experiment may underestimate the effect of elevated salinities on plant health.

7 The effect of inundation and salinity on the germination of seed banks from wetlands in South Australia

7.1 Introduction

In many regions, the hydrologic cycle is modified continually by land use and climate change. In Southern Australia the latter is likely to result in hotter, drier conditions and more variable wetland water regimes (Warwick and Brock 2003). Periods of low surface water availability often coincide with increased salinity (Hart, et al. 1990, Nielsen and Brock 2009, Nielsen, et al. 2003), because evapo-transpiration causes salts to accumulate in both surface water and sediment (James, et al. 2009).

In wetlands subject to wide environmental fluctuations, regeneration of plant communities depends upon a seed bank or vegetative propagules (Brock, et al. 1994). The maintenance of a propagule bank is a major factor in the continued existence of many plants common to Australia's temporary wetlands. Plants survive by building up a seed bank during favourable conditions, which acts as a refuge (Warwick and Brock 2003). Leck and Brock (2000) reported that seeds from Australian species all tolerated drying, and most germinated rapidly on re-flooding; a prerequisite for survival in temporary wetlands.

Both salinity and water regime influence germination (Casanova and Brock 2000, Keddy and Constabel 1986). Increasing salinity may: reduce viability of the seeds, thus reducing seed bank potential, block the cues that trigger emergence, leaving propagules alive but dormant in the seed bank, or not affect emergence but seedling death follows quickly (Nielsen, et al. 2003). Under high salt conditions, this may act as a pre-selection pressure such that species whose seeds are intolerant to such conditions are selected against. At salinities above 1500 μS cm^{-1} species richness and abundance of freshwater aquatic plants decreases (Brock, et al. 2005, James, et al. 2009, Nielsen, et al. 2003, Smith, et al. 2009). Different water regimes, and the duration of flooding events in particular, result in different assemblages of species (Casanova and Brock 2000) and previous environmental conditions experienced by a wetland may also influence emergence from the seed bank (Britton and Brock 1994, Brock, et al. 2005). Warwick and Clarke (1993) demonstrated a pronounced increase in variability among replicate samples from perturbed treatments in a variety of environmental impact studies. They suggest that variability in itself may be an identifiable symptom of perturbed situations.

The Upper South East (USE) region of South Australia has over 200 ephemeral wetlands; however dryland salinity has lead to the construction of a network of deep groundwater drains, often running adjacent to wetland complexes. This has lead to a drop in local groundwater levels, impacting the hydrology of many wetlands. This, in combination with drought conditions, has resulted in many of the wetlands experiencing extended dry periods; up to eight years in some cases. Consequently the USE is a region in which water for environmental purposes is becoming scarce and it is therefore important to know how wetland seed banks respond to re-wetting under a range of salinities and water regimes after experiencing extended periods of dry conditions at varying soil salinities.

This study investigates the combined stressors of previous hydrology and salinity regimes on seedling emergence from the seed bank under different water regimes and concludes whether or not saline drainage water should be used to stimulate germination. The following hypothesis was tested: as the length of time for which a wetland seed bank has experienced drought and salinities > 1500 μS cm^{-1} increases, the diversity of species emerging from the seed bank will decrease, irrespective of the water regime or salinity treatment imposed. In this study, sediments from three wetlands in the USE representing a gradient of past water regimes and salinity, were subjected to water of four salinities: <735 μS cm^{-1}, 1500 μS cm^{-1}, 4400 μS cm^{-1}; and 7350 μS cm^{-1}, under two water regimes: drained and flooded conditions. The germination response; the number of individuals germinating and the number of species present, was examined.

7.2 Materials and Methods

7.2.1 Site description and soil collection

Three wetlands; Rocky Swamp, Hanson Scrub and Bunbury CP, were selected on the basis of their previous salinity and hydrologic characteristics (Table 7-1) so that previous surface water salinities ranged from 4100 to 29400 μS cm^{-1} and the time since last inundation from 2 to 5 years.

Table 7-1: Summary of site characteristics for Rocky Swamp, Hanson Scrub and Bunbury CP.

Characteristics	Rocky Swamp	Hanson Scrub	Bunbury CP
Location (WGS84, 54H)	426424E, 5952589N	411078E, 5952230N	406922E, 6002632N
Last Inundated (SEWCDB unpublished)	2005	2004	2002
Salinity at last Inundation (μS cm^{-1})	8800	4100	29400
Approximate Avg Winter Rainfall (mm)	233	187	165
2003, 2004, 2005 & 2006 Winter Rainfall	325, 261, 260, 74	267, 241, 189, 61,	212, 195, 181, 82
Soil Type	Light Clay	Sandy Loam	Heavy Clay
Soil Salinity$_{SE}$ (μS cm^{-11})	12000±7350	1950±600	691000±24000

At each wetland, soil samples were collected from five locations at three elevations: the deepest part of the wetland; midway between the deepest part of the wetland and the high water mark; and at the high water mark, a total of 15 samples per wetland. Each sample consisted of a 15 cm × 15 cm × 5 cm deep soil core. Samples were collected on the 11th and 12th of April 2007. The soil samples were oven dried to a constant weight at 35°C. The soil was sieved to break up sediment and gross organic matter was removed. The composition of the seed bank may differ depending on elevation however in this study the response of the total wetland seed bank was under investigation. For each wetland, five composite samples consisting of a sample randomly selected from each of the three different elevations were produced. This ensured that each sample contained sediment representative of the whole wetland.

7.2.2 Experimental design

Aluminium trays (19 × 11 × 7 cm) were filled with sandy loam to a depth of 6.5 cm. 150 g of dried, sieved sediment from each composite sample was spread out on top of the sandy loam. The large sandy loam to sediment volume ratio ensured that the influence of the initial soil salinity was minimised. A slow release fertilizer (Osmocote® and Osmocote Plus®) was added to give an equivalent nitrogen loading of 100g m^{-2} year^{-1}. To mitigate fungal infection each sample was treated with a fungicide (Fungaride®).

Samples were placed in a glasshouse to minimise temperature extremes (Britton and Brock 1994), under the assigned water regimes: drained and flooded; and salinity treatments: <735 μS cm^{-1}; 1500 μS cm^{-1}; 4400 μS cm^{-1}; and 7350 μS cm^{-1}. The treatments were imposed by placing samples in one of four water treatments, each treatment containing the water with a different salinity treatment. Holes were pierced in the bottom of each tray to enable the exchange of water and ions. The salt concentration and depth of the water in the treatments was monitored and maintained every two to four days. By sitting the sample on the bottom of the treatment a flooded regime (under two cm of water) was imposed. Trays placed on a step within the water treatment experienced drained conditions, as only the very base of the tray was exposed to the water. As seedlings emerged, they were counted and removed from the trays fortnightly. The seedling emergence technique used by Brock et al. (1994) was followed. An individual of each species was grown until it could be identified. Plants were identified by Rosemary Taplin from the South Australian State Herbarium. The experiment lasted 16 weeks (27th April 2007 to 20th August 2007) after which no more seedlings emerged.

7.2.3 Statistical analysis

Because salinity was not replicated the statistical analysis only compared the factors wetland (site) and water regime within each salinity treatment. The means and standard deviations of the total number of germinants and number of species in each treatment were calculated. A 2-way ANOVA was used to compare the effects of different water regimes and wetlands on the number of germinants and the number of different species germinating within each of the salinity treatments. A 2-way PERMANOVA was used to determine if significant effects occurred between treatments and if there were any interactive effects, within each of the different salinity treatments. The analysis was conducted using PRIMER 6 (V6.1.10) + PERMANOVA (PRIMER-E). Using the same input data as for the PERMANOVA, a 2-D NMS ordination was produced using the PC-Ord 5.0 software (McCune and Mefford 1999).

7.3 Results

7.3.1 Seedling Emergence

A total of 25 species were recorded in the seed banks of the three wetlands; 21 species in Rocky Swamp, 19 in Hanson Scrub and 13 in Bunbury Conservation Park. Five taxa could not be identified as attempts to grow them to a stage where they could be identified were unsuccessful. The mean number of seedlings emerging ranged from 1270 m^{-2} in Rocky Swamp under drained conditions at 1500 μS cm^{-1} to 0 m^{-2} in Hanson Scrub under flooded conditions at 7350 μS cm^{-1} (Figure 7-1). Within each salinity treatment, only water regime had a significant effect on the total number of germinants, (735 μS cm^{-1}; F=18.45, p=0.0001, 1500 μS cm^{-1}; F=12.13, p=0.0012, 4400 μS cm^{-1}; F=15.21, p=0.0004 and 7350 μS cm^{-1}; F=5.70, p=0.0220) with a higher number of germinants recorded under drained conditions (Figure 7-1).

For the total number of species germinating within each salinity treatment there was an interaction between water regime and site in the 735 μS cm^{-1} treatment (F=4.63, p=0.016). Water regime had a significant effect with higher total number of species recorded under drained conditions (1500 μS cm^{-1}; F=28.73, p<0.0001, 4400 μS cm^{-1}; F=32.68, p<0.0001 and 7350 μS cm^{-1}; F=6.02, p<0.019). Site was significant in the 1500 μS cm^{-1} treatment (F=5.63, p=0.007).

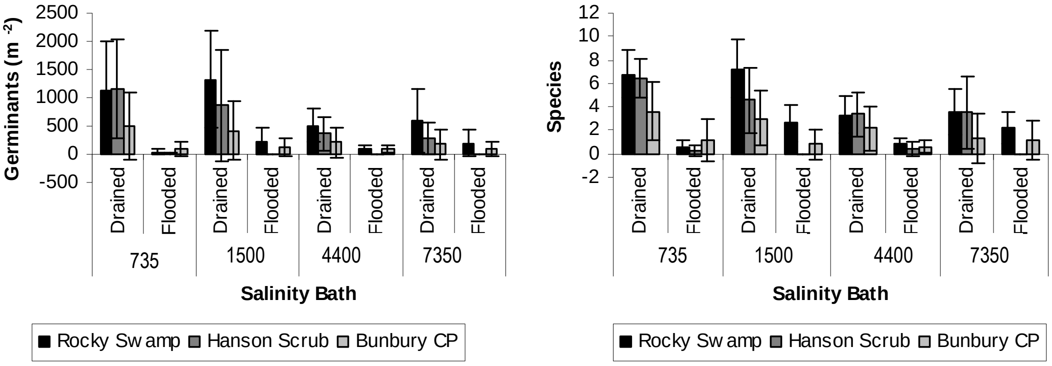


Figure 7-1: (a) Mean number of seedlings m^{-2} and (b) the number of species; that emerged from the three different wetland sediments under drained or flooded water regimes in each of the different salinity treatments (μS cm^{-1}). Bars represent standard deviations.

Numerically, Rocky Swamp was dominated by floodplain species which were greater than five times more abundant than aquatic species. For Hanson Scrub, exotic terrestrial species were the most abundant with aquatic species accounting for less than a fifth of the total number of species. Similarly, for Bunbury Conservation Park, exotic terrestrial species were most abundant while the native terrestrial species had the smallest number of individuals. For both Hanson Scrub and Bunbury Conservation Park exotic grasses (*Avena sp.* and *Hordeum sp.*) accounted for a majority of the exotic terrestrial individuals.

7.3.2 Two-way PERMANOVA

The results for a 2-way PERMANOVA examined the influence site (the previous hydrologic and salinity history of a wetland) and the effect of the imposed water regime within each salinity regime on the number of individuals of each species that germinated. There were significant interactions between the water regimes and site at all salinity treatments. At 735, 1500 and 4400 $\mu S\ cm^{-1}$at Rocky Swamp (F=2.61, p=0.003, F=2.29, p=0.003 and F=1.93, p=0.004) and Hanson Scrub (F=2.41, p=0.001, F=2.09, p=0.001 and F=1.97, p=0.001) there were significant differences between germination response under drained and flooded conditions, but there was no difference at Bunbury CP. For 7350 $\mu S\ cm^{-1}$ the interaction only occurred at Hanson Scrub (F=2.20, p=0.002).

7.3.3 Ordination

A 2-D ordination (stress=9.48) shows a separation of sites (Figure 7-2) with the majority of the Rocky Swamp samples located in the upper right hand corner of the ordination, Hanson Scrub in the upper left, and Bunbury CP samples in the lower left hand corner. The vector representing water regime had an $r^2=0.5$ however the vector representing salinity was only significant at an $r^2=0.1$. No germination response was recorded for Hanson Scrub samples under flooded conditions and therefore they cannot be shown on the ordination.

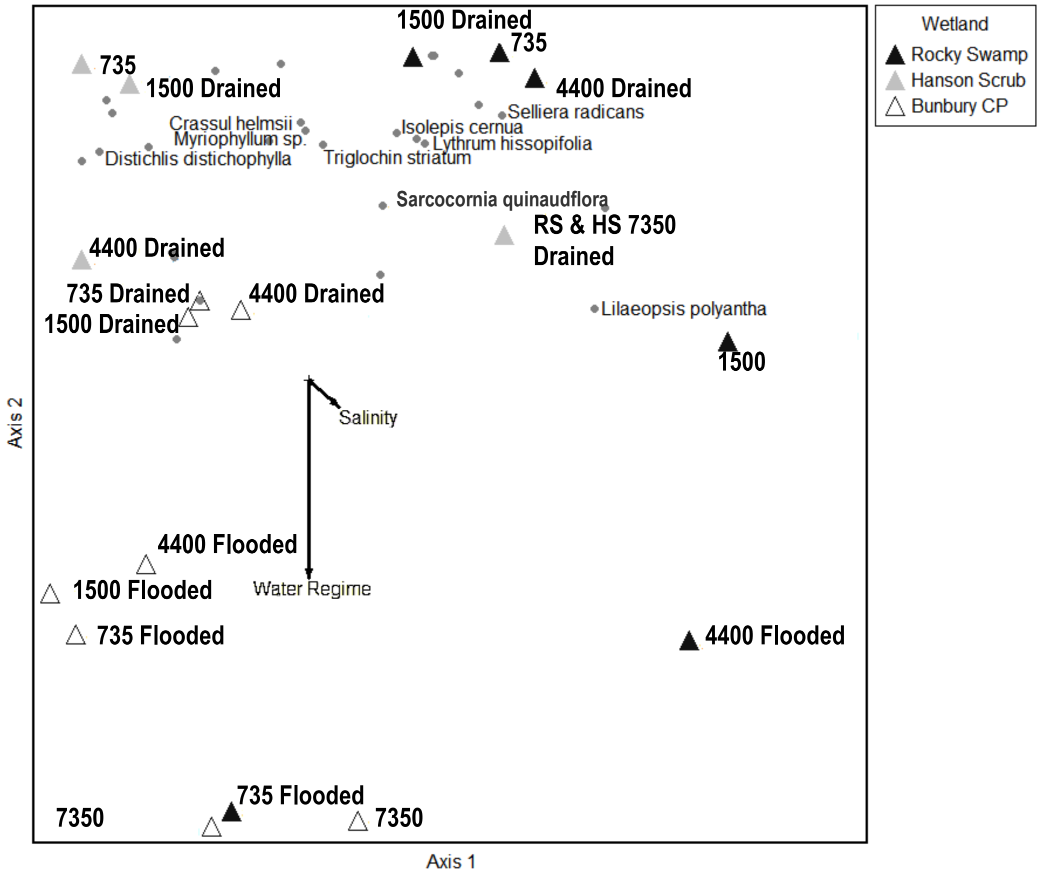


Figure 7-2: 2-D Ordination (stress =9.48) of samples (labelled triangles) in relation to the associated species (dots) with the vectors representing water regime ($r^2=0.5$) and salinity ($r^2=0.1$) displayed.

7.4 Discussion

The position of the drained and flooded sites on the ordination are consistent with the vector representing water regime (r^2=0.5) with the drained samples situated towards the top of the ordination and the flooded samples at the bottom. Various studies have demonstrated higher germination rates and diversity under drained rather than flooded conditions (Boedeltje, et al. 2002, Brock, et al. 2005, Robertson and James 2007, van der Valk and Davis 1978) and this work supports these findings. The positions of the native aquatic and riparian species which are common in wetlands in the South East region of South Australia are associated with the samples from Rocky Swamp and Hanson Scrub, the wetlands with the fresher pre-history, which were exposed to drained conditions.

Previous work by Brock et al. (2005) and Nielsen et al. (2003) has demonstrated that as salinity increases above 1500 μS cm^{-1} there is a decrease in species richness and abundance of the plants germinating. Site was a significant factor under salinity treatments of 735 and 1500 μS cm^{-1} but not at 4400 or 7350 μS cm^{-1} which suggests that there is a critical salinity between 1500 and 4400 μS cm^{-1} at which the benefits of a fresher previous history is negated by the salinity treatment imposed. This would need further experimentation using a replicated design.

The interaction detected in the 2-way PERMANOVA between site and water regime in the 735, 1500 and 4400 μS cm^{-1} indicates that Rocky Swamp and Hanson Scrub have an intact seed bank which is responsive to water regime whereby more species occur under drained conditions than flooded. At Bunbury CP where there was no difference between the drained and flooded treatments, the composition of the seed bank has been influenced by past history such that the only seeds which are left are those which are tolerant to a wide range of salinity and water regimes. The interaction in the 7350 μS cm^{-1} treatment was a result of no germination occurring under flooded conditions in Hanson Scrub, the wetland with the freshest pre-history.

Nicol et al. (2003) recorded between 22 000-78 000 seeds m^{-2} and 12-23 species in the seed bank of Bool Lagoon in the South East of South Australia. It has an average annual rainfall of 578 mm (BOM 2010) and a history of freshwater flows and had dried out during summer for the 5 years prior to the study. Brock et al. (1994) reported between 6 400-22 300 seeds m^{-2} and 11-26 species in seed banks in wetlands on the New England Tableland of New South Wales with a freshwater history but with different water regimes and histories of modification and an average annual rainfall of 791 mm (BOM 2010). Leck and Brock (2000) recorded between 8 870–21 210 seeds m^{-2} and 14-27 species in the seed bank of the Hamilton-Trenton Marsh, a freshwater tidal marsh in New Jersey, USA. Compared to these studies the numbers of seeds in the seed banks in this study were very low. Except for 2006 (Table 7-1) the winter rainfall in the other years is likely to have stimulated germination during the winter/early spring due to moisture in the topsoil from rain. However, without the presence of surface water, the seedlings would have died shortly after, preventing them from maturing and completing their lifecycles by flowering and setting seed. Over time this would lead to a depletion of the seed bank as the germinating seeds are not replaced.

For Rocky Swamp and Hanson Scrub, the number of species in the seed bank is comparable to that of the other studies suggesting that despite unfavourable previous conditions, the total number of seeds may decline but the diversity within each wetland (not across all wetlands) remains the same. However the composition of species comprising the diversity may change and there were fewer species at the higher salinity treatments. Bunbury CP had reduced diversity at all salinity treatments. The previous salinity regimes experienced may act as a pre-selection pressure such that under higher salinity conditions such as those at Bunbury Conservation Park, the seeds of glycophyte species are not viable and eventually disappear. Germination of exotic terrestrial grasses dominated in Hanson Scrub and Bunbury CP. However at Hanson Scrub and Rocky Swamp, the two wetlands with the freshest pervious history, indigenous wetland species germinated in the 735 and 1500 μS cm^{-1} treatments.

The previous drought and salinity conditions experienced by a wetland did affect the seed bank however the water and salinity regime imposed mitigated these impacts. This study provides evidence that extended periods of drought conditions may lead to a seed bank which has a reduced abundance of seeds and repeated exposure to high salinity changes the species composition of the seed bank and reduces the overall diversity. However it is likely that if favourable water and salinity regimes are provided over the next few seasons, in Rocky Swamp and Hanson Scrub, common wetland species which are still present in the seed bank may resurge. If drought conditions continue and repeat exposure to elevated salinities occurs, it would be predicted that the number of seeds and the species diversity of the seed bank of these two wetlands would follow a trajectory towards a seed bank similar to that of Bunbury CP.

8 General Discussion and Conclusions

A central goal of community ecology is to predict changes in species composition in time (succession) and in space (gradient analysis) (Shipley, et al. 1989) and this was the aim of Chapter 3. Changes in the composition of species in wetlands in the South East were comparable to those reported in relation to increasing secondary salinisation in the Wheatbelt region of Western Australia (Lyons, et al. 2004). The loss of freshwater species and replacement by halophytic species occurred over a period of two decades and this suggests that changes in composition caused by salinity effects are not likely to result from a once off salinity stress but are more likely to occur in areas where salinity has been acting for an extended (years) period of time. The results of the pond experiments (Chapter 5 and Chapter 6), which were run for 13 months and up to 10 weeks respectively, and other short term salinity studies (James and Hart 1993, Morris 1998, Salter, et al. 2007, Warwick and Bailey 1997, Warwick and Bailey 1998) support this. Although sublethal effects including reduced growth rates, lowered biomass (particularly of above ground biomass) and reduced length of morphological features such as leaf/stem length and number, and mortality rates were greater at the higher salinities, individuals of many common aquatic macrophyte species survive at salinities well above 6250 μS cm^{-1}.

It would appear that the native species of Australian lowland rivers have a level of tolerance and resilience to salinity increase, perhaps resulting from the selective pressure of the climatic history of the continent which has produced higher salt levels than the current natural level (James, et al. 2003). The conceptual model (Chapter 0) describes the process by which wetlands in areas affected by secondary salinisation, accumulate salt. Prior to land use changes, the salt that accumulated in the sediment of wetlands would have been removed by flushing during the next high-flow event (Mensforth 1996, Nielsen, et al. 2003), however climate change predictions indicate that these events are becoming less frequent (DFW 2010). This has resulted in a present rate of change that is unprecedented, and this is of concern as it is likely to be too fast for most biota to adapt (James, et al. 2003).

It is widely recognised that species richness of most biological groups shows an inverse relationship with salinity. This was demonstrated for invertebrates and waterbirds (Halse, et al. 2003, Figure 9) and Figure 4-1 depicts this same relationship for plants. Many salinity based studies and reviews have referred to a salinity of

1500 µS cm^{-1} as a level below which little ecological change and negligible sublethal effects for floral communities occur, and 6250 µS cm^{-1} as a cut off salinity above which few common freshwater macrophytes survive (Brock 1981, Hart, et al. 1991, James, et al. 2003, James and Hart 1993, Nielsen, et al. 2003, Smith, et al. 2009). The probability of occurrence curves (Chapter 4, Figure 4-3) of the freshwater species conform in general to the above guidelines, however it is clear that the wetland in which they were found had a considerable effect on the salinity tolerance of the plants. This suggests that there are between-wetland differences in the environmental conditions (including factors such as water quality and hydrology regime) that promote or suppress the salinity tolerance of aquatic plants. The effect of wetland on the salinity tolerance was also detected in the evapoconcentration experiment (Chapter 5) and the seed bank trial (Chapter 7). There were initial differences in the chemistry of the soils used from the two wetlands (Table 5-1), and this is reflective of differing previous salinity regimes.

The factor ‘wetland’ had a significant effect on the number and diversity of germinants in the seed bank trial; the native aquatic and riparian species which are common in wetlands in the South East region of South Australia were associated with the wetlands with a fresher pre-history. This is supported by Brock, et al. (2005) who reported that variable effects among wetlands may occur with both differences in the number of species and number of individuals present at low salinities and differences in the responses to salinity The specific responses and species composition varied among the seven sediments, as would be expected with local differences in wetland morphology, catchment characteristics and the history of wetting and drying and other human induced changes, varying between catchments and wetlands. Casanova and Brock (2000) found that a large proportion (60%) of the terrestrial species establishing from the intermittent wetland seed bank were exotic and suggested that invasion of exotic terrestrial species may be a consequence of the longer dry periods that are part of the natural water regime of this wetland. It has been proposed that the effects of salinity on plants germinating from a seed bank will be more pronounced at wetland edges or in temporary wetlands where water levels fluctuate, than from the same seed bank germinating in the permanently flooded areas of wetlands. This may be useful in the interpretation of first signs of salinity effects on wetland biota (Brock, et al. 2005).

Therefore, overall the salinity tolerances mentioned in previous reviews provide good general guidelines, however they do not offer information about the individual tolerances of the more salt sensitive species nor how the interaction of salinity with other factors that may vary between wetlands, including length and timing of exposure, rate of increase of salinity and repeated exposure to salinity affect the salinity tolerance of aquatic plants. The pond experiments isolated the effects of two possible hydrology and salinity regime scenarios: evapoconcentration and salinity pulses. In both experiments, the combined changes in hydrology and salinity had significant effects, however over the course of the relatively short term experimental treatments, some plants still managed to survive in salinity treatments that exceeded 25000 μS cm^{-1}, four times the widely accepted limit of 6250 μS cm^{-1} for freshwater aquatic plants. These results again suggest that it is the long term effects of extended and repeated exposure to salinity rather than a once off salinity stress that results in the loss of freshwater aquatic macrophyte species. Therefore, in an environment where surface water is a scarce resource, using pulsed releases of saline water for short periods of time may be an acceptable management tool (Chapter 6). However, repeated releases may have detrimental impacts.

Salinity is often treated as an ecological factor as if only the direct effects of the ions were significant, however it can also influence, for example, pH, dissolved oxygen and the nutrient balance of plants. It may be that biota respond as much to these indirect effects as to the direct ionic effects of increased salt (Morris, et al. 2009).

8.1 Knowledge Gaps

Many studies have focused on the effects of elevated salinity on germination from the seed bank and on plant survivorship, often in combination with standing water levels (Brock, et al. 2005, James and Hart 1993, Macek and Rejmánková 2007, Morris and Ganf 2001, Nielsen, et al. 2003, Nielsen, et al. 2007). However, fluctuations of salinity, depth and permanence (presence of water) are common and may occur on a seasonal or longer time scale (Brock and Lane 1983). Extended drought predicted as a consequence of climate change (lower rainfall and higher temperatures) combined with human induced changes to the natural hydrological regime will lead to reductions in the amount of water available for environmental and anthropogenic uses and it is predicted that rain will fall in short high intensity bursts, resulting in quicker but decreased run-off (Nielsen and Brock 2009).

Recent studies have started to reflect this with aspects of how the salinity concentration may change during the growing season (through evapoconcentration) or aspects of how altered hydrology may influence the timing of how wetlands receive water being incorporated into experimental design. *Vallisneria australis* showed tolerance to salinity when growing under conditions of permanent submersion (survival at 18000 μS cm^{-1} for 26 weeks) but results show clearly that high salinity strongly compromised the species’ ability to recover from an episode of sediment desiccation (Salter, et al. 2008). Inflorescence production by *Potamogeton cheesmanii* was strongly suppressed by evapoconcentration effects that increased salinities to 8500 μS cm^{-1} and no seed set was observed on plants that were salt-treated (James, et al. 2009). For communities developing from the seed bank, it did not make a difference to the final community whether the salinity was delivered as a sudden challenge or as a gradual change from fresh to saline over a 6 month period (Nielsen, et al. 2008). Ecological impacts of salinity are influenced by a range of factors including the sensitivity of species to salinity (including sublethal and lethal effects), rates of rise, length of time and time in a species' life cycle that salinity is experienced, combined stresses of associated factors, river regulation and flow regimes and location of a site within a catchment (Clunie, et al. 2002). There are clearly many more important combinations of hydrology and salinity regimes that need investigation, and as highlighted by work in Chapter 5 and Chapter 7, the previous hydrology and salinity history of the wetland will also impact the response of freshwater species. Before generalised outcomes on the impacts of salinity and water regime can be prepared for management application, further investigations into the levels of salinity, the interaction of salinity and hydrology, and whether the impacts of salinity can be generalised for wetlands across a wide geographic area are necessary (Brock, et al. 2005).

Separating the impacts due to flow regime change from other factors, such as water quality change and rising groundwater levels, is very difficult (Young, et al. 2000). This is coupled with the considerable temporal variation that exists in saline systems. Classifying salinity states on single values alone is clearly inappropriate for highly variable systems, and it is the frequency and range of variation, together with maximum and minimum values that are likely to be of much greater ecological significance (Davis, et al. 2003). It has been suggested that the most important characteristics of water regimes are threshold events such as extremes of depth and dry periods and that these may not be captured in measures of water regime such as average water levels (Smith and Brock 2007). A similar argument can be made for salinity regimes; that it is the threshold events in terms of extremes in salinity values lengths of exposure that are most important in determining the tolerance of halophytic species. While there are some indicative laboratory and field data on the lethal effects of increased salinity on particular species, this information is limited because it covers only short-term, acute effects, and a relatively small number of test species (mostly adults) have been used. Additionally, there are few (if any) studies on sub-lethal or long-term effects or on possibly more sensitive life stages. (Hart, et al. 1991).

Groundwater – surface water interactions in wetlands are highly dynamic, both temporally and spatially, yet poorly understood (Jolly, et al. 2008). Currently there is a lot of emphasis on the groundwater dependence of wetlands in the South East. There is clear evidence that groundwater dependent ecosystems are at risk in the South East as a result of falling groundwater levels (DFW 2010), and their requirements are being considered for the new and future Water Allocation Plan of the Lower Limestone Coast (SKM 2010). Modelling shows that as the groundwater levels decline the wetland habitat is progressively lost, with no wetlands remaining when the groundwater level is 1.5 m (DFW 2010), however there has not been any modelling done to predict the associated increase in salinity that is likely to occur.

Due to their complexity, James *et al.* (2003) suggest many of the remaining knowledge gaps can only be addressed through a multidisciplinary approach carried out in an adaptive management framework, utilising decision-making and ecological risk assessment tools. Innovative experimental science, together with imaginative predictive management can work together to underpin salinity management issues on both broad and local scales (Nielsen, et al. 2003).

8.2 Conclusions

As salinity levels rise, biotic communities respond in two fundamental ways. First, the most intolerant species within the community are lost from the system and secondly, tolerant species begin to competitively dominate (James, et al. 2003). Management of biodiversity can only take place in a whole-of-landscape context (Cocks 2003), however the hydrology of individual wetlands has important consequences for the development of increased salinity levels. Wetlands that form terminal systems (those that hold water after flood flows have receded) are potentially at greater risk than flow-through systems, as evapotranspiration from terminal systems will result in extremely high salt concentrations in the remaining body of water and in the surrounding soil (Cramer and Hobbs 2002). Given the importance of freshwater systems in the provision of ecological services and diverse habitats for a huge range of species, there is a clear need for restoration that can maintain sustainable ecological services whilst reinstating ecosystem function and habitat range (Giller 2005).

Finally, our knowledge of wetland plants, habitats, individual wetlands and their pattern in the landscape enables interpretation of how wetland plants have changed and will change in the landscape. The challenge is to predict what future wetland landscapes might look like under different management or development scenarios and to decide what is sustainable (Brock 2003).

Appendix I. Copy of publication of work from Chapter 6

Goodman, A.M., Ganf, G.G, Dandy, G.C., Maier, H.R. & Gibbs, M.S. (2010). The response of freshwater plants to salinity pulses.
Aquatic Botany, v. 93(2), pp. 59 -67

NOTE:
This publication is included on pages 105-113 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

http://dx.doi.org/10.1016/j.aquabot.2010.03.004

Appendix II. Copy of publication of work from Chapter 7

Goodman, A.M., Ganf, G.G, Maier, H.R. & Dandy, G.C. (2011). The effect of inundation and salinity on the germination of seed banks from wetlands in South Australia.
Aquatic Botany, v. 94 (2), pp. 102 -106

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